Current Science International
Volume: 11 | Issue: 03| July – Sept.| 2022

EISSN:2706-7920 ISSN: 2077-4435
DOI: 10.36632/csi/2022.11.3.24
Journal homepage: www.curresweb.com
Pages: 329-341

Morphology of Peltate Glandular Trichomes in Healthy Leaves of Field-Grown Sweet Basil Plants, Propagated Plantlets *In vitro*, and Naturally Infected by *Alfalfa Mosaic Virus*

Eman M. EL-Abagy and Samah A. Mokbel

Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, 12619, Egypt.

Received: 25 July 2022 **Accepted:** 30 August 2022 **Published:** 10 Sept. 2022

ABSTRACT

The variations in the peltate glandular trichome (GT) structures on the leaves of healthy *Ocimum basilicum* and infected ones with the *Alfalfa mosaic virus* (AMV) in the open field, as well as the propagated plantlets under *in vitro* conditions, were examined using scanning electron microscopy (SEM). Infected basil leaves with AMV showed severe malformations of peltate GT on leaves, which differed structurally by viral infection. A double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to assess the elimination of AMV using three apical meristem sizes (0.2, 0.3, and 0.4 mm). All the used sizes produced AMV-free *O.* basilicum explants (100%). The apical meristem sizes (0.3 and 0.4 mm) had a pronounced effect on the maximum establishment or ratio of survival rates (76.6 and 80 %). The AMV-free plants derived from the meristem size (0.4 mm) were multiplied on MS medium supplemented with 0.5 mg/l of BA and 0.5 mg/l of JA. Micrographs of *in vitro* cultures displayed broad secretory glands and trichomes of type non-glandular, indicating somewhat significant homogeneity among the adaxial and abaxial leaf surfaces of the regenerated plants *in vitro* and the healthy basil leaves in the open field. In conclusion, GT structure in diseased leaves might serve as a sign of viral disease stress. Based on the findings of this study, the food, cosmetics, and pharmaceutical industries also benefit significantly from the *in vitro* method.

Keywords: Scanning electron microscopy, Glandular trichomes, Meristem culture, Virus control, *In vitro* vegetative propagation.

1. Introduction

Sweet basil (*Ocimum basilicum*) is one of several aromatic herbs of the genus *Ocimum* and belongs to the family Lamiaceae with 250 genera and more than 7,000 species (Dosoky and Setzer, 2018). Members of the family Lamiaceae possess both non-glandular and glandular trichomes (GT) which are classified according to a head of a glandular in different plant parts, like the foliar vegetative parts (Meer *et al*., 2022). The GT structure act in plant taxonomy to differentiate among species or as discriminative characters at the subfamily level (Atalay *et al*., 2016; Azzazy, 2019). GT *O. basilicum* also reflects the leaf development process and acts as a specialized structure for storing volatile and semi-volatile compounds (Mofikoya *et al*., 2019) or biosynthesis of Essential oils (Eo) that result from the general phenylpropanoid biosynthetic pathway (Gang *et al*., 2002; Biswas *et al*., 2015; Azzazy, 2019; Hazzoumi *et al*., 2019).

The *Alfalfa mosaic virus* (AMV) is the *Alfamovirus* belonging to the family of Bromoviridae that can lead to symptoms including yellow mosaic (Calico) or whitish mosaic symptoms on leaves of basil plants (El-Attar *et al*., 2019). Several species of aphid vectors can spread the virus (AMV) in a non-persistent manner to the plant leaves on the surface of various plant species (Khalil *et al*., 2020).

Nowadays, as in the past, the severe problem is the spreading of many different plant viruses that can naturally infect *O. basilicum* plants and are responsible for symptoms on the leaves (Holcomb *et al*., 1999; Sanz *et al*., 2001; Davino *et al*., 2009; Poojari and Naidu, 2013; Ammara *et al*., 2015; Marei

Corresponding Author: Eman M. EL-Abagy, Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, 12619, Egypt.
E-mail: eman.abagy@gmail.com

and Magdy, 2017; El-Attar *et al*., 2019). Thus, the marketable product should include healthy leaves used as raw materials in the pharmaceutical and cosmetic industries (Baczek *et al*., 2019).

Tissue culture is an important tool *in vitro* to produce viral-free materials and plant-based compounds on a large scale. *In vitro* meristem-culture technique can solve this problem by isolating or culturing the small apical or lateral meristems from plants. The meristem is a vital tissue, developing from the leaf itself (Cortez *et al*., 2022), and is the common strategy for producing a completely healthy plant out of it. Besides, meristem culture maintains germplasm and high genetic stability *in vitro* under a controlled environment (Li *et al*., 2019; Mokbel and Kheder, 2020). However, the meristem size can affect the success of viruses' elimination (Panattoni *et al*., 2013). Robledo-Paz and Manuel (2012) recommended the isolation of a small meristematic dome with one leaf primordium for eliminating viruses.

