

# FINAL REPORT

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## **TITLE OF PROJECT:**

Disease-indexing and mass propagation of superior strawberry cultivars

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### **3. Executive Summary**

Strawberry is cultivated commercially throughout the year in Egypt and in Israel. It is an important economic crop for local consumption and for export as a high value fresh fruit in the winter to the European markets. Strawberry plants are obtained through vegetative propagation. Fungal and viral pathogens are transmitted via infected plants, therefore, the use of certified strawberry planting material is a prerequisite and the primary means for preventing these diseases and producing a high quality crop. Classical methods for identification of fungal pathogens on selective media are not always reliable, therefore, molecular tools for accurate and rapid diagnostic assays for viral and fungal pathogens of strawberry were assessed. The purpose of the project aimed at establishing reliable diagnostic methodologies for detection of the major fungal pathogens, viruses and phytoplasma affecting strawberry production; developing an efficient supply of disease-free planting material and plants of superior cultivars; and establishing a supply system of disease-free plants to local farmers.

By virtue of the USAID-MERC funds, the Egyptian laboratory was fully equipped to conduct state-of-the-art molecular detection of virus and phytoplasma diseases of strawberry. Training to this effect was conducted by the US and Israeli researchers on virus detection at Ain Shams University in Cairo from 16-20<sup>th</sup> April, 2007. Thereafter, a fungus detection training workshop was conducted by the Israeli principal investigator at Ain Shams University in Cairo, from 9-15<sup>th</sup> November, 2007. During the lifetime of the project, growers meetings were conducted 1-2 times every year by the Egyptian PI and staff to update local Egyptian farmers in virus and fungal detection methods in strawberry.

Joint Israeli-Egyptian work that stemmed from the research project was presented at two international conferences where these studies received worldwide recognition. An audience of more than 350 researchers, extension specialists and farmers were exposed to the scientific results that were published in two proceedings articles.

A peer-reviewed article co-authored by the Israeli PI and co-workers, and Egyptian PI and co-workers was published in the reputable scientific journal *Plant Pathology*, further accrediting the scientific work conducted in the project.

The research teams from Israel, Egypt and the US met at least five times within the lifetime of the project to plan, discuss, and conduct their mutual research. A concerted effort to deal with the current diseases of strawberry on a regional basis, with the expertise of the U.S. collaborator, promoted a very fruitful interaction. This will lead to continued efforts to deal with future problems beyond the duration of this research project.

## **4. Research Objectives**

Strawberry is cultivated throughout the year in Egypt and Israel and is an important economic fresh fruit crop for local consumption and winter export from the region to European markets. Fungal and viral pathogens are transmitted via infected plants, therefore, the use of pathogen-free, certified strawberry planting material is a prerequisite and the primary means for preventing diseases and producing a high quality crop.

### **The main goals of the research**

- (i) Develop, apply and establish a reliable diagnostic system based on molecular techniques for detection of the major fungal pathogens affecting strawberry production in the region.
- (ii) Develop, apply and establish a reliable diagnostic system based on molecular techniques for detection of the major viruses and phytoplasma affecting strawberry production in the region.
- (iii) Develop an efficient procedure from 'tube to field' for the supply of disease-free, high quality, planting material of selected cultivars, suitable for the region. This will be based on micro-propagation; short-term hardening and transfer of plantlets to field nurseries for daughter plant production and subsequently to strawberry fields within one year.
- (iv) Establish the initial tools for a supply system of disease-free plants in Egypt and Israel.

Cultivated strawberry (*Fragaria X ananassa* Duch.), grown commercially in various countries across the globe, is considered the most important crop among the 'small (soft) fruits' both for consumption as a fresh fruit and for processing. Like many other plant species, strawberry plants are propagated vegetatively. The majority of planting stocks are traditionally cultured from mother plants through runners and occasionally by *in vitro* propagation. Although vegetative propagation has numerous horticultural advantages it also has the disadvantages of pathogens (fungi, viruses, bacteria and phytoplasma) being transferred from one generation to the next through the propagation chain. However, despite extensive measures to maintain the propagation material pathogen and insect free, bacterial and fungal contaminations are frequently detected (Khayat et al., 1997). Certified plants are the primary means for preventing these diseases in fruit production fields. A certification scheme for strawberries was established in Israel in the mid 80s (Spiegel, 1998) and did not exist in Egypt before this project was begun.

### **Standard cultivation procedures**

Strawberry is cultivated year-round in Egypt and Israel. Certified, disease-free plants are obtained following traditional protocols based on subjecting selected pre-nucleus plants to a thermotherapy step; excision of meristem-tips from terminal growth and regenerating new plants on a nutrient medium. Following hardening, these plants are potted, maintained in insect-proof conditions and tested extensively for fungi and viruses. Plants found free of pathogens and true to type in horticultural evaluation, become the source material (nucleus stock) providing daughter plants for foundation nurseries. The latter produce plants for field nurseries which in turn produce plants for fruiting fields. Currently, this propagation chain takes approximately 2.5 – 3 years during which plants might be exposed at some of these stages to re-infection by pathogens (Spiegel, 1998). Plant multiplication rates are approx. 100 to 150 daughter plants per mother plant. In Egypt, growers obtain virtually all their planting stock from local nurseries. There are about 100 hectares of licensed strawberry nurseries in Egypt (2001) producing about 300 million plants annually. In Israel, there are approximately 30 hectares of strawberry nurseries. Farmers obtain their mother plants for open field nurseries from two major sources licensed nurseries.

### Phytosanitary regulations

Fundamental to the establishment of a sustainable export industry is the utilization of appropriate cultivars. The Egyptian strawberry industry predominantly uses cultivars from California and Florida although throughout this research two Israeli cultivars were used in both Israel and Egypt, Tamar and Yael. Current Egyptian regulations stipulate that university or government research institution personnel must test all new strawberry cultivars for two years before commercial sale to the private sector. The Ministry of Agriculture and Land Reclamation will not issue an import permit to private nurseries or growers until the research data on cultivar suitability for Egypt is obtained (Pichia, 1999; 2000). One of the reasons for this regulation is to protect against introduction of foreign pathogens (e.g., *Colletotrichum* spp., *Phytophthora* spp. etc.). Egyptian phytosanitary laws are excessively strict and impede the availability of new cultivars. Protocols (such as those devised within the framework of this project) should be set up for more rapid and accurate phytosanitary screening of recently introduced strawberry cultivars to permit more rapid access to new and improved cultivars. In Israel, nucleus and foundation nurseries are tested, by law, twice a year for the major strawberry pathogens. The methods used for fungi are morphological and for virus diseases grafting on indicator plants is being employed. No molecular assays have yet been implemented, however, this project was aimed to adapt these techniques and apply them for pathogen detection under local cultivation conditions.

### Major fungal and viral diseases in the Middle East region

The major fungal pathogens affecting strawberry in the Mediterranean region, include *Phytophthora cactorum* causing Phytophthora crown rot (Ahmed et al., 1984; Gamal El- Din et al., 1981; Wright et al., 1966), *Colletotrichum acutatum* and *C. gloeosporioides* causing anthracnose (Freeman et al., 2002; Freeman and Katan, 1997; Horowitz et al., 2002), *Verticillium dahliae* causing Verticillium wilt (Howard et al., 1982; Maas, 1998) and *Rhizoctonia fragariae* causing crown and root rot (Freeman and Nicoli, 1999; Howard et al., 1992; Khafagi et al., 1993; Mostafa et al., 1992; Razik et al., 1989). Recently, *Macrophomina phaseolina* has been extensively diagnosed both in Israel and Egypt, due to the eradication of methyl bromide (Zveibil and Freeman, 2005; Zveibil et al., 2012). These soil-borne pathogens cause similar symptoms of wilting and, therefore, it is very important to be able to distinguish among pathogens for effective control. Classical methods for identification of fungal pathogens on selective media are not always reliable, therefore, molecular tools are being developed. For example, species-specific primers based on sequence of internal transcribed spacer (ITS) regions of ribosomal DNA are available for detection of *Colletotrichum* species (Freeman et al., 2001), *Phytophthora* species (Bonants et al., 1997; Lacourt et al., 1997) and *Verticillium* (Dan et al., 2001), however, not yet for *Rhizoctonia* pathogenic to strawberry. No standard protocols exist yet for the implementation of these tools for reliable diagnosis of the above pathogens *in planta* in strawberry.

In spite of their economical importance, many strawberry viruses have only been partially characterized due to low virus titers, presence of interfering compounds in the plants and lack of alternative hosts. The methods used currently for detection of strawberry viruses and phytoplasma are based on biological (indexing), serological (ELISA) and molecular (PCR) techniques (Converse et al., 1988; Martin, 2001). The most reliable single detection method applied for the major strawberry viruses is grafting leaflets excised from infected plants onto sensitive indicators. Clones of *F. vesca* and *F. virginiana* are used as indicators for strawberry viruses. Symptoms produced on these indicators are often used to characterize strawberry viruses. Often only 14-21 days are required for symptom expression. This bioassay, known as 'indexing', is time - consuming, requires a temperature-controlled greenhouse and can be inaccurate. However, its broad detection spectrum is extremely valuable for poorly characterized viruses. Recent advances in diagnostics

and characterization of several major strawberry viruses have provided serological and molecular assays. The development of the enzyme-linked immunosorbent assay (ELISA) provided a much-needed technique for the detection of strawberry-specific viruses such as *strawberry mild yellow edge virus* (SMYEV) (Quail et al., 1995) and *fragaria chiloensis ilarvirus* (FCIV) (Spiegel et al., 1993). Nematode-borne viruses and other viruses with a wide host range like *tobacco streak virus* (TSV) have been detected by ELISA (Converse et al., 1988). Serological detection methods are lacking for major strawberry viruses.

The development of the reverse transcriptase – polymerase chain reaction (RT-PCR) provides a molecular tool for detection of viruses and phytoplasma. Broad spectrum assays using degenerate primers for detection of a range of pathogens including previously uncharacterized ones in a single test and specific assays for pathogen identification are developed based on RT-PCR and immunocapture (IC)-RT-PCR (Martin et al., 2000). The complete sequence or RT-PCR detection has been published for one strain of SCV (Posthuma et al., 2002; Schoen et al., 2003), SMYEV (Jelkmann et al., 1992), SVBV (Honetslegrova et al., 1995) and SMoV (Thompson et al., 2002). This information is available in the nucleic acid databases. Primer pairs have been developed for these four viruses and used in a limited study (Babini et al., 2003). Additionally, efforts have been made to develop laboratory based assays for all known strawberry viruses. Recent efforts have resulted in RT-PCR based assays for Strawberry pallidosis associated virus (Tzanetakis et al., 2004), Beet pseudo yellows virus (Tzanetakis and Martin, 2004), *Fragaria chiloensis* virus (Tzanetakis and Martin, 2005), Strawberry necrotic shock virus (Tzanetakis et al., 2004), Strawberry chlorotic fleck virus (Tzanetakis and Martin, 2007) and Apple mosaic virus (Tzanetakis and Martin, 2005). At this time, laboratory based detection methods are lacking for Strawberry latent C virus and Strawberry pseudo mild yellow edge virus. The description of primers for RT-PCR detection and ELISA for serological detection of viruses in strawberry has been reviewed recently (Martin and Tzanetakis, 2006). These tests need to be evaluated on a broader scale to ensure that they will not result in false negatives when used in quarantine and certification programs. As part of this project, RT-PCR tests were developed for the four major aphid-borne viruses (SMoV, SCV, SMYEV and SVBV) to detect strains present in the Middle East and the USA. The tests were adapted for use on field grown strawberries as well as strawberries grown under protected cultivation or in tissue culture.

The methods developed and applied in this project offer alternatives for the expensive and time-consuming biological indexing procedures (Martin, 2001).

#### Mass production of strawberry propagation material

Phytopsanitary measures and induction of flowering of daughter plants are two main factors paramount in strawberry propagation. An effective and convenient way to eliminate contaminants from the stock to be planted in the field can be achieved by using plants that are directly propagated by means of tissue culture in aseptic conditions. Several protocols for *in vitro* mass propagation have been reported in the literature, including safe procedures that do not elicit genetic or epigenetic variation (Boxus, 1998; Kinet and Parmentier; 1989, Jemmali et al., 1994).

Using a precocious protocol developed by Rahan Meristem and used in the project, a large population of mother plants that did not deviate from the mother clones were obtained. Expert growers and farm advisers evaluated this material in Israel for the third consecutive year. The results validated the hypothesis that besides a high genetic fidelity, the plants produced *in vitro* were highly productive, showed normal flowering and fruit set characteristics, and were "cleaner"

from contaminants when compared to plants produced through the conventional protocol (Khayat et al., 1997).

