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SUMMARY - Thirty-six trees of nine varieties were selected as basic material for the certification programme in the framework of the project for the obtention of virus and virus-like disease-free, true-to-type budwood and seedlings. Besides trueness-to-type assessment, pomological and technological tests, indexing for virus diseases was made with biogical tests (grafting to indicators), accompanied by ELISA and electrophoresis. Clones were sanitated by shoot tip grafting in combination with thermotherapy. In Turkey, certified propagating material must be free from tristeza, stubborn, exocortis, cachexia and psorosis.

Key words: citrus, virus, virus-like, certification, diagnosis, project, Turkey

RESUME *- Trente-six arbres de neuf variétés ont été sélectionnés comme matériel de base pour un programme de certification dans le cadre du projet d'obtention de matériel végétal indemne de maladies à virus et de type viral et authentique. A part des essais visant à vérifier l'authenticité variétale, les caractéristiques pomologiques et technologiques, on a aussi effectué l'indexage pour les maladies à virus en ayant recours à des tests biologiques (greffage sur les indicateurs), à l'ELISA, à l'électrophorèse et à la culture des agents pathogènes. Les clones ont été assainis par la technique du microgreffage associée à la thermothérapie. Le matériel de multiplication certifié doit être indemne des maladies suivantes: tristeza, stubborn, exocortis, cachexie et psorose.*

Mots-clés: *agrumes, virus, virus-similaires, certification, diagnostic, projet, Turquie*

Introduction

The total citrus producing area of Turkey is about 120,000 ha which equals 2% of the total country's horticultural area. The major citrus growing area is the Mediterranean and the Aegean region as well as the Eastern Black Sea region. As to tree distribution, 75,18% is grown in the Mediterranean, 23,57% in the Aegean Sea and 1,25% in the Black Sea region respectively. According to 1992 data, citrus trees are about 26,000,000 and the overall production totals 1,670,000 tons. Turkey exported 18% of this production as fresh fruit in 1992 (Anonymous, 1993). The production has rapidly increased, particularly in the Eastern Mediterranean region (Çukurova plain). In the near future, with the irrigation of the GAP Region (South-eastern Anatolian Irrigation Project), some industrial crops e.g. cotton and maize, will be replaced by horticultural crops, especially citrus, in the Çukurova region. At present, the Çukurova region yields more than half the total production of the country and its potential to increase citrus production is six-fold higher.

The Turkish citrus industry

The average yield is about 20 ton/ha with peaks of 40-70 ton/ha in some orchards. The production was about 60,000 tons in the 1950s, 520,000 tons in 1965 and 1,500,000 tons in the 1990s (Ozsan, 1970; Anonymous, 1993).

Sour orange is widely used (95%) as a rootstock throughout the Mediterranean and in a part of the Aegean region. Trifoliate orange and Troyer citrange are used as rootstocks in some areas in the Aegean and everywhere in the Black Sea regions.

The most represented citrus species are sweet orange followed by mandarin, lemon and grapefruit (Table I and Figure I) (Ozsan, 1970; Tuzcu, 1991).

Table I – Citrus varieties grown in Turkey under the Programme

Commercial varieties	Growing Regions	SFREC	ACRI
Oranges			
• Washington Navel	Med., Ag.	+	+
• Skaggs Bonanza	Med.	-	-
• Atwood Navel	Med.	+	+
• Lanelate	-	+	-
• Nave Late	-	+	+
• Navelina	-	+	+
• Valencia	Med., Ag.	+	+
• Shamouti	Med., Ag.	+	+
• Moro	Med.	+	-
• Sanguinelli	Med.	+	-
• Some Local Variety	Med., Ag.	-	-
Mandarins			
• Satsuma	Med., Ag., BS	+	+
• Fremont	Med.	+	-
• Clementine	Med., Ag.	+	+
• Nova	Med.	+	+
• Robinson	Med.	+	+
• Lee	-	+	-
• Minneola	Med.	+	+
• Encore	Med.	-	-
• Marisol	-	+	-
• Okitsu	-	+	-
• Ellendale	-	+	-
• Fairchild	Med.	+	-
• Fortune	Med.	+	-
• Some Local Variety	Med., Ag.	-	-
Lemons			
• Interdonato	Med., Ag.	+	+
• Italian	Med.	+	+
• Femminello St. Teresa	Med.	-	-
• Lamas	Med.	-	-
• Monachello	Med.	-	-
• Kütdiken	Med.	+	+
• Yediveren Kütdiken	Med.	+	+
• Kibris	Med.	+	+
• Meyer	Med.	+	-
Grapefruits			
• Marsh-seedless	Med., Ag.	-	-
• Red Blush	Med.	-	-
• Star Ruby	Med.	+	+
• Rio Red	Med.	+	-
• Henderson	Med.	+	+
• Flame	-	+	-