Despite the importance of peltate GT, no described studies or comparisons of their morphology and structure under viral infection stress had performed to date, that performing the first goal of this research. The second objective aimed to solve the viral problem and evaluate the effect of three sizes of meristem tip (0.2, 0.3, and 0.4 mm) on eliminating the AMV and the survival rate. Besides, confirmation-possibility of the induction of GT in the healthy leaves in vitro to use by producers of pharmaceuticals and cosmetics as a storing structure of essential oils in the personal care industry.

2. Materials and Methods

This study includes two parts: The first part compares the changes in the glandular peltate trichome structures among healthy *O. basilicum* leaves, naturally infected ones by AMV, and the developed plantlets *in vitro* using SEM at the Applied Center for Entomo-nematodes (ACE), Experimental Research Station at the Faculty of Agriculture, Cairo University. The second part evaluates the effect of the meristem culture technique on the elimination of AMV from the *O. basilicum* using a double-antibody sandwich (DAS)-ELISA assay at the Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Center.

2.1. The first part concerns the morphology of leaves

2.1.1. The source of AMV-infected basil plants

The healthy, as well as infected basil branches (*O. basilicum* leaves) by AMV symptoms were previously studied in 2019 (El-Attar *et al*., 2019). The infected plants by AMV showed leaf curling and yellow mosaic (Fig. 1). The detected virus in *O. basilicum* leaves was done using double-antibody sandwich (DAS)-ELISA, reverse transcription (RT)-PCR, and then molecularly characterized or deposited in GenBank under the accession number MH625710. Examined plant branches were kept in the greenhouse out from insects until performing the following experiments.

2.1.2. Morphological analysis using SEM

Three little fresh leaves (approx. 1 cm in length) from the healthy and naturally infected with AMV as well as the healthy developed plantlets from the successful size were mounted independently on SEM brass stubs and then adhesive with a layer of Carbon tape, followed by spraying with a thin layer of gold/palladium in Sputter Coater (Erdtman, 1960). The diameter of peltate glandular trichomes and their number on both adaxial and abaxial surfaces of leaves were determined in healthy field-grown and propagated plants *in vitro*. The glands' measurements and the specimen's micrographs were recorded and viewed using an SEM microscope (a JEOL GM 5200).

2.2. The second part concerns the plant tissue culture

2.2.1. Surface sterilization

The collected field-grown branches from healthy and naturally infected *O. basilicum* plants with AMV were washed thoroughly with running tap water for 30 minutes, followed by rinsing three times in distilled water for 3 min each. The washed branches were immersed in 20% Sodium hypochlorite solution (commercial bleaching compound, Clorox) with two drops of the detergent Tween 20 for 20 min. Later, these branches were rinsed in sterile distilled water multiple times (3-4 times, 3 min each) to remove traces of sterilizing agent and remained in it until the excision of meristems.

2.2.2. In vitro meristem excision and AMV-elimination

Ten meristem tips of three sizes (0.2, 0.3, or 0.4mm) were excised from the apical buds using a zoom binocular microscope (Nikon SM2745T, Japan). The apical dome surrounded by 1-2 young leaf primordial was *in vitro* established in culture tubes (Pyrex 25x150mm) containing 15 ml hormone-free solid MS (Murashige and Skoog, 1962) medium. The pH was adjusted to 5.7 before MS-medium autoclaving at 121◦C for 20 min. These were incubated under 16/8h lighting/dark conditions and at 25±2ºC for 60 days. The experiment was repeated twice under the same conditions. The status of the explants was regularly observed every week. Six to eight weeks after initiation, survival was recorded as a percentage of the number of explants in the initial culture, counting only green explants with 1-2 leaves (% of survival = (N survival/N explants to initial culture) × 100). The DAS-ELISA tested the virus status or the virus-free percentage.

2.2.3. Multiplication of virus-free shoots and the trichome induction

After the initial culture, individually meristems (that showed the highest survival percentage) were transferred further to a fresh culture MS medium every four weeks in a culture jar containing 40 ml MS medium supplemented with 0.5 mg L^{-1} (BA) Benzyl adenine (Saha *et al*., 2010) and modified by adding 0.5 mg L^{-1} of (JA) Jasmonic acid according to literature. Explants were subcultured twice on the same modified medium (pH 5.7). The cultures were incubated under 16/8h lighting/dark conditions and at 25±2ºC. After three months of incubation, the *in vitro O. basilicum* plantlets were assayed again by DAS-ELISA then the peltate glandular trichomes (on both adaxial and abaxial surfaces) were examined using SEM at one time.