### Rationale

Fungal and viral pathogens may be transmitted via infected strawberry propagation material, therefore, using certified plants is the primary means for preventing these diseases in fruit production fields. A certification scheme for strawberries was established in Israel in the mid 80s (Spiegel and Frank, 1998) but does not exist in Egypt. The current propagation chain (from meristem to fruit production plants) takes approximately 2.5 – 3 years during which plants might be exposed at various stages to re-infection by pathogens (Spiegel and Martin, 1993;1998; Khayat et al., 1997).

In the proposed research it was intended to develop and apply protocols for certifying, disease-free mass-produced propagation material. Development of rapid, robust, sensitive and standardized methods for the detection of the major fungal pathogens and viruses affecting strawberry would facilitate: (i) efficient production of disease-free plant material; (ii) the completion of the certification program; (iii) shorten post-entry quarantine procedures for imported material and permit validation and implementation of the diagnostic methods for large scale testing of propagation material, including *in vitro* material. The proposed, efficient propagation procedure from ‘tube to field’ will shorten the process to about 1 year.

The outcome of this project will provide a source for much needed research information to quarantine and certification agencies, to germplasm repositories, experimental stations, government administrative agencies in Israel and Egypt and the small fruit industries. Implementing the new protocols will increase farmers’ income and reduce environmental hazards resulting from excessive pesticide and fungicide spray regimes.

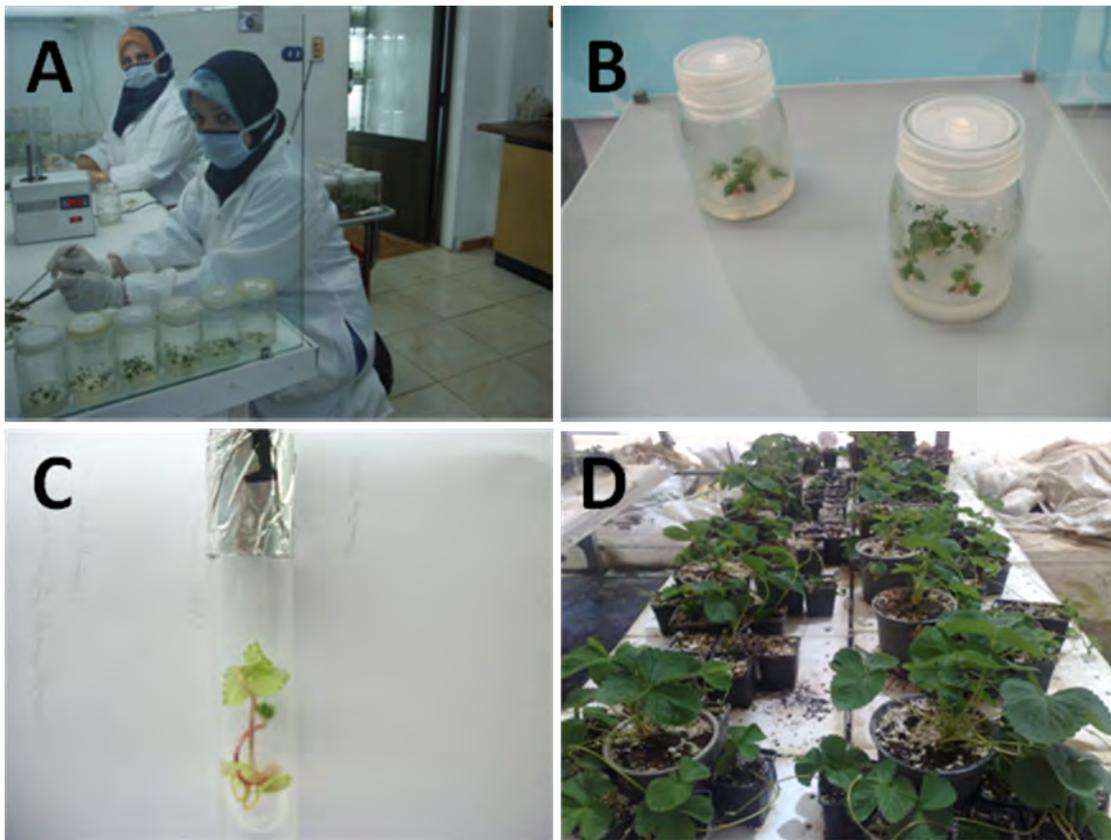
An interdisciplinary multinational collaboration embracing academia and the private sector to advance the strawberry growing industry especially will benefit local, small farm holders in Egypt, and will promote a fruitful long-term interaction throughout the duration of this research project.

## **5. Methods and Results**

### **A. Mass Propagation**

#### **The objective of the research**

The objective of the research was to evaluate the mass propagation procedure (Fig. 1) as a commercial method to produce strawberry mother plants for field nurseries in Israel and Egypt. The commercial runner-propagation process in authorized nurseries in Israel and Egypt is generally similar. The process involves propagation of nucleus daughter plants in the foundation greenhouse (May – Dec), chilling at 2<sup>o</sup>C (Dec – March), and rooting these plants in small pots in controlled greenhouses (Mar – May). Although this procedure is well established, this long and expensive method to produce thousands of mother plants very often limits the propagation process, especially for the introduction of a new breeding cultivar to the commercial fields. Tissue culture for mass propagation is not season-dependent and in some cases could be helpful in maintaining or up-scaling particular plant material or new breeding lines. However, the performance of mother plants originating from tissue culture has not been widely analyzed. In this work, the performance of mother plant developed from tissue culture (mass propagation), nucleus stock and commercial resources in the farmer's nurseries were compared and evaluated. In addition long term effects, such as yields and fruit quality in the production fields were analyzed.



**Fig. 1.** Mass propagation (tissue culture) procedure. A. Selection of the appropriate meristems for tissue culture, mass propagation; B and C, preparation of medium and propagation of plantlets; and D, multiplication and acclimatization of plantlets in greenhouse.

Plant material preparation – For the nursery season (May), 5-6 mother plants of the Israeli cultivars “TAMAR and YAEL” from three different sources were prepared:

- 1) Mass propagation (tissue culture) – hardened plants after 3 cycles of apex culture propagation in high cytokinins media and rooting in hormone-less culture (Figs. 1 and 2).
- 2) Nucleus stock – Rooted runners from the nucleus greenhouse (Fig. 3) that did not go through a chilling period.
- 3) Commercial (certified) – Rooted plants from the commercial authorized nursery.



**Fig. 2.** Hardened tissue-culture plants



**Fig. 3.** Runners from the nucleus greenhouse

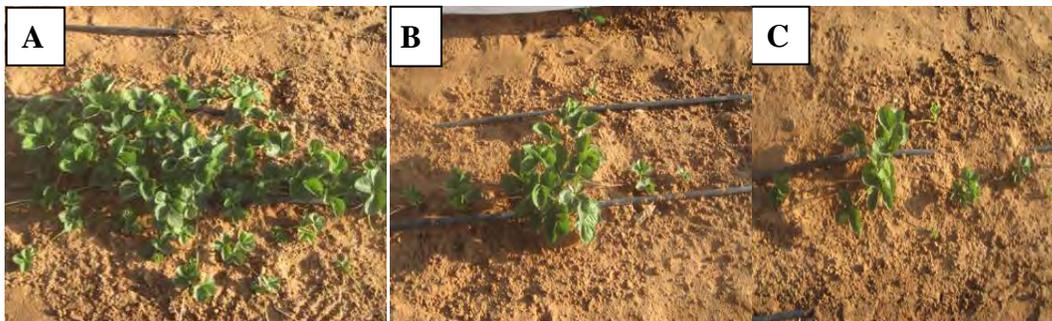
### Israel

Mother plants were propagated on May 2<sup>nd</sup> 2008, in Yemini's nursery, Tzofit, Sharon region, Israel. The plant material consisted of 4 mother plants (cv. TAMAR) from each of the following sources (below), and were planted at 2 x 2.5m distance:

- 1) Mass propagation – tissue culture plants after hardening.
- 2) Nucleus stock – Rooted runners from the nucleus greenhouse.
- 3) Commercial – Rooted plants from a commercial authorized nursery.

### **The nursery agricultural practice**

Combined overhead and drip irrigation with addition of fertilizers as used in practice. The nursery was treated frequently with pesticides and fungicides as needed. Runners appear from June, which produce young daughter plants till mid September. Propagation quality and number of runners were evaluated from each of the three planting stocks (Fig. 4).



**Fig. 4.** View of the field nursery 45 days after planting, June 16<sup>th</sup>.

- A. Mass propagation of plants from tissue culture
- B. Propagation of plants from nucleus stock
- C. Propagation of plants from commercial nurseries.

**In Israel, the propagation process was successful for all treatments. The mass propagation, tissue-culture plants maintained their high reproduction and were first to fill their plot area as shown in figure 5 (E and F).**



**Fig. 5.** View in nursery of the different planting groups, 4 months after planting, at the end of August. (E) Plants grouped from nucleus stock (bottom 2 plots), tissue culture (middle 2 plots) and commercial (last 2 plots). (F) Mass propagation from tissue culture source (left) and nucleus stock (right).



**No differences were observed regarding the degree of powdery mildew susceptibility. In comparison to other cultivars in the nursery, cv. Tamar remained highly susceptible to powdery mildew.**

### Egypt

#### **Plant material:**

Two field nursery experiments were conducted in 5<sup>th</sup> May 2009 at Ismailia and Qalubia to compare the performance of mass propagation plants, foundation plants and classical breeding plants, from Tamar cultivar, 10 plants were planted in the nursery from each order in each replicate. Data were recorded on number of runners, number of leaves, plant height and number of transplants in late August. Results are shown in table 1 as follows:

**Table 1.** Effect of different strawberry plant orders on runner formation and transplant production of Tamar cultivar

Plant Orders	Growth Characteristics			
	No. of runners	No. of leaves	Plant height (cm)	No. of transplant
Mass propagation	19.71a	16.78a	17.11a	26.67a
Nucleus plants	19.63a	16.89a	18.80a	27.44a
Commercial plants	11.22b	13.89b	17.66a	28.44a

Data show that number of runners and leaves increased significantly in mass propagation (TC) and nucleus plants compared with that of the commercial order, while no significant differences were observed between orders for transplants recorded on 28<sup>th</sup> August.

**In summary, in both Israel and Egypt, data show that in Tamar cultivar numbers of runners and leaves increased significantly in mass propagation and nucleus orders compared to commercial order plots. However, no difference was observed between orders for number of transplants. Yael cultivar reacted similarly as that of Tamar.**

### Field Experiments

#### **Planning experiments in the fruiting fields in both Israel and Egypt**

In September 2009, 40 plants from each treatment plot (see above) were planted in 4 replicates at a commercial field in Tzofit, Israel. Yield was estimated from 16 weekly harvests, from December to March. Number of fruit per plant and the average fruit weight was analyzed at two to four harvesting dates. Fruit quality i.e., firmness, TSS, shelf-life and acidity, was examined in fruits from the different selected harvests.

### Israel

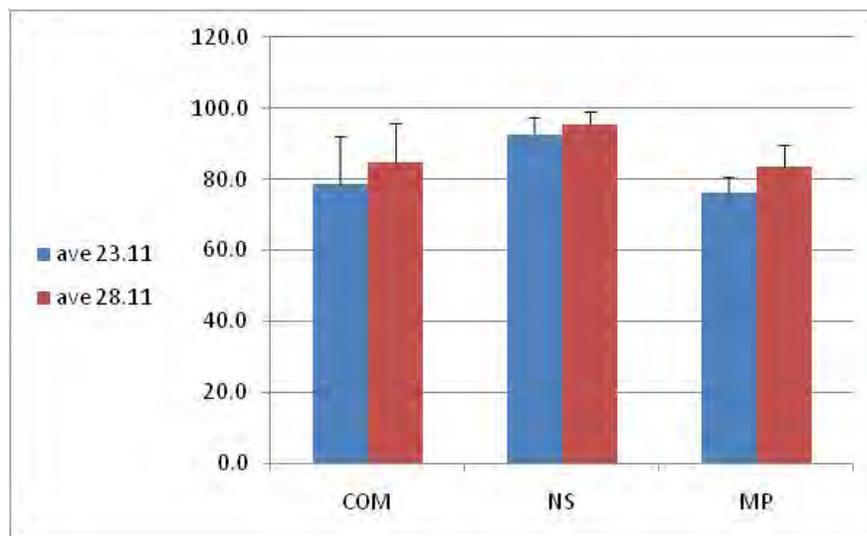
#### **Field performance (2008-2009 season)**

##### **Field agricultural practice**

Irrigation was by continuous drip with additional fertilizers as used in commercial practice. The nursery was treated with pesticides and fungicides as needed. Plants initiated flowering by the second week of Nov. and were fully flowered by the end of this month.