SFREC: Subtropical Fruits Research and Experiment Center,
ACRI: Antalya Citrus Research Institute
Med.: Mediterranean Region
Ag.: Aegean Region
BS.: Black Sea Region

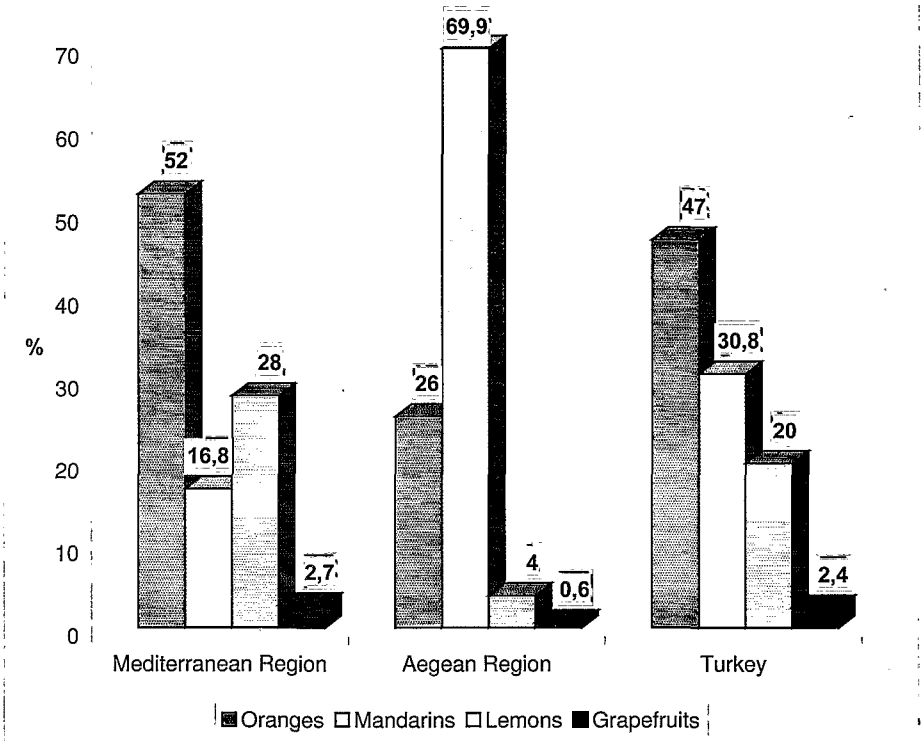

Figure I – Citrus species grown in Turkey (1992)

Citrus trees are infected by a number of virus and virus-like diseases, the major being the psorosis complex, tristeza, stubborn, exocortis, cachexia-xyloporosis, impietratura, gummy bark, crinkly leaf, cristacortis, and satsuma dwarf. The most economically important are psorosis and exocortis. Tristeza is a potential threath to the Turkish citrus industry since sour orange is widely used and the vectors of tristeza virus (CTV) are present in the country. Natural transmission is suspected for, apparently, there is no virus transmission by vectors in the field. Because of the use of sour orange, exocortis does not induce visible symptoms. Most of sweet orange trees are infected with stubborn and most of mandarin trees with cachexia-xyloporosis. Finally impietratura is one of the most important diseases of grapefruit (Azeri and Heper, 1973; Azeri, 1979; Baloglu, 1988; Caglayan, 1987; Chapot, 1961; Chapot and Bahcecioglu, 1969; Cinar *et al.* 1993; Gullu, 1990; Korkmaz *et al.* 1994; Norman, 1963; Onelge, 1994; Salibe, 1986; Sen and Baloglu, 1994; Yilmaz *et al.*, 1990).

Citrus research, training and improvement programmes

Research in citrus virus diseases started in the 1950s with the help of foreign scientists. In 1964, with the collaboration of the Research Institute of Citrus in Antalya and the Faculty of Agriculture, University of Ankara, the first indexing works started and CTV was identified (Anonymous, 1978).

In 1970, the World Bank supported the governmental project "Improvement of Fruit and Vegetable Farming in Turkey", named MEYSEB. Many farmers, growers and nurserymen were supported to establish new orchards between 1970-1980 in the framework of this project. Since technicians and growers were little aware of the presence of virus and virus-like diseases, many disorders were disseminated in the new groves, particularly in the Eastern Mediterranean region (Cinar, 1993a).