2.2.4. Virus indexing

A double-antibody sandwich enzyme-linked immune-sorbent assay (DAS-ELISA) was performed, according to Clark and Adams (1977), to test all plantlets derived from cultures *in vitro* against AMV. LOEWE Biochemica GmbH (Germany) supplied ELISA kits. The samples were assessed visually and calorimetrically at 405 nm in a Clindiag Microplate Reader (MR-96). Data of samples testing negative for the virus based on values lower than the OD of the negative control read.

2.3. Statistical analysis

Statistical software package (Agri. Stat., ICAR) and the independent samples t-test had used to compare the mean values at a significance level of 5% for all research data types (measured and numerical data in triplicate).

3. Results

3.1. The first part

3.1.1. The AMV symptoms on *Ocimum basilicum* leaves

The AMV-characteristic symptoms appeared as leaf curling and yellow mosaic on the leaves (Fig.1) with an aromatic fragrance or a strong scent enough to be noticeable.

Fig. 1: Symptoms caused by natural infection by *Alfalfa mosaic virus* on basil

3.1.2. Glandular trichomes investigations

In the healthy leaves of basil plants, there was a healthy peltate GT of a broad head or large secretory head filled with oily contents (Fig. 2A and B). In contrast, the secretory gland in the infected leaf with AMV appears as a round and smooth gland (Fig. 3A). Ruptured the secretory glands in the infected leaf sample integrally (Fig. 3B and C), the release of exudate and forming a cavity or a hole (Fig. 3D), and an accumulated secretion in the subcuticular space with different forms (Fig. 3E). By examining the yellow and green parts from the infected leaf-sample with AMV, the appearance of a rupture of two peltate GT in the symptomatic yellow part, but one swollen mature gland in the asymptomatic green part of the same sample (Fig. 4.). In addition, tissue of the healthy leaf sample appears normal and regularly arranged (Fig. 5A), while the infected tissue appears malformed and irregular (Fig. 5B), with the disappearance of non-glandular hairs in tissues of healthy or the infected leaf by AMV.

Conversely, under *in vitro* conditions in the development-healthy basil plantlets derived from the successful size of 0.4 mm, Fig.7 shows a broad head of peltate GT and normal tissues without damage to non-glandular trichomes (tapered hairs).

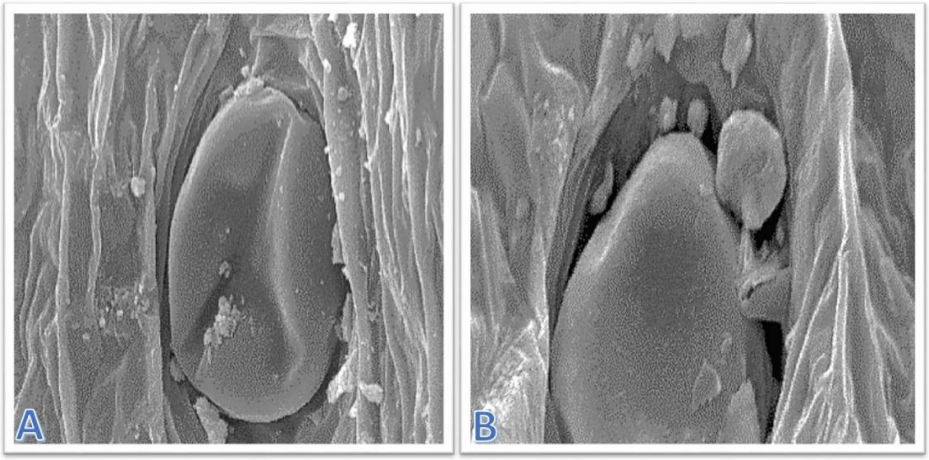

Fig. 2: Scanning electron micrographs show the size and shape of the peltate glandular trichome in the naturally healthy *Ocimum basilicum* leaf. A broad head **(A)** and a filled secretory head **(B)**. X = 750.