##### **Flowering time**

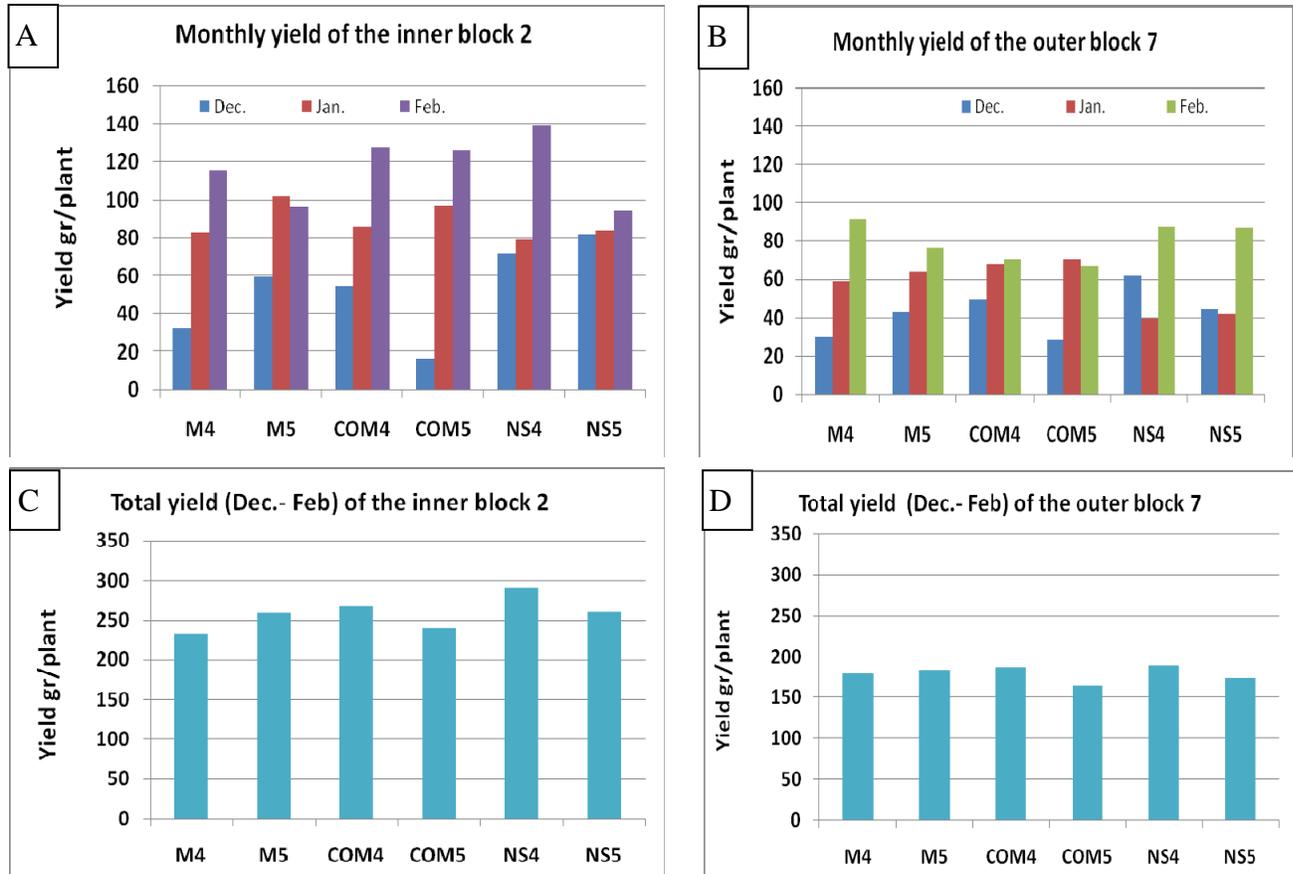
The flowering percentage was calculated as number of plants that initiate flowers from the total plants in each plot. There were some differences between the plots but none of them were attributed to the plant origin. As shown in figure 6, the plants derived from nucleus stock (NS) were the first to reach 100% flowering, but no significant differences was determined between the mass propagation (MP) and the commercial treatment (COM).



**Fig 6.** Flowering percentage in the trial plots. Column values represent average of four plots  $\pm$  stdev.

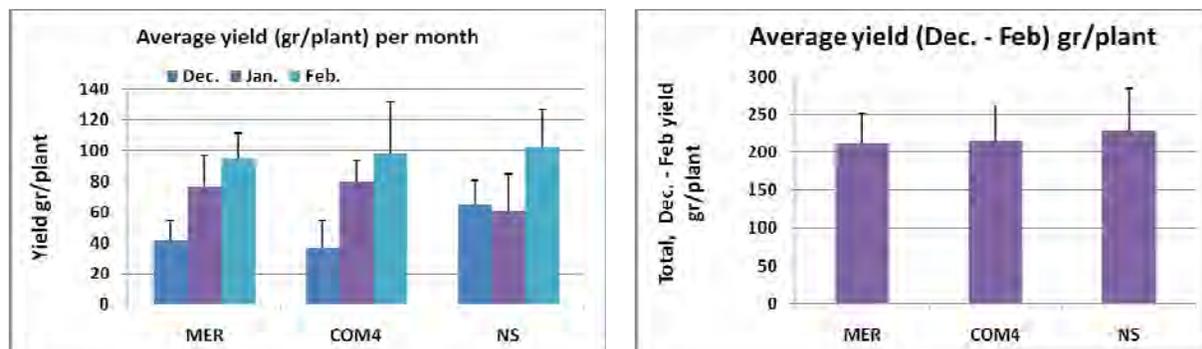
### Fruit yield

The strawberry plots were harvested weekly and yields were measured. Monthly yields of the outer blocks (line7) differed from the inner blocks (line2) and were continuously 25% lower as shown in figures 7, A and B. However, no significant differences were found in the total yield calculated till the end of February between the tested plots (Fig. 7, C and D).



**Fig 7.** Monthly (A, B) and total (C, D) yields of all plots from the three different plant origins.

**Yield summary** – Combining results from the two distinct field blocks (inner and outer) showed that there were minor changes between the plant treatments. The mass propagation plants (MER) did not differ from the commercial ones (COM), however plants from the nucleus stock (NS) increased early yield in Dec. but produced lower yields in January (Fig. 8).



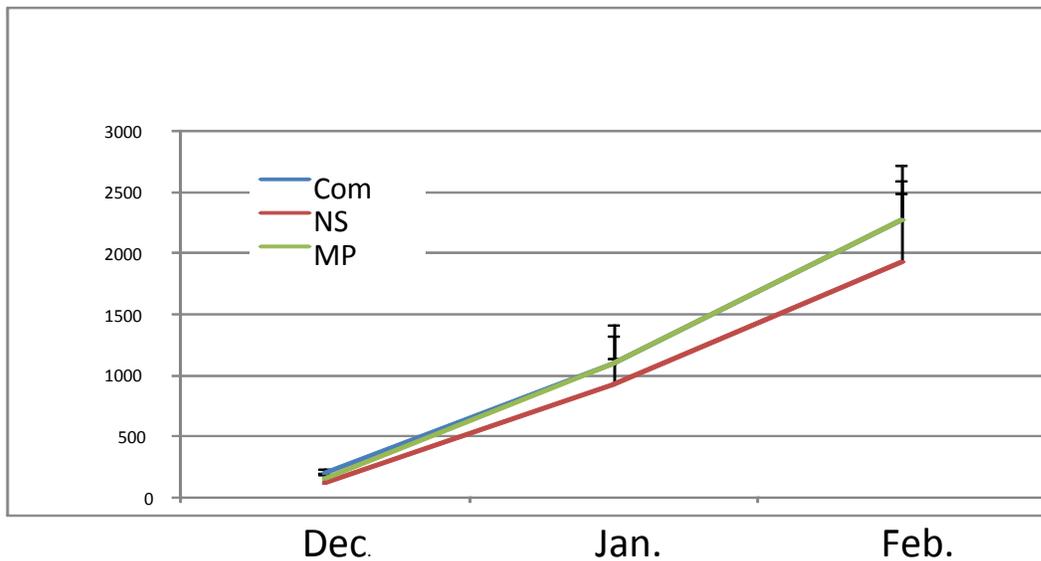
**Fig 8.** Strawberry monthly and average yields. Column values represent average of 4 plots  $\pm$  stdev.

The total summarized yield for 2009 for each plant order showed minor changes that were not significant for each order (Fig. 8). It was concluded that the origin of the mother plants for the nursery field has no effect on berry yields or earliness.

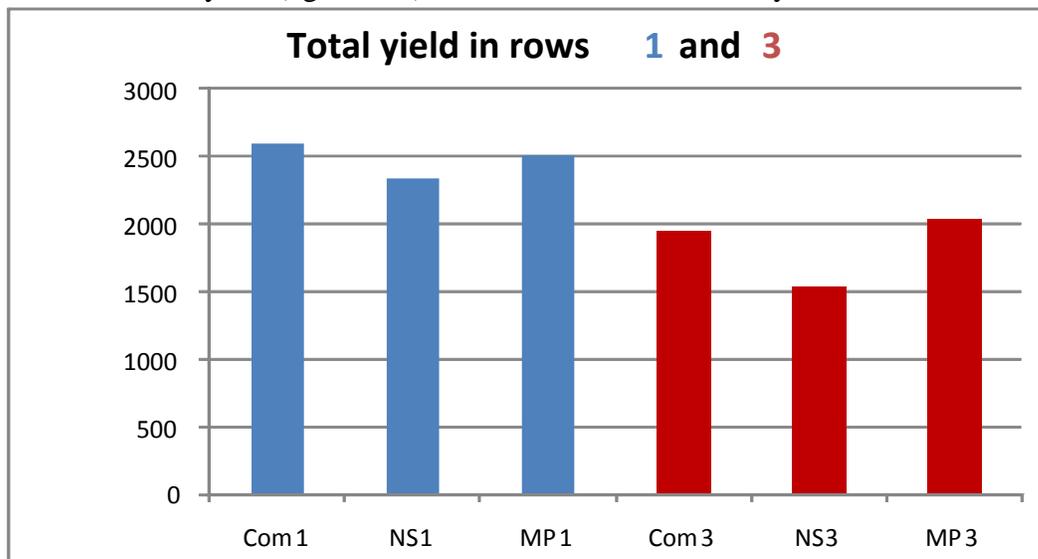
#### Field performance (2009 -2010 season)

**Plant material:** 36 plants (cv. Tamar) from each of the above sources were planted on September 22<sup>st</sup> 2009 in Moshav Tzofit. The plants were set as two blocks in rows 1 and 3 in the fruiting field.

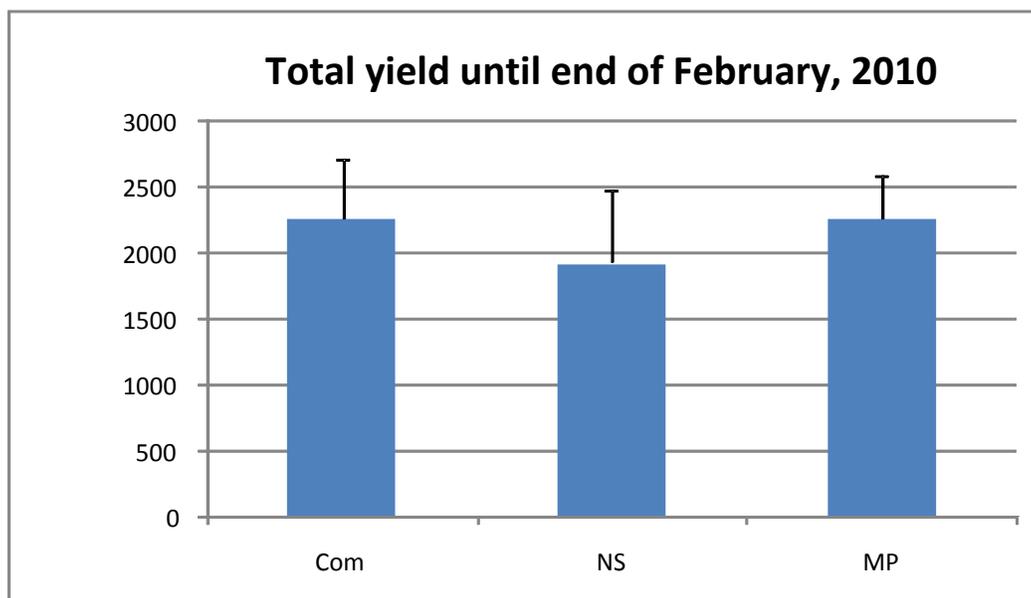
**Fruit yield** – The strawberry plots were harvested weekly and the plots yield were measured. The accumulated yield is an average of two plots each summarized, containing 4 harvest points per month. The yield of mass propagation (MP) plots was similar to those measured in the commercial (COM) plots and was not significantly different from the nucleus stock (NS) yield in kg (Fig. 9).