In 1978, in collaboration with the University of Çukurova (Adana), the Citrus Research Institute (Antalya), Alata Horticultural Research Institute (Icel) and the Aegean Agricultural Research Institute (Izmir), a new project (Citrus Research and Training Project) started. One of the objectives of this project was to obtain propagating material and to increase quality and quantity through selection, certification and tests for citrus viruses (Anonymous, 1978). Under this project, plant breeding, growth characteristics, storage, agricultural machinery, plant protection, technology, economy, plant genetic resources and education-demonstration were addressed, and the following actions planned:

- To determine the level of damage and incidence of virus diseases
- To identify virus disease vectors
- To obtain virus-free propagation material by thermoterapy and tissue culture.

This project pioneered the work on this subject in Turkey: 275 trees of 9 varieties were inspected and 16 trees selected as candidates by using different kinds of selection methods in the Eastern Mediterranean region. With the same methods, candidate trees were also selected in two other regions. Eventually a total of 36 trees were selected in the three Turkish regions (Table II) (Anonymous, 1984; Goral *et al.* 1991).

Table II – Citrus varieties selected under the Citrus Research and Training Project between 1979-1983

Varieties	Size	Varieties	Size
Interdonato lemon	8	Clementine mandarin	4
Lamas lemon	4	Satsuma mandarin	5
Kütdiken lemon	3	Washington Navel	4
Yediveren lemon	1	Shamouti orange	3
Kibris lemom	1	Valencia orange	3

* These varieties are used in the "Turkey Citrus Variety Improvement Programme"

In the second phase of this project, these 36 trees were evaluated for adaptation and yield in the Eastern and Western Mediterranean regions, thus qualifying it as basic material (Cinar, 1993 a; Goral *et al.*, 1991; 1993).

After 1980, virus and virus-like diseases caused serious damages on citrus. Therefore the Ministry of Agriculture and the University of Çukurova established a cooperative project that lasted from 1982 to 1987. During this period, indexing procedures, transmission modes and identification of virus and virus-like diseases of citrus were investigated. The techniques necessary for obtaining virus-free plant material were developed and a new laboratory established in the Department of Plant Protection, Faculty of Agriculture, University of Çukurova, Adana (Cinar, 1993b). The collaboration ended in 1988. The Ministry of Agriculture did not support the Department any more, and the University started to work alone. Currently, there are two certification programs in Turkey, the first one named SFREC (Subtropical Fruits Research and Experimental Centre, University of Çukurova, Adana) and the second ACRI (Antalya Citrus Research Institute, Antalya).

These programmes have the following facilities

SFREC has a computerized greenhouse (about 3,5 da), and screenhouses, well equipped laboratories, administrative buildings and glasshouses (about 9 da, which are directly used to reproduce healthy and registered material). Within the framework of SFREC and in the Department of Plant Protection of the University of Çukurova, purification, isolation, serological tests, indexing and electrophoresis tests are performed routinely.

ACRI has the same facilities and has a good technical collaboration with FAO (Goral *et al.*, 1993) .

The Institutions involved in the citrus variety improvement program are:

1) Subtropical Fruits Research and Experimental Centre (SFREC), University of Çukurova

With the collaboration between the Ministry of Agriculture and the University, the Ministry of Agriculture built the facilities described above in the University campus. These greenhouses and screenhouses were established in 1988 (Cinar, 1993b).

The main goal of the SFREC is the production of virus-free and true-to-type propagating material (budwood and seedlings) and to support all the research works on citrus. All kinds of indexing in citrus, serological tests, culture assays and electrophoresis tests are made routinely. Besides the research works, SFREC has produced 40-50,000 seedlings/year and released budwood to the private nurseries for production of virus-free seedlings in the region. The citrus budwood improvement programme was initiated in 1986. This programme envisages:

a. **Surveys.** Surveys started in 1982 to identify the viruses present in the region. To this aim, all citrus orchards in the Eastern Mediterranean region were surveyed.

b. **Selection of source trees.** Sanitation techniques for obtaining virus-free budwood were applied to: (Table I, II).

The 36 trees previously selected through the Citrus Research and Training Project

New varieties directly imported and planted in breeding plots in the University orchards.

c. **Indexing and re-indexing works.** Biological indexing is routinely used and is made in the climatized greenhouses.

d. **Serology, electrophoresis and culturing.** ELISA test is used for identification of CTV and stubborn disease. Polyacrylamide gel electrophoresis (PAGE) is used to identify citrus exocortis and cachexia-xyloporosis viroids. The causal organism of citrus stubborn disease (*Spiroplasma citri*) is determined by *in vitro* culture.

e. **Sanitation.** The previously selected 36 candidate trees were indexed, then subjected to thermoterapy, *in vitro* shoot tip grafting (STG), and the combination of STG and thermoteraphy. Plants obtained through this procedure were multiplied by micrografting and re-indexed for virus and virus-like diseases.

f. **Production and release of virus-free budwood and seedlings.** Virus-free plants were multiplied by micrografting on rough lemon and sour orange and used as the source of virus-free budwood. Some of this virus-free budwood is used for seedling production under SFREC and supplied to private nurseries for the same purpose (all private nurseries are working under the control of SFREC).