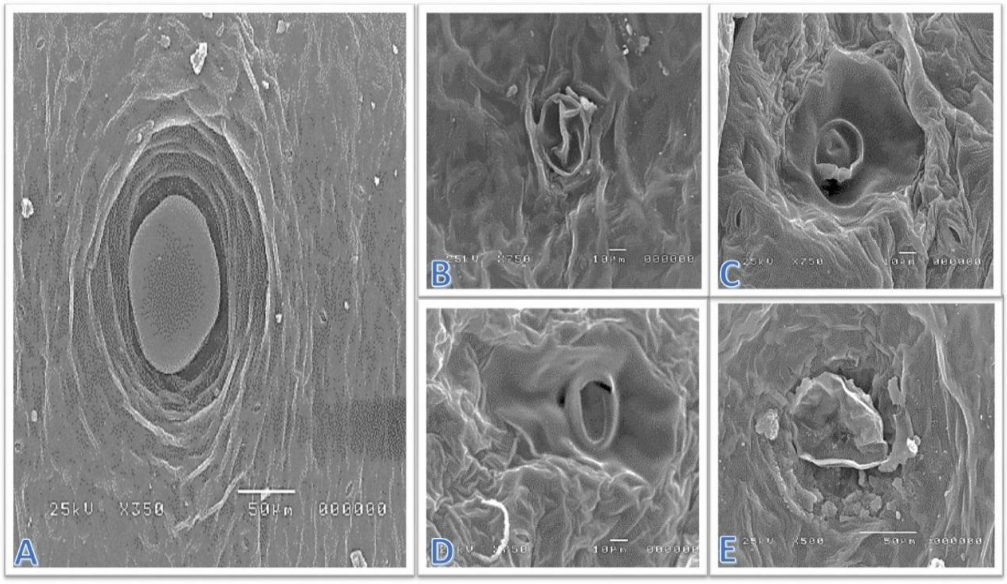

Fig. 3: Scanning electron micrographs show the peltate glandular trichome of the naturally infected *Ocimum basilicum* leaf with the *Alfalfa mosaic virus*. A swollen-smooth round head **(A)**, ruptured the secretory glands **(B and C)**, a cavity in the extruded head **(D)**, and an accumulation of thickened secretions in the subcuticular space **(E)**.

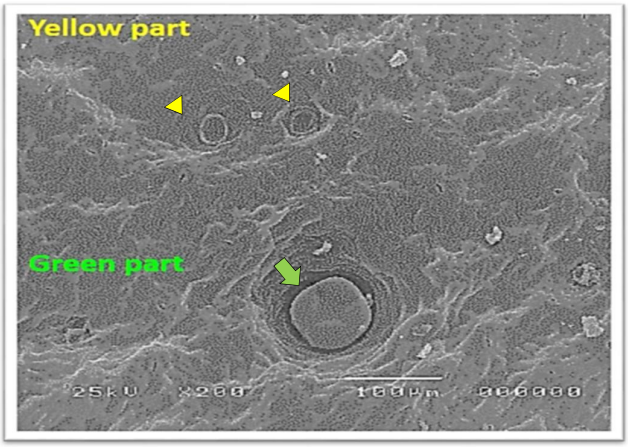

Fig. 4: Scanning electron micrograph shows the rupturing of the two peltate glands in the yellow part of the infected *Ocimum basilicum* leaf with *Alfalfa mosaic virus* (yellow arrowheads) and one non-rupturing peltate gland with a broad secretory head in the green part of the same diseased leaf (green arrow).

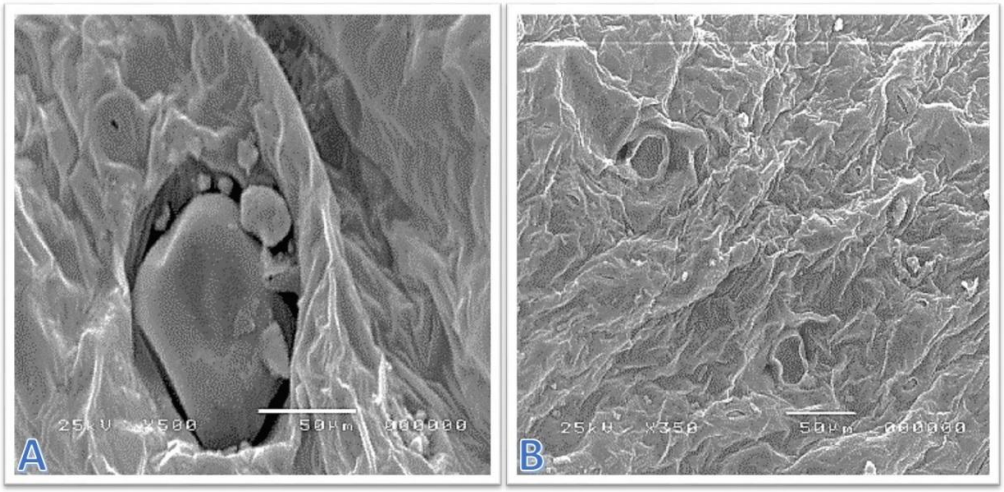

Fig. 5: Scanning electron micrographs show the difference between the tissue shape of the healthy and infected *Ocimum basilicum* leaf with *Alfalfa mosaic virus*. Normal healthy or arranged orderly tissues **(A)**, and malformed or irregular tissues **(B)**.