**Fig. 9.** Accumulated yield (kg/dunam) from Dec. 2009 to February, 2010.



**Fig. 10.** Total yield (kg/dunam) of plots from the three different plant origins planted in rows 1 (blue) and 3 (red).



**Fig. 11.** Total yield (kg/dunam) of the three plant origins by the end of February, 2010.

Approximately 20% lower yield was measured in the repeats of row 3 compared to row 1, in each treatment (Fig. 10). However, this difference is due to field variations and not due to plant origin. The average total yield represented in Fig 11. is not significantly different between treatments.

**These results therefore confirm the results achieved during 2009 and conclude that the source of mother plant origin has no effect on plant performance and productivity in the fruiting field.**

## Egypt

### Field experiments (2008-2009)

Two field experiments were set up on 17<sup>th</sup> September 2008 in Ismailia and Qalubia to compare the growth, fruit production and fruit quality of transplants originating from commercial breeding, mass propagation (tissue culture) and nucleus plants of cv. Tamar. Approx. 200 plants from each order were planted in a four-row bed system. A sprinkler irrigation system was used in the first month after planting then drip irrigation was used thereafter under plastic mulch. Data were recorded on number of leaves, plant height, no. of secondary crowns and number of inflorescences. Early yield was calculated from late Nov. (first harvest) till late January (Table 2).

**Table 2.** Effect of different plant orders on growth and early yield of Tamar strawberry fruits

	No. of leaves	Plant height (cm)	No. of secondary crowns	No. of inflorescences	Early yield (gm / plant)
Nucleus	7.50 a	13.67 ab	3.86 a	5.26 a	109.13 a
Mass propagation	6.41 b	14.67 a	2.29 b	4.79 b	103.46 b
Commercial	5.92 b	13.36 ab	2.46 b	4.36 b	107.02 b

Results indicate that transplants from nucleus plants showed significant increase in no. of leaves, no. of secondary crowns and no. of inflorescences compared to mass propagation as well as commercial plants without significant difference between them. Mass propagation plants were higher than those from nucleus and commercial origins without significant difference between them. Nucleus transplants showed a significant increase in early yield compared to that of mass propagation plants. However, plants from commercial source did not significantly differ from mass propagated ones.

Various fruit characteristics i.e. average fruit weight, fruit firmness, total soluble solids (TSS), ascorbic acid and total acidity were determined in mid January (Table 3).

**Table 3.** Effect of different plant orders on physical and chemical fruit characteristics of Tamar strawberry fruits

Plant source	Average fruit weight (g)	Fruit firmness (g/cm <sup>2</sup> )	TSS (%)	Tetratable acidity (mg/100g)	Ascorbic Acid (mg/100g)
Nucleus	27.28 a	325.0 c	10.00 a	0.147 a	69.50 a
Mass propagation	26.65 a	416.7 a	9.27 a	0.163 a	61.49 b
Commercial	23.71 b	396.4 b	9.33 a	0.130 a	54.08 c

Results indicate that nucleus and mass propagation plants showed significant increase in average fruit weight compared to commercial source. With respect to fruit firmness, Mass propagation plants possessed the highest values of fruit firmness while fruits from nucleus plants had the lowest values of firmness. Concerning total soluble solids (TSS) and tetratable acidity (TA) as affected by plant order, no significant differences were detected. As for ascorbic acid content, nucleus plants exhibited the highest value followed by mass propagated plants while commercial plants showed significantly lower values among the three tested orders.

#### **Field experiments (2009-2010)**

Two field experiments were set up on 14<sup>th</sup> September 2009 in Ismailia and Qalubia to compare the growth, yield and fruit quality of transplants originating from commercial, nucleus and mass propagation plants of Tamar cultivar. Approximately 200 bare rooted plants from each order were planted in four-row bed system. Data on yield appear in table 4. Mass propagated plants produced earlier yields compared with both of the nucleus and commercial plant orders. Nucleus plants recorded higher average fruit weight values compared to those from mass propagated and commercial plants, while no significant differences were detected between mass propagated and commercial ones. Fruit TSS in commercial plants were the highest while nucleus plants produced the lowest. Ascorbic acid content was lowest in fruits from commercial plants compared to nucleus and mass propagated ones, but no significant difference was detected between the last two orders.

**Table 4.** Effect of different plant orders on early yield and some traits of fruit characteristics of Tamar strawberry cultivar.

Plant order	Early yield (g/plant)	Fruit weight (g)	TSS (%)	Ascorbic acid mg/100g
Nucleus	138.36 b	30.37 a	6.533 c	85.16 a
Mass propagation	154.18 a	27.77 b	6.867 b	81.27 a
Commercial	133.87 b	26.84 b	7.333 a	67.48 b

#### **Summary of horticulture results from Israel and Egypt**

**It appears that the transplants originating from the nucleus source produced flowers earliest in the season. Therefore, in the beginning of the season the yield from these plants was greater**

than that of plants from mass propagated and commercially produced nursery sources. Average weights also appear to be higher in the nucleus produced plants although they were not significantly higher than mass propagated and commercially grown nursery plants.

These results therefore confirm those recorded during two consecutive growing seasons (2009 and 2010) in Israel and Egypt, and thus we conclude that the source of mother plant origin has no detrimental nor beneficial effect on plant performance and productivity in the fruiting fields in both countries.

## B. Fungal diagnostic assays

### Objectives of the research

Develop, apply and establish a reliable diagnostic system based on biological and molecular techniques for detection of the major fungal pathogens affecting strawberry production in Israel and Egypt.

### Israel

Cultural identification of *Colletotrichum*, *Phytophthora*, *Fusarium*, *Verticillium*, *Macrophomina* and *Rhizoctonia* from plants is standard practice in Israel. For further characterization of the pathogens, molecular methods are available and routinely used in the Freeman lab. Infected plants are routinely received in the Freeman laboratory and diagnosed for the above pathogens on specific and semi-selective media according to the technical workplan. Detection of fungal diseases from diseased nursery plants of various cultivars (Tamar, Hadas, Malach, Yael, Yuval, Festival, Tamir, Orly and others) were sampled from farmers who notified Freeman of plant wilt problems during the strawberry growing seasons from May to Sept. 2006 – 2010 on a regular basis. Summary of the major wilt causing fungal pathogens isolated from affected strawberry plants in Israel appears in table 5.

**Table 5.** Detection of the major wilt causing fungal pathogens isolated from affected strawberry plants in Israel

Year	Farms <sup>1</sup>	Plants <sup>2</sup>	Pathogen				
			Col <sup>3</sup>	Rhiz <sup>4</sup>	Fus <sup>5</sup>	Macro <sup>6</sup>	Phyto <sup>7</sup>
2006	22	60	0 (0%)	12 (21%) <sup>8</sup>	17 (30%)	13 (22%)	2 (3%)
2007	28	119	13 (11%)	10 ( 8%)	33 (28%)	24 (20%)	1 (0.8%)
2008	25	98	13 (13%)	12 (12%)	21 (21%)	49 (50%)	3 (3%)
2009	11	30	0 (0%)	9 (30%)	5 (17%)	10 (30%)	6 (20%)
2010	11	24	10 (42%)	0 (0%)	24 (50%)	2 (8%)	0 (0%)

<sup>1</sup>No of farms from where wilted plants were sampled. <sup>2</sup>No of wilted plants. <sup>3</sup>*Colletotrichum* spp. <sup>4</sup>*Rhizoctonia* spp. <sup>5</sup>*Fusarium* spp. <sup>6</sup>*Macrophomina phaseolina*. <sup>7</sup>*Phytophthora cactorum*. <sup>8</sup>No of positive isolations followed by percentage from total wilted plants

In all isolations over a five-year period (2006-2010) four of the major fungal pathogens (*Colletotrichum*, *Phytophthora*, *Rhizoctonia* and *Macrophomina*) were detected in strawberry field nurseries however, no plants from nucleus, foundation greenhouses exhibited disease symptoms nor were any diseased plants detected from these sources. It should be noted that although *Fusarium* can be a major pathogen of strawberry, it is likely that the isolates detected are saprophytes as to date no pathogenic Fusaria have been detected in Israel.

A training course was conducted in Ain Shams Univ., Cairo by Freeman for transfer of the protocols for detection of the 5 major soilborne fungal pathogens affecting strawberry to the Egyptian team in November 2007. Further to the workshop conducted, isolates of *Colletotrichum* species causing anthracnose disease, suspected of infecting strawberry in Egypt, were subsequently identified. The isolates were sent to Israel for molecular verification and one of each species, *C. acutatum* and *C. gloeosporioides*, were identified. This is the first report of such findings and served as a basis for a mutual peer-reviewed publication. See results and molecular identification below.

### Egypt

Strawberry plants (Tamer and Yael, cvs) were randomly collected from nucleus and foundation greenhouses and nurseries at three stages of the growth (nucleus, foundation and commercial transplants from cold storage), from the Ismailia and Nobarria experimental plots. *Colletotrichum*, *Rhizoctonia*, *Phytophthora*, *Macrophomina*, *Verticillium* and *Fusarium* species were not recorded on any tested samples throughout this study at the stage of the nucleus and foundation greenhouses. However, at the commercial stage, wilt disease was the most severe in all tested samples from nurseries (Fig. 12), with four of the major fungal pathogens (*Colletotrichum*, *Phytophthora*, *Rhizoctonia* and *Macrophomina*) being detected from the years 2007-2011 (Table 6). Similar to Israel, *Fusarium* can be a major pathogen of strawberry, however, in Egypt it is likely that the isolates detected are saprophytes as to date no pathogenic Fusaria have been detected in this country.



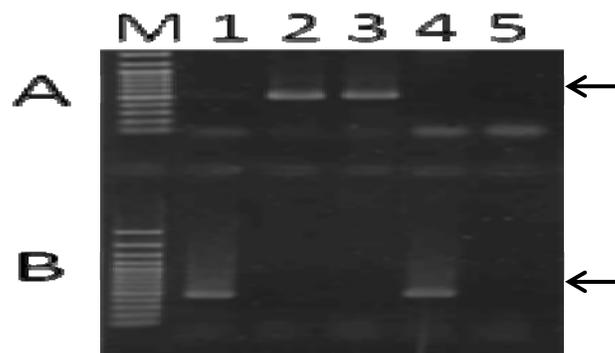
**Fig. 12.** Infected strawberry nursery in Ismailia, Egypt

**Table 6.** Detection of the major wilt causing fungal pathogens isolated from strawberry plants in Egypt

Year	Farms <sup>1</sup>	Plants <sup>2</sup>	Pathogen				
			Col <sup>3</sup>	Rhiz <sup>4</sup>	Fus <sup>5</sup>	Macro <sup>6</sup>	Phyto <sup>7</sup>
2007	2	%T	0.3%	1.1%	4.2%	0.4%	0.8%
2008	4	%IP	22.4%	7.9%	22.4%	17.1%	0%
2009	4	%IP	8%	11.8%	41.2%	35.3%	0%
2010	4	%IP	6%	23%	24%	53%	0%
2011	6	%IP	0%	14%	21%	63%	2%

<sup>1</sup>No of farms from where wilted plants were sampled. <sup>2</sup>% of infected plants of total (T) plants or % of infected plants (IP). <sup>3</sup>*Colletotrichum* spp. <sup>4</sup>*Rhizoctonia* spp. <sup>5</sup>*Fusarium* spp. <sup>6</sup>*Macrophomina phaseolina*. <sup>7</sup>*Phytophthora cactorum*.

Further to the workshop conducted, isolates of *Colletotrichum* suspected as infecting strawberry in Egypt were subsequently identified. The isolates were sent to Israel for molecular verification and one of each, *C. acutatum* and *C. gloeosporioides*, were identified (Fig. 13). For the first time in Egypt, *C. acutatum* and *C. gloeosporioides* were identified from infected wilted strawberry plants and verified according to species-specific primers for *Colletotrichum*. Two representative isolates of *C. acutatum* and *C. gloeosporioides* (confirmed by morphological and molecular methods) were used in Koch postulate tests to ensure infection using clean plant material from Tamar and Yael cultivars Fig. 14). Six transplants were used as replicates for each treatment with transplants inoculated by a spore suspension of the isolated fungi. Likewise, healthy detached fruit at the mature stage were inoculated by spraying with the same spore suspensions. Both fungi were pathogenic to both Tamar and Yael cvs and fruit alike (Fig. 14). This is the first report of such findings and served as a basis for a publication on this work. See results and molecular verification below.