The first virus-free budwood was released to the farmers in 1991 by SFREC, the 1994 release being of about 40,000 seedlings. About 75,000 virus-free, true-to-type plants were produced in private nurseries in the 1994-1995 growing season. This capacity will increase in the near future.

g. **Training.** Beside the production of virus-free budwood and seedlings, different kinds of research work related to citrus virus and virus-like diseases were conducted by SFREC, which also organizes national and international training courses.

SFREC publishes a journal "Turuncgil Bulteni = Bulletin of Citrus" periodically, which is distributed to 1000 farmers, and holds seminars, meetings and conferences for citrus growers in the region.

2) The Programme of Antalya Citrus Research Institute (ACRI)

The citrus budwood selection, certification and variety improvement project is a sub-project of the Citrus Research and Training Project started in 1978. At the end of the first phase of this project in 1984, the research stopped for some time (Anonymous, 1978).

Computerized greenhouses (about 7 da), screenhouses (about 1.8 da) and laboratories funded by the World Bank were established both in Antalya and in Adana. Later, Antalya Citrus Resarch Institute (ACRI) started to work alone by the appointment of the Ministry of Agriculture.

The citrus variety improvement program was started by the technical co-operation of FAO with ACRI in February, 1988 (Goral *et al.*, 1993).

The main purposes of this programme are as follows:

- Selection of trees
- Indexing
- Sanitation by STG
- Re-indexing of seedlings after STG
- Establishment of budwood reproducing plants and foundation blocks
- Reproduction of healthy stocks and multiplication of healthy propagative material.

The previously selected 36 candidate trees were used as primary sources (Table II). All selected trees were biologically indexed for virus and virus-like diseases. This project had two phases: the first for the obtention of virus and virus-like disease-free budwood, and the second for the establishment of mother blocks and distribution of disease-free budwood.

The varieties included in the ACRI programme are listed Table I. The diagram was prepared by ACRI. In the short term budwood reproduction and re-indexing works were done at the same time. The first fruits were harvested from re-indexed plants, 20,000 budsticks were found true-to-type and given to certified seedling reproducing units of ACRI in 1992. In 1993 ACRI sold 20,000 virus-free budsticks to private nurseries.

A collection plot was established with five different virus-free varieties grafted on sour orange rootstock in ACRI orchards. The present goals of the ACRI programme are:

- to produce more virus -free and true-to-type budwood
- to help official or private companies establishing nurseries for the production of virus-free budwood and distribution to the growers
- to promote a legislation for a mandatory certification programme
- to obtain and release more virus-free and true-to-type citrus varieties
- to obtain and improve the new varieties.

SFREC under the University and ACRI under the Ministry of Agriculture are working separately on the project for "The obtention of virus and virus-like disease-free true-to-type budwood and seedlings".

SFREC produced and released virus-free and true-to-type budwood to three private nurseries. The production of seedlings by these private nurseries is inspected by SFREC. In order to produce seedlings, the private nurseries must obtain permission from the Ministry of Agriculture. The Ministry of Agriculture gives blue labels to the nurseries, if they apply for them. The blue label shows that seedlings are produced from virus and virus-like disease free and true-to-type budwood. The production capacity of SFREC is about 50,000 seedlings/year. But the total capacity of private nurseries is of about 150,000 seedlings/year.

ACRI has released budwood to Governmental nurseries and only to one private nursery. Although the present production capacity of ACRI is about 20-30,000 seedlings/ year, its facilities are good enough to reproduce and release about 400,000-500,000 seedlings/year (Goral *et al.* 1993).

ACRI generally releases healthy material to the Western Mediterranean region, and SFREC usually to the Eastern Mediterranean area. The seedling requirement of Turkey is about 400,000-600,000 seedlings/year. At present 50% of the requirements are met, and in a near future all seedlings to be used in the citrus industry will be provided by the above Institutes.

The first private nursery which conforms to certification rules taken was established in 1992 and the first healthy seedlings produced were sold in 1994. The capacity of the nursery is about 100,000 seedlings/year. Greenhouses are air heated and may be used as screenhouse during summer. All the pots are drip-irrigated.

The price of the virus and virus-like disease-free and true-to-type seedlings is much higher than the common seedlings. In order to support the use of certified seedlings, the government subsidises citrus growers (25% of the total costs of a newly-established orchard).

On the other hand, the Ministry of Agriculture enforces quarantine measures regulating the distribution of «standard» grafted plants among provinces.

Provisions are being made to limit the production of citrus plants of «standard» category.

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