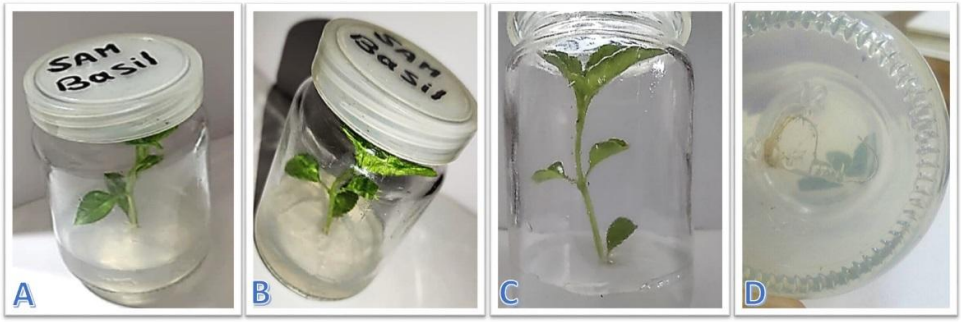

Fig. 6: A successful multiplication and elongation of sweet basil (*Ocimum basilicum*) plantlets within 90 days. The well-developed healthy *O. basilicum* plantlets from the meristem size (0.4 mm) on MS medium supplemented with 0.5 mg/l of BA and 0.5 mg/l of JA after three subcultures **(A-D)**.

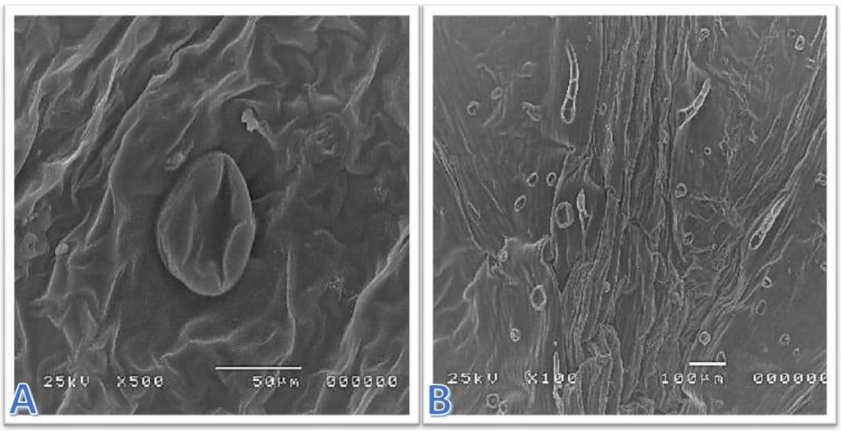

Fig.7: Scanning electron micrographs show a peltate gland of healthy leaf from *in vitro* propagated *Ocimum basilicum* plantlets (derived of the successful size, 0.4 mm) on a medium with 0.5 mg/l of BA and 0.5 mg/l of JA within 90 days. A broad-filled secretory head **(A)** and normal tissue with non-glandular trichomes **(B)**.

3.2. The second part

3.2.1. Effect of meristem size on the elimination of AMV and survival rates

The MS medium without any growth regulators has used for three meristem tips of different sizes (0.2, 0.3, and 0.4 mm) to obtain AMV-free basil plantlets. The results of DAS-ELISA confirmed the absence of AMV in all tested meristems-derived shoots (Table 2).

Table 2: Assess the effect of three sizes of meristem tips on the elimination of *Alfalfa mosaic virus* from basil plantlets *in vitro* by DAS-ELISA and T-test for the difference between mean values of survivals.

Size/ collected Results	Size 0.2mm	Size 0.3mm	Size 0.4mm	After 90 days	Negative Control
[a] O.D value at 405 nm	0.312	0.331	0.354	0.388	0.394
Results against AMV	-ve	-ve	-ve	-ve	-ve
The percentage of virus elimination	100%	100%	100%	100%	100%
Total survived meristems in an each size	16	23	24	-	-
Survival (%)	53.33	76.66	80	-	-

Statistical results	Sizes 0.2/0.3mm		Sizes 0.2/0.4mm		Sizes 0.3/0.4mm	
[b] The mean values of survivals	5.3	7.6	5.3	8	7.6	8
STD	1.15	0.58	1.18	1	0.58	1
T Value	3.13		3.024		0.5	
Statistical Value	0.035		0.039		0.64	
Statistical Significance	*		*		ns	

[a] O.D. is an average value from two wells at 405 nm reading taken within 30 min of adding the substrate solution; the Negative sample is lower than the healthy control sample. [b]Values are averages of thirty meristems survived in each size; ns = not significant.