**Fig. 13.** Species-specific identification of *Colletotrichum acutatum* (A) – (primer *CaInt2* in combination with primer ITS4; arrow denotes specific band of 490 bp) and *C. gloeosporioides* (B) – (primer *CgInt* in combination with primer ITS4; arrow denotes specific band of 450 bp) according to amplification products of genomic DNA: Lane 1 - *C. gloeosporioides* from strawberry (representative Cg-317); Lane 2 - *C. acutatum* (representative TUT-5954 from strawberry); Lane 3 – *C. acutatum* (Yael cultivar infected from Egypt); Lane 4 – *C. gloeosporioides* (Tamar infected from Egypt) and Lane 5 - water control. Lane M: DNA markers with sizes in kilobase (Kb).

For the first time in Egypt, *C. acutatum* and *C. gloeosporioides* were identified from infected wilted strawberry plants and verified according to species-specific primers for *Colletotrichum*.



**Fig. 14.** Infected plants and fruit of Tamar and Yael cultivars infected with both isolates of *Colletotrichum acutatum* and *C. gloeosporioides* isolates.

#### Summary of fungal pathogen diagnosis

In Israel and Egypt the major fungal soilborne pathogens that were identified were similar (*Macrophomina*, *Rhizoctonia* and *F. oxysporum*) in all the years when diseased plants were sampled from infected nurseries. This indicated that the Egyptian researchers have applied the diagnostic techniques acquired during the training periods conducted during the project period.

The nucleus and mass propagated plants, planted in the nurseries, were not more susceptible to disease than those originating from classical foundation plants. This indicates that they are not "weaker" or more susceptible to disease than those grown outdoors (classical foundation plants) that perhaps were exposed inadvertently to adverse conditions and pathogens. It should be noted that *Macrophomina* was isolated at increased frequencies indication escalation of this disease in both Israel and Egypt. This may be due to the elimination of methyl bromide as a soil fumigant, allowing *Macrophomina* to establish itself over the years. *Fusarium*,

**although isolated at high frequencies, was not shown to cause disease, therefore it is assumed that the fungi collected were saprophytic and not pathogenic.**

**For the first time in Egypt, *C. acutatum* and *C. gloeosporioides* were identified from infected wilted strawberry plants and verified according to species-specific primers for *Colletotrichum* which resulted in a mutual publication (Embaby et al., 2010).**

### **C. Development of virus and phytoplasma diagnostic assays**

#### **Objectives of the research**

Develop, apply and establish a reliable diagnostic system based on molecular techniques for rapid detection of the major viruses and phytoplasma affecting strawberry production in Israel and Egypt. An import permit for the below test control viruses was approved by both the Israeli and Egyptian teams from their relevant Ministries of Agriculture and the positive controls were imported from the US partner, Dr. R.R. Martin, and are currently kept as dried material in both labs.

#### **Israel**

(i) Establishing a molecular indexing system

Protocols were completed for the indexing system after improving the RNA extraction method from strawberry. The protocol was finalized and used successfully for the first virus detection workshop which was conducted in Cairo, from 16-20<sup>th</sup> April, 2007.

(ii) Evaluation of RNA-RT-PCR assays and testing nucleus plants

The detection protocols were evaluated on positive controls in Israel prior to the workshop that took place in Cairo from 16-20<sup>th</sup> April, 2007. Positive results were obtained for several viruses by RT-PCR and verified by cloning and sequencing the amplified product of the expected size. Reliable tests are currently available for the viruses underlined in the list below.

Testing nucleus stock plants maintained in the Volcani Center, Israel was conducted for all the viruses below. The viruses FrCLV and SVBV were detected in four and three plants, respectively. These results were verified by sequencing. All the suspect plants were destroyed.

List of viruses received include: Strawberry mottle (SMoV), Strawberry crinkle (SCV), Strawberry veinbanding (SVBV), Strawberry mild yellow edge (SMYEV), Strawberry chlorotic fleck (SCFV), Strawberry necrotic shock (SNSV), Strawberry latent ringspot (SLRSV), Apple mosaic (AMV), Fragaria chiloensis latent (FCLV), Strawberry pallidosis associated (SPaV) and Beet pseudo yellows viruses (BPYV).

**No viruses were detected in the fields during the 2007-12 cultivation seasons. This indicates that the detection protocols are accurate and reliable, as also visibly, no symptoms of virus infections were detection in strawberry in Israel.**

#### **USA**

(i) A molecular indexing system was established. An RNA extraction protocol that utilizes several properties of nucleic acids was developed and implemented at the virus detection workshop that

took place in Cairo from 16-20<sup>th</sup> April, 2007. Two new criniviruses that were recently identified in strawberry (Crini-3 and Crini-4) were added to the tests that were carried out at the workshop.

(ii) Dried samples of strawberry that were collected near Cairo during the first day of the workshop were initially identified as Strawberry vein banding virus and SMYEV. These tests were repeated and the PCR products sequenced to ensure the products indeed originated from viral sequences. Results were published in a proceedings article with all partners appearing as authors (see below).

Strawberry samples sent from Egypt to Corvallis tested positive for Beet pseudo yellows virus by RT-PCR and sequence comparison showed the strain from the Egypt strawberries were 98% identical at the amino acid level to the strawberry isolate in GenBank. Testing in Egypt identified SMYEV and SLRSV in production fields.

Exchange of information with Dr. Martin and retesting of samples indicated that dried strawberry plant samples were not a reliable source as positive controls. This could be due to degradation of the virus, a high level of phenolic compounds or both. These unexpected results are significant for the study, detection and identification of strawberry viruses especially in areas where no information is available. In order to overcome this problem, RNA extracted from known infected strawberry plants in Martin's lab in Corvallis were sent to Spiegel's lab and RT-PCR tests were conducted. Following sequencing of products and comparison to Martin's results a reliable system was established in Israel and in Egypt.

Deep sequencing of dsRNA from strawberry *Fragaria chiloensis* sp. *chiloensis* and *F. chiloensis* sp. *patagonica* infected with SMYEV, Strawberry crinkle, SVBV and SMOV yielded sequence information on all four viruses. This demonstrates the utility of this technique as a tool to look for novel viruses in declining plants in Egypt as the final part of the virology component of this project. Tissue samples from declining plants in Egypt were collected in spring of 2011 for this type of analysis, however, RNA and dsRNA extractions from these tissues upon arrival in Corvallis were unsuccessful.

In addition, when plants of *F. chiloensis* sp. *chiloensis* were graft inoculated with SMYEV the virus was detected by real-time RT-PCR three days post inoculation. Whereas, when *F. chiloensis* sp. *patagonica*, plants graft inoculated with the same source of SMYEV the virus was not detectable by real-time RT-PCR until six weeks post inoculation. Eight weeks after inoculation, the titer of SMYEV was comparable in two subspecies of *F. chiloensis*. This slow development of virus in the plants could be used to reduce its spread during the season if the low virus titers resulted in reduced or lack of aphid transmission. Basically, it would mean a six week lag period between when a plant became infected until it could serve as a source of inoculum. This should result in a very slow spread of SMYEV in the field and reduced requirements for vector control in areas where this virus is prevalent.

## Egypt

The first virus detection workshop which was conducted in Cairo, from 16-20<sup>th</sup> April, 2007 imparted the knowledge applicable for molecular diagnostics in Ain Shams Univ.

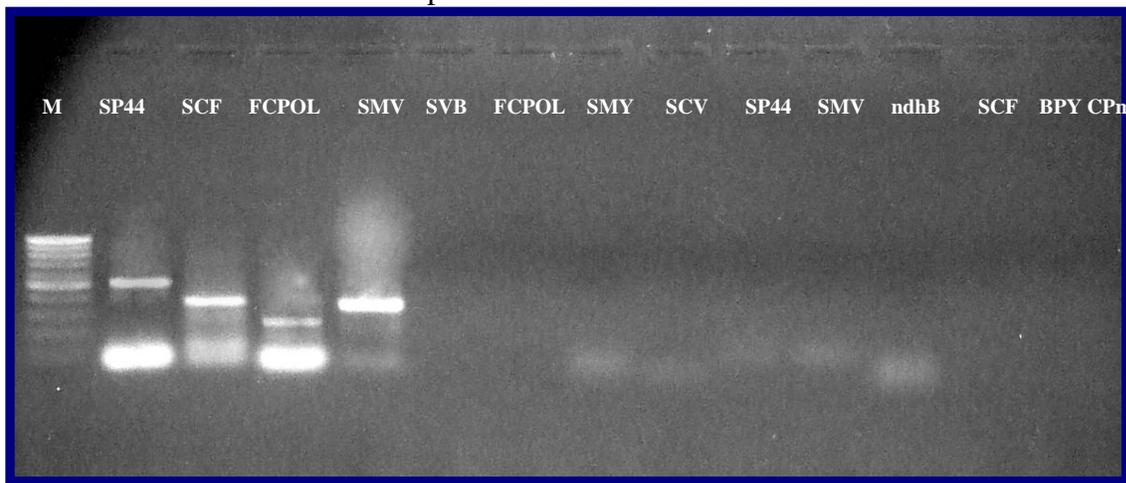
### A. Virus detection in nucleus plants

Ten leaves were sampled from the nucleus propagation material of Tamar and Yael cultivars produced through meristem tissue culture. The plants of these cultivars are maintained in a nucleus

greenhouse equipped with insect-proof nets. Samples were transferred to the Molecular Biology Lab, Ain Shams Univ. for virus detection according to the protocols adapted from the workshop in Cairo in 2007.

Specific PCR reaction was performed for detection of 9 viruses which may infect strawberry plants as follows: 1- Strawberry veinbanding virus (SVB); 2- *Fragaria chiloensis* latent virus (FCPOL); 3- Strawberry mild yellow edge virus (SMY); 4- Strawberry crinkle virus (SCV); 5- Strawberry pallidosis virus (SP44); 6- Strawberry mottle virus (SMV); 7- *ndhB*; 8- Strawberry chlorotic fleck (SCF) and 9- Beet pseudo yellows virus (BPY CPm). The PCR components and program used in this work was according to the method used in the Martin lab.

Data presented in Figs. 15 and 16 indicated that only positive controls produced positive results according to expected size for each control, while negative results were obtained with tested samples, which indicates that the tested samples were free of the 9 examined viruses.



**Fig. 15.** Agarose gel electrophoresis of specific PCR reaction for detection of 9 viruses in nucleus order of strawberry plants cv. Tamar derived from meristem culture. M, 100-bp DNA ladder.



**Fig. 16.** Agarose gel electrophoresis of specific PCR reaction for detection of 9 viruses in nucleus order of strawberry plants cv. Yael derived from meristem culture. M, 100-bp DNA ladder.

### Detection of SLRSV and RpRSV viruses

Leaf samples of infected strawberry plants showing distinct viral symptoms like mottle, crinkle, vein banding, vein necrosis, yellowing, dark green, vein black, and leaves malformation were collected from Qalubia. Strawberry latent ring spot (SLRSV) and Raspberry ring spot (RpRSV) viruses were detected in leaves samples using specific polyclonal antibodies specific for SLRSV and RpRSV by dot blot immunoassay (DBIA) as described by Lin *et al.* (1996). The viral antigens were serologically precipitated against specific polyclonal IgG-SLRSV and IgG-RpRSV by DBIA. The dot blot immunoassay was found to be sensitive in detecting SLRSV and RpRSV in all infected plants (Table 7).

**Table 7.** Detection of SLRSV and RpRSV in strawberry plants showing virus-like symptoms using DBIA (where + is positive and – negative).

Samples	SLRSV	RpRSV	Samples	SLRSV	RpRSV	Samples	SLRSV	RpRSV
Vesca	-	-	18	-	-	36	-	-
1	-	+	19	+	+	A	-	+
2	+	+	20	+	+	AR	+	+
3	-	+	21	-	+	C	-	+
4	+	+	22	+	+	D	-	+
5	+	+	23	+	+	E	+	+
6	-	+	24	-	+	1	+	-
7	-	-	25	+	-	2	-	-
8	+	-	26	+	-	3	-	-
9	-	-	27	+	-	4	-	-
10	+	-	28	-	-	5	+	-
11	-	+	29	+	+	6	+	+
12	-	+	30	+	+	102	+	+
13	-	+	31	+	+	103	-	+
14	+	+	32	-	+	104	-	+
15	+	+	33	-	+	107	-	+
16	-	+	34	-	+	109	-	+
17	-	-	35	-	-	110	-	-

The dot blot immunoassay (DBIA) was also used to detect RpRSV in strawberry plants cv. Festival. This cultivar was used to produce RpRSV free plants as well as micro propagated plantlets. Meristems were excised from RpRSV plants under a stereomicroscope, cultivated on MS medium and incubated under conventional conditions. After four subcultures the meristems developed to shoots. RpRSV infected shoot tips were cultivated on Ms medium to verify disease and infected plantlets were exposed to chemotherapy, thermotherapy and combination treatments.