Testing ten meristem cultures of each size affected the survival rate of explants in three individual *in vitro* experiments (Table 2). Results in Table (2) after two months of incubation showed only 53.3% of the survived meristems using tips of 0.2 mm in size. A high survival rate of 76.6% or 80% in the case of meristems tips of size 0.3 mm or 0.4 mm, respectively. Results of Table (2) confirmed that not all survival percentages were significantly different among sizes 0.3 mm and 0.4 mm, whereas size (0.2 mm) was different rather than other sizes (0.3 mm and 0.4 mm) at a 5% level of significance. The only shoots derived from size (0.4 mm) were used as an *in vitro* explants source for multiplication and plant

elongation. Shoots were subcultured three times on MS medium (supplemented with 0.5 mg/l of BA and 0.5 mg/l of JA) every four weeks (Fig. 6A-D). The development of plantlet leaves was achieved by refreshing the MS medium every 28 days, and the number of leaves ranged from four to six at the end of the last subculture (Fig. 6C). Plants remained virus-free for three months as confirmed by DAS-ELISA (Table 2).

Table (3) illustrates the results of measurement and numerical traits of the GT in field-grown and *in-vitro* healthy leaf samples, where the minimum diameters on the adaxial and abaxial sides were somewhat different, whereas the maximum diameters and numbers of the GT on both sides were not significantly different at a 5% level of significance.

Table 3: T-test results for the difference between the mean of Min/Max diameters and the number of peltate glands of both leaf adaxial and abaxial sides of field-grown and the *in vitro O. basilicum* propagated on a medium with 0.5 mg/l of BA and 0.5 mg/l of JA within 90 days.

[(a)] GTs Parameter	Field-grown		*In-vitro*	
	Leaf adaxial side	**Leaf abaxial side**	**Leaf adaxial side**	**Leaf abaxial side**
Mean/Min diameters (µm)	29.96	31.8	37.6	40.5
STD	**1.15**	**1.4**	**2.08**	**0.50**
T Value	5.57		9.99	
Statistical Value	0.005		0.001	
Statistical Significance	*		*	
Mean/Max diameters (µm)	48.56	47.8	45.1	52.0
STD	**3.9**	**3.9**	**2.1**	**4.1**
T Value	1.338		1.276	
Statistical Value	0.252		0.271	
Statistical Significance	**ns**		**ns**	
Mean/Number (in mm^2/cm^2)	8	9	7.3	8.3
STD	**0**	**1**	**0.57**	**0.58**
T Value	2		1	
Statistical Value	0.116		0.374	
Statistical Significance	**ns**		**ns**	

[(a)]Value represents the mean of three replicates; ns = non-significant.

4. Discussion

The pharmaceutical sector and sustainable agriculture production depend on a steady supply of virus-free plant materials. The current study highlights the negative changes in the glandular trichome structures (GTs) on the basil leaves infected by AMV compared to healthy ones. The GT in healthy leaves is the site of the production of volatile aromatic compounds (Biswas *et al*., 2015; Azzazy, 2019; Hazzoumi *et al*., 2019). The GT of representatives of the family Lamiaceae, particularly Sweet basil, has been described in detail in terms of their density and overall appearance (Azzazy, 2019). Some species of the family Lamiaceae have two types of GT known as capitate and peltate (Werker, 2000; Azzazy, 2019; Hazzoumi *et al*., 2019). Therefore, in agreement with the taxonomy of literature (Werker, 2000; Gang *et al*., 2001; Azzazy, 2019), the type of gland named peltate or secretory trichome, is found in healthy *O. basilicum* leaves characterized by a broad head (Fig. 2). Notably, the observed type of GT has been the most widely reported in all previous anatomical studies conducted in the family of Lamiaceae. However, there is no available information on the morphological features of GT in the leaves infected with AMV.

Anyways, the results confirm the structural damage or severe malformations of GT on the leaves under the stress of viral infection. The observed malformations suggest that the rupture of peltate GT is evidence of the detrimental consequences of viral infection for the basil plants. Since the peltate GT of *O. basilicum* stored essential oil, the release of oil or their exudate after gland rupture has represented the plant defense strategy (Mofikoya *et al*., 2019) to relieve both biotic and abiotic stressors (LoPresti

2015; Mofikoya *et al*., 2019). It is not surprising, that the defensive role of the GT probably makes plants tolerant to viral infections and helps protect the leaves from aphid attacks, overall, plant-insect interaction affected essential oil biosynthesis in Sweet basil (Shafiee-Hajiabad *et al*., 2015). Accordingly, under viral stress and through variations in GT-morphology in Sweet basil, these leaves lost the main constituents of oil like terpenoids and phenolics (Werker, 2000) and thus, lost its most advantage in the pharmaceutical and cosmetics industries. The current findings (SEM results) also documented the extent of damage to GT in the yellow part of the leaf with viral symptoms rather than the green part of the same symptomatic leaf. It is worth noting during the study that the fragrance emitted by infected plants was much stronger than that of healthy plants. Therefore, the SEM images helped to describe GT morphology and the sharp smells produced by basil leaves, in which viral infection stress affected basil plants severely through oil secretion. Therefore, there was no need to determine the amount of oil which outside the scope of this study due to the observed images of SEM. These findings corroborate the involvement of the leaf GT in the response of the plant's defense against viral infection, which also corresponds to the wall thickening of naturally infected plants with AMV shown in our previous study (El-Attar *et al*., 2019). Besides, GT together with non-glandular trichomes may also provide mechanical strength, against herbivores (Simmons and Gurr, 2005) or potentially improve defenses against pathogens (Guerrieri and Digilo, 2008). The current view is that no plant can serve as a unique reference to study the stress of natural viral infection on both types of GTs. Sometimes, a few studies have documented the impact of different viruses on the infected plant's GT artificially (Kontaxis and Schlegel, 1962; Angell and Baulcombe, 1995; Waigmann *et al*., 1997; Kogovsek *et al*., 2011). The results of these studies attributed to the genes involved in the biosynthesis of metabolites produced in GT or localization of the viral cytoplasmic inclusion bodies and viral particles near the GT. Therefore, our results provide the first evidence that AMV infection changes the peltate GT of *O. basilicum* leaves. Thus, the study needed to find alternative ways to eliminate AMV and produce healthy green leaves containing non-deformed GT structures.

Concerning the effect of meristem size on AMV elimination or survival rates, and based on the results of Table (2) using the DAS-ELISA, the complete elimination of the AMV from basil plants in the present study was conducted directly through the meristems excised from the apical buds of various sizes (0.2, 0.3, or 0.4mm) on a hormone-free MS medium. The DAS-ELISA is sensitive to detecting different viruses among various plant species *in vitro* (Khan *et al*., 2003; Taha *et al*., 2015). Regardless of the type of either pathogen or plant, meristem culture alone can eliminate impossible viruses to eradicate by thermotherapy (Smith *et al*., 1970). In addition, the *Iris mosaic virus* was eliminated from iris plants using meristem culture alone (Baruch and Quak, 1966). Similarly, Mokbel and Kheder (2020) confirmed that a meristem tip of size (0.2 or 0.3 mm) without thermotherapy is the optimum length for eliminating phyllody fruit phytoplasma from strawberry plants. Moreover, virus eradication depends on the size of excised-meristem tips because the rate of plant growth in the meristematic area is increasing compared to virus multiplication (Verma *et al*., 2004; Wang and Valkonen, 2008). The results of the study support the findings of numerous reports (Faccioli and Marani (1998), Fuglie *et al*., (1999), Singh *et al*., (2008), Kumar *et al*., (2009), AlKhazindar (2015). These reports point to the meristems with 0.2 or 0.3 mm in size have proven to be more effective in different virus elimination, and the using meristems including 1-2 leaf primordial increases the growth in many plant species. Furthermore, Whitehouse *et al*. (2011) confirmed the suitability of meristem culture via sizes ranging from 0.2 to 0.5 mm for producing strawberry plants free of fungal diseases. Ashnayi *et al*. (2012) stated that a meristem tip of 0.4 mm size is the optimum for *Carnation etched ring virus* elimination from infected carnations. Senula *et al*. (2000) also reported high regeneration rates (90–100%) from garlic meristem size (1.0 mm) with 2-3 leaf primordia. In contrast, Ramgareeb *et al*. (2010) found that thermotherapy combined with meristem size less than 0.5 mm eradicated both *Sugarcane mosaic virus* and *Sugarcane yellow leaf virus* successfully from sugarcane plants. Overall, meristem-culture plays a role in excluding viruses from plants through several mechanisms: (1) Cell injury during the excision operation causes loss of the enzymes required for viral replication and RNA degradation (Mellor and Stace-Smith, 1977). (2) The poor development of vascular tissue in the meristematic cells and slow movement of virus particles from cell to cell (Parmessur *et al*., 2002). (3) Virus shows a low concentration from the base of the plant towards the meristematic area (Wang and Valkonen, 2008).