Tissue samples from suspect plants were sent to the USA lab of Martin from Ragab of Egypt. The NADH Bsubunit RT-PCR was positive, which shows the RNA extraction and Reverse Transcription reaction worked well. The size of the PCR product was about 700 base pairs showing that RNA rather than DNA was amplified (would have been 1100 base pairs). The plants were tested for the following viruses by RT-PCR: Apple mosaic, Arabis mosaic, Beet pseudo yellows, *Fragaria chiloensis* latent, *Impatiens necrotic spot*, Raspberry ringspot, Strawberry chlorotic fleck, Strawberry crinkle, Strawberry latent ringspot, Strawberry mottle, Strawberry mild yellow edge, Strawberry necrotic shock (TSV), Strawberry pallidosis, Strawberry veinbanding, Tomato black ring

and Tomato ring spot viruses. Suspect material sampled from Egyptian fruiting fields was tested by the molecular methods in the USA and Egypt and the following viruses were detected: SMYEV, SVBV, BPYV and SPaV. All other virus tests were negative. Extraction of dsRNA from the tissue was also attempted, but did not yield any bands on the gels. Thus, all tests were negative.

### **Summary of virus diagnosis**

**Protocols were completed for the molecular indexing system for detection of strawberry infecting viruses after improving the RNA extraction method from strawberry. The protocol was finalized and used successfully for the first training workshop dedicated to virus detection which was conducted in Cairo, from 16-20<sup>th</sup> April, 2007.**

**No viruses were detected in the fields during the 2007-12 cultivation seasons. This indicates that the detection protocols are accurate and reliable, as also visibly, no symptoms of virus infections were detected in strawberry in Israel.**

**Strawberry material sampled from Egyptian fruiting fields was tested by the molecular methods in USA and Egypt. The following viruses were detected: SMYEV, SVBV, BPYV and SPaV while nucleus plants tested negative for all viruses. The first joint publication on virus detection in strawberry in Egypt was published following this work (Ragab et al., 2009).**

### **Literature cited**

Ahmed K.G.M.; A.A. EL-Deeb and T.S.Khafagi (1984). Reaction of strawberry fruit to leather rot caused by *Phytophthora cactorum* (Leb.&Cohn.) Schroet in relation to chemical constituent in different stages of growth. Proc. 9<sup>th</sup> Inter. Cong. For Stat. Computer Sci, Social Demographic Res;31 Mar.-10 Apr., Cairo Egypt, PP. 631-644.

Babini, R., Cieslinska, M., Karesova, R., Thompson, J., Cardoni, M., Malinowski, T., Paprstein, F. and Jelkmann, W. (2003). Occurrence and identification of strawberry viruses in some European countries. Presented at the 10<sup>th</sup> International Symposium on Small Fruit Virus Diseases, Valencia, Spain.

Bonants, P., Hagenaar-Weerd, M., Van Gent-Pelzer, M., Lacourt, I., Cooke, D., and Duncan, J. (1997). Detection and identification of *Phytophthora fragariae* Hickman by the polymerase chain reaction. *Eur. J. Plant Pathol.* 103:345-355.

Boxus P. (1998). Micropropagation of strawberry via axillary shoot proliferation. In: Methods in Molecular Biology. Vol. 111: Plant Cell Culture Protocols. Ed. Hall R. D. Humana Press Inc. Totowa NJ, USA.

Converse, R.H., Adams, A.N., Barbara, D.J., Clark, M.F., Casper, R., Hepp, R.F., Martin, R.R., Morris, T.J., Spiegel, S. and N. Yoshikawa. (1988) Laboratory detection of viruses and mycoplasma-like organisms in strawberry. *Plant Dis.* 72:744-749.

Dan, H., Ali-Khan, S.T., and J. Robb. J. (2001). Use of quantitative PCR diagnostics to identify tolerance and resistance to *Verticillium dahliae* in potato. *Plant Dis.* 85:700-705.

- Embaby, E.M., Ragab, M.E., Doug Doug, Kh. A. Al., Ahmed, R., Zveibil, A., Maymon, M., and Freeman, S. (2010). First report of *Colletotrichum acutatum* and *C. gloeosporioides* causing anthracnose diseases on strawberry in Egypt. *Plant Pathology* 59:808.
- Freeman, S., Horowitz, S., and Sharon, A. (2001). Pathogenic and non-pathogenic lifestyles in *Colletotrichum acutatum* from strawberry and other plants. *Phytopathology* 91:986-992.
- Freeman, S. and Katan, T. (1997). Identification of *Colletotrichum* species responsible for anthracnose and root necrosis of strawberry in Israel. *Phytopathology* 87:516-521.
- Freeman, S., and Nicoli, G. (1999). Implementation of IPM: Strawberries. In: *Integrated Pest and Disease Management in Greenhouse Crops*, (eds. L. Gullino, J. van Lenteren and Y. Elad) pp. 454-472. Kluwer Academic Publishers, The Netherlands.
- Freeman, S., Shalev, Z., and Katan, J. (2002). Survival in soil of *Colletotrichum acutatum* and *C. gloeosporioides* pathogenic on strawberry. *Plant Dis.* 86:965-970.
- Gamal El-Din, I. F.; K.G.M. Ahmed; Nawal A. Eisa; F. A. Fadl and Y. S. Khafagi (1981). Physiological studies and host range of *Phytophthora cactorum* (Leb. & Cohn.) Schroet, the casual organism of leather rot of fruits and collar rot of strawberry in Egypt. Annual Agric. Res. Bull., No. 1465, Fac. Of Agric., Ain Shams Univ.
- Honetslegrova, J. Mraz, I. and Spak, J. (1995). Detection and isolation of strawberry veinbanding in the Czech Republic. *Acta Hort.* 385:29-32.
- Horowitz, S., Freeman, S., and Sharon, A. (2002). Use of GFP-Transgenic strains to study pathogenic and non-pathogenic lifestyles in *Colletotrichum acutatum*. *Phytopathology* 92:743-749.
- Howard, C.M., and Albrechts, E.E. (1982) Outbreaks of Verticillium wilt of strawberries in central Florida, *Plant Dis.* 66:856-857.
- Howard C.M., Maas, J.L., Chandler, C.K., and Albrechts, E.E. (1992) Anthracnose of strawberry caused by the *Colletotrichum* complex in Florida, *Plant Dis.* 76:976-981.
- Jelkmann, W., Maiss, E. and Martin, R.R. (1992). Sequence of a potyvirus associated with strawberry mild yellow edge virus. *J. Gen. Virol.* 73:475-479.
- Jemmali A., Boxus P. H., Kevers C. L. and Gaspar T. H. (1994). Flowering abundance of strawberry depending on the number of subcultures In Vitro. In *Physiology, Growth and Development of Plants in Culture*. P. J. Lumsden, J. R. Nicholas and W. J. Davis eds. 356-362. Kluwer Academic Publishers, The Netherlands.
- Khafagi, Y.S.; M.A. Beshir and W. M. Assal (1993). Chemical constituents of some strawberry cultivars in relation to their reaction to *Rhizoctonia* root-rot disease. *Egypt. J. Appl. Sci.*, 11: 95-106.
- Khayat E., Abdul Razek A., Halevy D. and Guntmacher T. (1997). Effective cleaning of strawberry plants from pathogenic fungal and bacterial contaminants. *Acta Hort.* 439:369-372.

- Kinet J. M. and Parmentier A. (1989). The flowering behavior of micropropagated strawberry plants cv. 'Gorella': the influence of number of subcultures on the multiplication medium. *Acta Hort.* 265:327-334.
- Lacourt, I., Bonants, P.J.M., Van Gent-Pelzer, P.M., Cooke, D.E.L., Hagenaar-De Weert, M., Surplus, L., and Duncan, J.M. (1997). The use of nested primers in the polymerase chain reaction for the detection of *Phytophthora fragariae* and *P. cactorum* in strawberry. *Acta Hort.* 439:829-838.
- Maas, J. (1998). Compendium of strawberry diseases. APS Press. 98 pp.
- Martin, R.R. (2001) Recommended procedures for detection of viruses of small fruit crops. *Acta Hort.* 551:113-116.
- Martin, R.R., James, D. and Levesque, C.A. (2000). Impacts of Molecular Diagnostic Technologies on Plant Disease Management. *Annu. Rev. Phytopathol.* 38:20
- Martin, R.R. and Tzanetakis, I.E. 2006. Characterization, detection and management of strawberry viruses. *Plant Dis.* 90:384-396.
- Mostafa, A.M.; Y.S. Khafagi; W.M. Assal and H.A. Mohamed (1992). Integrated control for root-rot and wilt diseases of strawberry. *Egypt. J. Appl. Sci.*, 7 (9): 277-291.
- Picha, D.P. (2000). Strategic options for the development of strawberry export industry, subsector study. Agricultural Technology Utilization and Transfer ATUT/RONCO, Website: <http://www.atut.gov.eg> .
- Pichia, D.P. (1999). Strawberry diseases and their control. Agricultural Technology Utilization and Transfer ATUT/RONCO, Website: <http://www.atut.gov.eg>.
- Posthuma, K.I., Adams, A.N., Hong, Y. and Kirby, M.J. (2002). Detection of Strawberry crinkle virus in plants and aphids by RT-PCR using conserved L gene sequences. *Plant Pathol.* 51: 266-274.
- Quail, A.M., Martin, R.R., Spiegel, S. and Jelkmann, W. (1995). Development of monoclonal antibodies specific for strawberry mild yellow edge potexvirus. *Acta Hort.* 385:39-45.
- Ragab, M.E., Dogdog, K., Attia, A., Sobolev, I., Spiegel, S., Zeidan, M., Freeman, S., Tzanetakis, I.E., and Martin, R.R. (2009). Detection of strawberry viruses in Egypt. *Acta Hort.* 842:319-322.
- Razik, A., Grinstein, A. and Katan, J. (1989). Rhizoctonia disease in propagation material and field grown strawberry. *Acta Hort.* 265:579-585
- Sami, A. (2000). Strawberry take-off? <http://weekly.ahram.org.eg/2000/473/ec3.htm>
- Schoen, C.D., Limpens, W., Moller, I. Groeneveld, L. and Lindner, J.L. (2003). The complete genomic sequence of strawberry crinkle rhabdovirus, a member of the *Rhabdoviridae*. Presented at the 10<sup>th</sup> International Symposium on Small Fruit Viruses, Valencia, Spain, July 21-25, 2003.

- Spiegel, S. (1998) Virus certification of strawberries. In "Plant Virus Disease Control", pp. 320-324. Eds. A. Hadidi, R.K. Khetarpal and H. Koganezawa, APS Press, U.S.A.
- Spiegel, S. and Frank, A. (1982). Virus and virus-like diseases in strawberries in Israel. *Acta Hort.* 129:99-101.
- Spiegel, S. and Martin, R.R. (1998) Virus and viruslike diseases. In "Compendium of Strawberry Diseases" pp. 62-72. Ed. J. Maas, APS Press, U.S.A.
- Spiegel, S. and Martin, R.R. (1993). Improved detection of potato leafroll virus in dormant potato tubers and microtubers by the polymerase chain reaction and ELISA. *Ann. Appl. Biol.* 122:493-500.
- Spiegel, S., Martin, R.R., Legget, F., ter Borg, M. and Postman, J. (1993). Characterization and geographical distribution of a new ilarvirus from *Fragaria chiloensis*. *Phytopathology* 83:991-995.
- Thompson, J.R., Leone, G., Lindner, J.L., Jelkmann, W. and Schoen, C.D. (2002). Characterization and complete nucleotide sequence of Strawberry mottle virus: a tentative member of a new family of bipartite plant picorna-like viruses. *J. Gen. Virol.* 83: 229-239.
- Tzanetakis, I.E. and Martin, R.R. 2004. Complete nucleotide sequence of a strawberry isolate of *Beet pseudo yellows virus*. *Virus Genes* 28:239-246.
- Tzanetakis, I.E. and Martin, R.R. 2005. New features of the genus *Iilarvirus* revealed by the nucleotide sequence of *Fragaria chiloensis latent virus*. *Virus Research* 112:32-37.
- Tzanetakis, I.E. and Martin, R.R. 2007. Strawberry chlorotic fleck: Identification and characterization of a novel *Closterovirus* associated with the disease. *Virus Res.* 124:88-94.
- Tzanetakis, I.E. and Martin, R.R. 2005. First report of strawberry as a natural host of *Apple mosaic virus*. *Plant Dis.* 89:431.
- Tzanetakis, I.E., Halgren, A.B., Keller, K.E., Hokanson, S.C., McCarthy, P.L., Maas, J.L. and Martin, R.R. 2004. Identification and detection of a virus associated with strawberry pallidosis disease. *Plant Dis.* 88:383-390.
- Tzanetakis, I.E., Mackey, I.C. and Martin, R.R. 2004. Strawberry necrotic shock virus is a distinct virus and not a strain of *Tobacco streak virus*. *Arch. Virol.* 149:2001-2011.
- Wright, W.R., Beraha, L., and Smith, M.A. (1966) Leather rot on California strawberries, *Plant Dis. Rep.* 50:283-287.
- Zveibil, A., and Freeman, S. 2005. First report of crown and root rot in strawberry caused by *Macrophomina phaseolina* in Israel. *Plant Dis.* 81:1014.
- Zveibil, A., Mor, N., Gnayem, N., and Freeman, S. 2012. Survival, host-pathogen interaction, and management of *Macrophomina phaseolina* on strawberry in Israel. *Plant Dis.* 96:265-272.