For the formation of GT, explants derived from meristem tips 0.4 mm long were multiplied successfully on an agar-solidified MS medium (Saha *et al*., 2010), modified by adding 0.5 mg/L^{-1} of

JA. According to the results in Table (3), adding 0.5 mg/L^{-1} of JA supported the normal development of GT, which showed no significant difference in gland measurements between healthy field-grown and propagated plantlets *in vitro*. Incorporated BA and JA in the culture media improved the subsequent *in vitro* multiplication (Pattnaik and Chand, 1996; Sahoo *et al*., 1997; Saha *et al*., 2010) and the generation of more glandular trichomes (Zhang *et al*., 2019). Besides non-glandular trichomes that distinctively occurred on the abaxial surface of basil leaves *in vitro*, they were not observed on the surface of the healthy leaves of field-grown plants, suggesting that the use of phytohormone (JA) is a suitable media component in the production of GT (Gomi, 2020) and non-glandular trichomes (Fig.7). Under *in vitro* conditions, a similar response has been reported (Andrys *et al*., 2018) on other medicinal plant species like *Lavandula angustifolia* of the Lamiaceae family toward GT production in MS medium in the presence of JA. In addition, after applying JA or its derivatives, long-stalk glandular trichomes were observed on the surface of the basil leaf as on the tobacco plant (Zhang *et al*., 2019). Like Cytokinin, which also plays a crucial role in controlling the density of GT in tomatoes, the application of Cytokinin 6-benzyl-amino purine led to much higher densities (Maes and Goossens, 2010). Furthermore, trichomes (non-glandular) serve also as a protective mechanism against insects (Kaur *et al*., 2022) and solar radiation besides reducing leaf evaporation (Wagner *et al*., 2004; Dalin *et al*., 2008). Indeed the application of JA positively affects a great variety of morphological or physiological responses in plants and is involved in a specific signaling network of interactions among several pathways of phytohormone signaling (Yang *et al*., 2019). The success of GT formation *in vitro* could depend on the impact of several functions of JA (Traw and Bergelson, 2003; Bosch *et al*., 2014; Yang *et al*., 2019). (1) Directly involved in developmental plant processes. (2) Promoting the participation of transcription factors in the glandular trichomes. (3) Triggering significant increases in glandular trichome production of leaves. Moreover, peltate-glands of sweet basil produce volatile terpenes, mainly terpenes (like linalool, geraniol, and geranial) and phenylpropanoids (like estragole and methyl cinnamate) upregulated by JA (Iijima *et al*., 2004), and identified as the dominant volatile compounds in the leaves of basil plants that determined its distinctive aroma (Klimankova *et al*., 2008). Finally, the results of this study can help plan future large-scale pharmaceutical and cosmetic industries to obtain virus-free material with healthy leaves under changing climatic conditions.

5. Conclusions

The first part of this study is the first of its kind that primarily focuses on the leaves' GT morphology of *O. basilicum* under the stress of AMV infection.

The stress of viral infection affects the morphology of the GT and leads to structural damage in the secretory glands of *O. basilicum*.

The second part of this research includes the method of solution to AMV elimination and GT formation problems.

a) Virus indexing using a DAS-ELISA assay confirmed the absence of the AMV in all *in vitro* cultures of *O. basilicum* from all used sizes (0.2, 0.3, and 0.4 mm) of meristems.

b) Meristems of a size of 0.4 mm achieved high survival rates, and a method described here is helpful for large-scale production of *O. basilicum* by meristem tip culture.

C) The GT development occurs through MS media containing 0.5 mg L^{-1} of each Benzyl adenine and Jasmonic acid.

6. Acknowledgment

The authors would like to thank Prof. Dr. Hoda. E.A. Ibrahim, Central Laboratory for Design and Statistical Analysis Research, ARC, Giza, Egypt., for her assistance in conducting the statistical analysis of research data.

Abbreviations:

Alfalfa mosaic virus; **AMV**, Glandular trichome; **GT**, Scanning electron microscopy; **SEM**, Double-antibody sandwich; **DAS,** Murashige and Skoog; **MS**, Optical density; **OD,** Benzyl Adenine; **BA**, Jasmonic acid; **JA**.

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