## **6. Impact Relevance and Technology Transfer**

Considerable cooperation was achieved during the lifetime of the project. All necessary equipment for the successful implementation of the research project was purchased; a gradient PCR machine and refrigerated centrifuge in Israel, and all equipment for the implementation of molecular detection of viruses in strawberry and for other aspects of the project for Egypt (see list below), according to the workplan. In addition, a greenhouse to contain disease-free nucleus strawberry plants was constructed in Ain Shams University, Egypt, to guarantee the basis for disease-free planting material in the future.

The Strawberry Improvement Center in Ain Shams University is now capable of screening strawberry propagation material for fungal and virus pathogens of strawberry after successful completion of the virus detection workshop (from 16-20<sup>th</sup> April, 2007) and fungal detection workshop (9-15<sup>th</sup> November, 2007) which were conducted in Cairo. A nucleus greenhouse was constructed in the Ain Shams University institute for maintenance of disease-free propagation material.

In Egypt, approximately 2 field days each year were held with strawberry nurserymen in Kalubia, Ismailia and Nubaria Governorates. Attendance was between 20 to 40 growers per field day. Strawberry specialists from the ministry of Agriculture and pathologists as well as five project staff attended and described the factors affecting quality and quantity of transplants in the nurseries and how to avoid disease infection in the nurseries.

The project team in Egypt prepared a pamphlet for the small farmers indicating that cheap, clean strawberry mother plants will be available to them from the Strawberry Center of the Ain Shams University program. The pamphlet also includes recommendations for reducing and avoiding fungal diseases in strawberry nurseries and production fields which is a direct result from experimental work conducted during this project.

The information derived from the project has been disseminated by the Israeli and Egyptian Ministries of Agriculture and by their bulletins. People directly involved in the project, researchers and students alike, have been regularly in direct contact with growers and the grower's organizations, and have disseminated information by lectures, visits and articles in trade and international journals. Impact of the project's results will have a direct effect on production by elevating yields, producing disease-free propagation material and contributing to extensive new field planting.

### Equipment purchased in Egypt:

TC-312 Thermal Cycler (Techne); Power Supply, PS 304 Apelex; Unitwest Orbital Shaker; vertigel range for proteins electrophoresis; Recirculating Cooler; Bench pH. Meter; Electrical Analytical Digital Balance; Refrigerator Variable Speed Centrifuge with rotors; Water Distiller; varigel Horizontal gel Electrophoresis equipment; UVI transilluminator; liquid nitrogen container; compact vortex; laptop computer; data show projector.

## **7. Project Activities/Outputs**

### Meetings within the context of the project:

1. Freeman met in January 2006 with Ragab in Egypt to initiate the project. Martin visited Israel in May, 2006 and met with the Israeli partners in Volcani and at RAHAN to coordinate future research.
2. Freeman met Ragab in Cairo November 2006 to discuss mutual research progress.
3. Freeman met Ragab in Cairo in February 2007 to discuss the workshop plans.
4. The first annual meeting was held in conjunction with the first strawberry virus diagnostic workshop took place at Ain Shams University in Cairo, from 15-20<sup>th</sup> April, 2007.
5. The first fungal detection workshop was conducted by Freeman in Cairo between 9-15<sup>th</sup> November, 2007 at Ain Shams University.
6. The second annual meeting of the project took place on 2<sup>nd</sup> March 2008, in concert with the 6<sup>th</sup> International Strawberry Symposium held from 3-7<sup>th</sup> March, 2008.
7. Freeman visited Ragab in Egypt from 22<sup>nd</sup> – 25<sup>th</sup> August 2008 to discuss the workplan, the field trials and mutual publications.
8. The third annual meeting was held in Cairo, Egypt during the period: 21<sup>st</sup> – 23<sup>rd</sup> April, 2009.
9. Freeman visited Ragab in Egypt from 2<sup>8th</sup> to 3<sup>1st</sup> May, 2010, to discuss the current and future workplan, and the annual meeting of 2010.
10. The fourth annual MERC meeting took place in Lisbon, Portugal on 24<sup>th</sup> August 2010, jointly with a symposium titled "Berries: From genomics to sustainable production, quality and health", as part of the International Horticulture Congress (IHC2010), held between 22-27<sup>th</sup> August, 2010.
11. Freeman visited Ragab in Egypt from 11<sup>th</sup>-15<sup>th</sup> November 2010 to discuss the current and future workplan for 2011.

### International training

Two training workshops were conducted among the researchers of the project.

1. The first, a virus detection workshop was conducted in Cairo between 16-20<sup>th</sup> April, 2007 at Ain Shams University with the participation of Dr. S. Freeman, Dr. S. Spiegel, Ms. Eppie Lotem (Rahan R&D) and Dr. M. Zeidan from Israel, Dr. M. Ragab from the Univ. of Ain Shamas, and 20 participants from various institutes in Egypt. All participants from Egypt were trained by Dr. Martin in a hands-on detection workshop with equipment purchased by Dr. Ragab for this purpose. In addition to his professional expertise, Dr. M. Zeiden served as an interpreter that greatly enhanced the communication at the workshop. Following the acquisition of this knowledge it is envisaged that a mutual study will be presented on molecular detection of viruses in strawberry at the 6<sup>th</sup> International Strawberry Symposium to be held from 3-7<sup>th</sup> March, 2008. We were very fortunate that Dr. Cheryl L. Wojciechowski, AAAS Diplomacy Fellow from the MERC & CDR Programs U.S. Agency for International Development, was able to participate in most of the workshop training. This further demonstrated the importance and priority dedicated by the program to foster the cooperation between scientists and participating countries at the workshop.
2. The first fungal detection workshop was conducted by Dr. S. Freeman in Cairo between 9-15<sup>th</sup> November, 2007 at Ain Shams University. All participants from Egypt, including 15 persons (project staff) and others from various institutes, were trained by Dr. Freeman in a very fruitful hands-on detection workshop with equipment purchased by Dr. Ragab for this purpose.

Diseased plants were isolated in Egypt using the identification protocols imparted at the fungal diagnostics workshop. The pathogens were further analyzed and the responsible pathogen species of *Colletotrichum* was verified in the Freeman lab. This will lead to future mutual publications.

In addition, local training was conducted in disease detection for 3 weeks in February for the project staff which was also provided by some university and ARC staff associated with the project.

Following the knowledge acquired for virus detection during the at the April 2007 training workshop, a mutual study was presented on “Detection of strawberry viruses in Egypt” by Mohamed Ragab, Khaled Dogdog, Amany Attia, Irena Sobolev, Sara Spiegel, Mouhammed Zeidan, Stanley Freeman, Ionnis E. Tzanetakis and Robert R. Martin at the 6<sup>th</sup> International Strawberry Symposium held from 3-7<sup>th</sup> March, 2008.

Following the first fungal detection workshop which was conducted by Dr. S. Freeman in Cairo between 9-15<sup>th</sup> November, 2007 at Ain Shams University, regular sampling and detection of fungal pathogens from diseased plants are being conducted in Egypt. Likewise, virus detection is also being conducted, however, no diseased plants were isolated with visible virus infections in Israel from various sources.

#### Local training in Egypt

Two grower meetings were conducted in late April 2009 in Qalubia and Ismailia for local Egyptian nurserymen whereby 57 and 41 attendants from the two respective governorates attended. Specialists from the ministry of Agriculture and plant pathology institute participated with the project staff. The meeting focused on the importance of the clean plants from foundation stock for nursery establishment and the root rot disease during nursery growth period.

In Egypt, 3 field days were held with strawberry nurserymen on 23<sup>rd</sup> May, 16<sup>th</sup> June and 17<sup>th</sup> July 2010 in Kalubia, Ismailia and Nubaria Governorates, respectively. Attendance was 23 to 40 growers per field day. Strawberry specialists from the ministry of Agriculture and pathologists as well as five project staff attended and described the factors affecting quality and quantity of transplants in the nurseries and how to avoid disease infection in the nurseries.

A field day for the growers at Qalubia Governorate, Egypt, was conducted on 25<sup>th</sup> February 2011 and many of the strawberry fields were visited. The project staff discussed the reasons for appearance of weak plants, infected field plants with root and crown rot diseases and the following implementations directly associated with the project goals were recommended to the growers: Growers should use foundation plants for their nurseries; fumigate the nurseries and fields by appropriate means; check their fields regularly for infection in roots and crowns and send to the lab and suspect plants, particularly when they use canal water for irrigation; follow a crop rotation and do not grow strawberry close to other vegetables such as bean or cucurbits that may carry diseases that can infect strawberry (such as *Macrophomina*). A pamphlet describing these methods was distributed to the growers (see below).



جامعة عين شمس  
كلية الزراعة

## مشروع تحسين الفراولة ( ميرك )



Joint, co-authored publications

1. A joint research effort directly related to the MERC project was presented at the 6<sup>th</sup> International Strawberry Symposium held from 3-7<sup>th</sup> March, 2008:

M. Ragab, K. El-DougDoug, S. Mousa, A. Attia, I. Sobolev, S. Spiegel, S. Freeman, M. Zeidan, I.E. Tzanetakis and R.R. Martin. 2009. Detection of strawberry viruses in Egypt. *Acta Horticulturae* 842:319-322.

### Detection of Strawberry Viruses in Egypt

M. Ragab, K. El-DougDoug, S. Mousa and  
A. Attia  
Strawberry Improvement Center  
Faculty of Agriculture  
Ain Shams University, Cairo  
Egypt

I. Sobolev, S. Spiegel and S. Freeman  
Agricultural Research Organization  
The Volcani Center  
Department of Plant Pathology  
Bet-Dagan 50250  
Israel

M. Zeidan  
Plant Protection and Inspection Services  
Ministry of Agriculture and  
Rural Development  
PO Box 78, Bet-Dagan 50250  
Israel

I.E. Tzanetakis  
Dept. of Plant Pathology  
University of Arkansas  
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USA

R.R. Martin  
USDA-ARS  
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Research Lab  
Corvallis, OR  
USA

**Keywords:** RT-PCR, SMYEV, SVBV, BPYV, SPaV, *Fragaria*

#### Abstract

As part of a USAID-MERC funded project, 'Disease-indexing and mass propagation of superior strawberry cultivars', an effort was made to evaluate the virus status of strawberries in Egypt. Diagnostic reverse transcription-polymerase chain reaction (RT-PCR) tests for *Strawberry mottle virus*, *Strawberry crinkle virus*, *Strawberry vein banding virus* (SVBV), *Strawberry mild yellow edge virus* (SMYEV), *Strawberry chlorotic fleck virus*, *Strawberry necrotic shock virus*, *Strawberry latent ringspot virus*, *Apple mosaic virus*, *Fragaria chiloensis latent virus*, *Strawberry pallidosis associated virus* (SPaV) and *Beet pseudo yellows virus* (BPYV) were developed and/or evaluated at the USDA-ARS laboratory in Corvallis, Oregon. Positive controls for the testing consisted of dried leaf samples of strawberries infected with each of the above viruses, shipped to Egypt and Israel under import permits. Collection of strawberry test samples was done in production fields (cultivars unknown) and from nuclear stock plants grown in Egypt ('Tamar' and 'Yael' originating from Israel). RNA extraction was carried out using Qiagen kits or a previously described protocol. Reverse transcription and polymerase chain reactions were carried as per manufacturer's recommendations. Amplicons were visualized after separation on an agarose gel and staining with ethidium bromide. The RT-PCR detection of viruses from RNA extracted from positive controls that were vacuum dried was successful for several but not all of the viruses used in this study. Thus, extracted RNA that can be shipped under ethanol may be a better positive control than dried tissue samples. Material carried back to the USA from Egypt as extracted RNA under ethanol was not impacted by storage at room temperature during transport. Initial testing of strawberry material from fields in Egypt showed that SMYEV, SVBV, BPYV and SPaV were detected in field samples. The plants tested from the nuclear stocks tested negative for each of the viruses tested.

#### INTRODUCTION

The USAID-MERC program funds projects to foster cooperation between Israel and Arab nations by supporting joint research programs and technology transfer. The objectives of this project are to improve the efficiency of strawberry production in Egypt through improved plant health of nursery stocks and increased efficiencies in plant production. This report presents information on initial efforts to assess the virus status of strawberries in Egypt and the development of the resources and expertise to operate a strawberry virus indexing program there.

Strawberry (*Fragaria* spp.) can be infected by more than 30 virus and virus-like

2. Two species of *Colletotrichum* in strawberry were identified for the first time in Egypt and this new data was published in the British journal "Plant Pathology":

E.M. Embaby, M.E. Ragab, Kh. A. Al. Doug Doud, R. Ahmed, A. Zveibil, M. Maymon, and S. Freeman. 2010. First report of *Colletotrichum acutatum* and *C. gloeosporioides* causing anthracnose diseases on strawberry in Egypt. *Plant Pathology* 59:808.



*Plant Pathology* (2010) 59, 808

Doi: 10.1111/j.1365-3059.2010.02262.x

### First report of *Colletotrichum acutatum* and *C. gloeosporioides* causing anthracnose diseases on strawberry in Egypt

E. M. Embaby<sup>a</sup>, M. E. Ragab<sup>b</sup>, Kh. A. Al. Doug Doug<sup>b</sup>, R. Ahmed<sup>b</sup>, A. Zveibil<sup>c</sup>, M. Maymon<sup>c</sup> and S. Freeman<sup>c\*</sup>

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During the growing seasons of 2007 and 2008, typical anthracnose symptoms were observed in cultivated strawberry (*Fragaria × ananassa*) fields in Kalubia and Ismailia governorates, Egypt. Disease symptoms on immature and ripe fruits contained circular, sunken, and dark-coloured lesions (1–12 mm) bearing salmon-coloured masses of conidia, with lesions also appearing in necrotic petioles and stolons (Freeman & Katan, 1997). A representative *Colletotrichum acutatum* culture (isolate 4), from infected fruit (cv. Yael), possessed hyaline, cylindrical conidia attenuated at both ends, measuring 12.6 (11.8–15.4) × 4.1 (3.3–5.1) µm (Gunnell & Gubler, 1992). Additional symptoms of wilted plants resembled those of anthracnose crown rot caused by *C. gloeosporioides* (Freeman *et al.*, 2002). A representative *C. gloeosporioides* culture (isolate 1), isolated from necrotic roots (cv. Tamar), possessed hyaline, oblong conidia with obtuse ends, measuring 15.5 (14.3–17.3) × 4.5 (4.3–5.0) µm (Gunnell & Gubler, 1992).

Symptoms typical to those observed in the field were obtained 3 weeks after inoculation on 2-month-old potted strawberry transplants (six replicate plants each for isolate 1 and isolate 4), sprayed with conidial suspensions (10<sup>6</sup> conidia per mL) and maintained in a moist chamber for 48 h at 25°C. Water-inoculated plants remained healthy. Re-isolations were made from infected fruit, petioles, stolons and crowns, verifying the causal agents of disease.

Species-specific PCR amplification was conducted on the two representative *Colletotrichum* isolates. The identities of the pathogens were confirmed as *C. gloeosporioides* (isolate 1) resulting in a single amplified DNA fragment of 450 bp using primers ITS4 and CgInt; and *C. acutatum*

(isolate 4) with an amplified product of 490 bp using primers ITS4 and Calnt2 (Freeman *et al.*, 2002). This is the first reliable and accurate report, based on molecular identification, of *C. acutatum* and *C. gloeosporioides* causing anthracnose on strawberry in Egypt, although a record on occurrence of strawberry anthracnose was published previously in a local journal (Khafagi, 2006).

#### Acknowledgements

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#### References

- Freeman S, Katan T, 1997. Identification of *Colletotrichum* species responsible for anthracnose and root necrosis of strawberry in Israel. *Phytopathology* 87, 516–21.
- Freeman S, Shalev Z, Katan J, 2002. Survival in soil of *Colletotrichum acutatum* and *C. gloeosporioides* pathogenic on strawberry. *Plant Disease* 86, 965–70.
- Gunnell IS, Gubler WD, 1992. Taxonomy and morphology of *Colletotrichum* species pathogenic to strawberry. *Mycologia* 84, 157–65.
- Khafagi YS, 2006. First record of strawberry anthracnose in Egypt. *Egyptian Journal of Applied Science* 21, 397–402.

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3. Within the context of the third annual MERC meeting that took place in Lisbon, Portugal on 24<sup>th</sup> August 2010, jointly with a symposium titled "Berries: From genomics to sustainable production, quality and health", as part of the International Horticulture Congress (IHC2010), held between 22–27<sup>th</sup> August, 2010, three studies were presented:

- A. Identification of *Colletotrichum acutatum* and *C. gloeosporioides* on strawberry in Egypt. E.M. Embaby, Plant Pathology Dept., National Research Centre, Cairo; M.E. Ragab, Kh. A. Al. Doug Doug, R. Ahmed, Faculty of Agriculture, Ain Shams Univ., Cairo, Egypt.; A. Zveibil, M. Maymon, and S. Freeman, Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel.
- B. Molecular detection of some strawberry viruses in Egypt. Khaled al-Doug Doug<sup>1</sup>, Mohamed Ragab<sup>2</sup>, Stanley Freeman<sup>3</sup>, Sara Spiegel<sup>3</sup>, Robert R. Martin<sup>4</sup> and Amani Attia<sup>5</sup> Plant Microbiology Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt; <sup>2</sup>Hort. Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt; <sup>3</sup>Agric. Res. Organization, The Volcani Center, Bet Dagan Israel; <sup>4</sup>Horticultural Crops Research Laboratory., USDA-ARS, Corvallis, Oregon USA; <sup>5</sup>Horticulture Research Inst., ARC, Giza, Egypt
- C. Evaluation of mass propagation application as a commercial practice for producing strawberry mother plants. Nir Dai<sup>1</sup>, Zecharia Tanami<sup>1</sup>, Sara Slotzky<sup>1</sup>, Amany Attia<sup>2</sup>, Abo-El-Ezz Omran<sup>2</sup>, Mohamed Ragab<sup>3</sup>, and Stanley Freeman<sup>4</sup>. <sup>1</sup>Institute of Plant Sciences and <sup>4</sup>Plant Protection, ARO, The Volcani Center, Bet Dagan 50250, Israel; <sup>2</sup>Horticulture Research Institute ARC, Cairo, Egypt; <sup>3</sup>Strawberry Improvement Center, Fac. of Agric., Ain Shams Univ., Cairo, Egypt

## **8. Project Productivity**

All the proposed goals of the project were accomplished.

### Summary of virus diagnosis

Protocols were completed for the molecular indexing system for detection of strawberry infecting viruses after improving the RNA extraction method from strawberry. The protocol was finalized and used successfully for the first training workshop dedicated to virus detection which was conducted in Cairo, from 16-20<sup>th</sup> April, 2007. Strawberry material sampled from Egyptian fruiting fields was tested by the molecular methods in USA and Egypt. The first joint publication on virus detection in strawberry in Egypt was published following this work (Ragab et al., 2009). No viruses were detected in the Israeli strawberry fields during the 2007-12 cultivation seasons which indicates that the detection protocols are accurate and reliable, as also visibly, no symptoms of virus infections were detected.

### Summary of fungal pathogen diagnosis

In Israel and Egypt the major fungal soilborne pathogens that were identified were similar (*Macrophomina*, *Rhizoctonia* and *F. oxysporum*) that were detected over the years during the project when diseased plants were sampled from infected nurseries. This indicated that the Egyptian researchers have learned to apply the diagnostic techniques acquired during the training workshop period conducted in Cairo, between the 9-15<sup>th</sup> November 2007, at Ain Shams University. For the first time in Egypt, *Colletotrichum acutatum* and *C. gloeosporioides* were identified from infected wilted strawberry plants and verified according to species-specific primers which resulted in a mutual publication (Embaby et al., 2010).

### Summary of horticultural, mass propagation aspects of the project

Transplants originating from the nucleus source produced flowers earliest in the season. Therefore, in the beginning of the season the yield from these plants was greater than that of plants from mass propagated and commercially produced nursery sources. Average weights also appear to be higher in the nucleus produced plants. Therefore, we conclude that the source of mother plant origin has no effect on plant performance and productivity in the fruiting fields in both countries.

## Appendices

### **Freeman (Israeli PI) and Ragab (Egyptian PI)**



**First strawberry virus diagnostic workshop which took place at the Strawberry Improvement Center, Ain Shams University, Cairo, between 16-20<sup>th</sup> April, 2007**

**Participants at workshop and during field trip**





**Hand-on workshop**



**Discussions and lectures**

**The first fungal detection workshop was conducted in Cairo between 9-15<sup>th</sup> November, 2007 at Ain Shams University.**



**Local training associated with strawberry fungal diseases and propagation**



The second annual MERC (M24-022) meeting took place in Huelva, Spain on March 2<sup>nd</sup> in conjunction with the 6<sup>th</sup> International Strawberry Symposium, held from March 3<sup>rd</sup>-7<sup>th</sup>.



Participants at the 2<sup>nd</sup> Annual MERC meeting in Huelva, Spain, in concert with the VI International Strawberry Symposium that was held between 3<sup>rd</sup> to 7<sup>th</sup> March, 2008. L to R: N. Gnayem (Israel), N. Dai (Israel), S. Freeman (Israel), M. Ragab (Egypt), A. Attiya (Egypt), R. Martin (USA), K. Doug-Doug (Egypt).

The third annual meeting took place in Lisbon, Portugal on 24<sup>th</sup> August 2010, jointly with a symposium titled "Berries: From genomics to sustainable production, quality and health", as part of the International Horticulture Congress (IHC2010), held between 22-27<sup>th</sup> August, 2010



L to R: K. Doug-Doug (Egypt), A. Attiya (Egypt), N. Dai (Israel), S. Freeman (Israel), M. Ragab (Egypt), R. Martin (USA)

## **Direct beneficiaries from the project (personnel, students, farmers)**

All the below researchers, technicians and students participated in the study of all project objectives i.e., development, application and establishment of a reliable diagnostic system based on classical and molecular techniques for detection of the major fungal, virus and phtoplasma pathogens affecting strawberry production in the region; development of an efficient procedure for the supply of disease-free, high quality, planting material of selected cultivars, based on micro-propagation, short-term hardening and transfer of plantlets to field nurseries for daughter plant production and subsequently to strawberry fields within one year. A list of participating farms is also provided.

### **Israel**

#### **Personnel:**

##### **Technicians:**

Mrs. Marcel Maymon: Identification of *Colletotrichum* species affecting strawberry in Israel and Egypt.

Mrs. Aida Zveibil: Isolation and identification of soilborne pathogens affecting strawberry in Israel.

Mrs. Irena Sobolev: Isolation and detection of viruses of strawberry in Israel.

Mr. Zecharia Tanami: Propagation of strawberry in the lab, greenhouse and field evaluation.

##### **Students:**

Mrs. Sigal Horowitz (Ph.D): Host range of *Colletotrichum* affecting strawberry and other crops in Israel.

Mr. Tomer Gershon (Ph.D): Factors affecting pathogenicity of *Colletotrichum* of strawberry in Israel.

#### **Farms where field experiments were conducted:**

Yemini, Sharon region, Central Israel

Nachshon, Sharon region, Central Israel

Porat, Sharon region, Central Israel

Volcani Experimental Station, Central Israel

### **Egypt**

#### **Personnel:**

##### **Researchers:**

Dr. El-Saied Embaby: Isolation and identification of soilborne pathogens affecting strawberry

Dr. Abo El-Ezz Shehata: Nursery propagation and field evaluation

Dr. Amany Attia: Fruit production evaluation

Dr. Sabry Mossa: Virus detection of strawberry and isolation

Dr. Khaled Dougdoug: Virus identification and isolation

##### **Technicians:**

Propagation of strawberry in the greenhouse and lab:

Mrs. Rehab Fawzy

Mrs. Randa Gamil,  
Mrs. Azza Khalifa  
Mrs. Rehab Marzok  
Mrs. Hala El Saied  
Mrs. Iman Yahia  
Mrs Nadia Salah

Nursery propagation and fruit production evaluation:

Mr. Khaled Abdalla  
Mr. Mahmoud Abdelaziz  
Mr. Ahmed El-Saied

Students:

Mrs. Reda El-Saied Ahmed (PhD): "Factors affecting yield and quality of strawberry genotypes".

Mrs: Heba Mohamed: "Isolation and identification of soil borne pathogens of strawberry".

Mrs: Yassmer Mahmoud: "Isolation and detection of viruses of strawberry in Egypt".

Farms where field experiments were conducted:

- 1- Ahmed El-Sadek (Elroda, Ismailia)
- 2- Refat Rezkana (Toukh, Kalubia)
- 3- Strawberry Improvement Center greenhouse (Shoubra El Kheima)
- 4- Khaled Zein El-Dein (ElKatta , Giza)
- 5- Saad Abdelaty (Elekhsas, Giza)