Development of a Citrus Certification Program in Egypt

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ABSTRACT. An account is given on the evolution of a disease-free citrus certification program in Egypt after 2 yr of activities within the framework of the Egyptian-German Citrus Cooperation. Fifty-six candidate trees of the main citrus varieties were selected and indexed, and many were found to be positive for psorosis, exocortis, cachexia, and oak leaf pattern diseases. This material will undergo shoot-tip grafting for the production of healthy primary sources. As a first step, three varieties and one rootstock of these candidate trees will be sanitized and released to the program in 2002 as virus-tested healthy stocks. A Citrus Center has been established at Bahteem, located 10 km from Cairo, for the conservation and pre-multiplication of healthy propagating plant materials. A laboratory and an indexing greenhouse have been constructed and equipped for serological, electrophoresis and biological indexing. While waiting for the enforcement of the national legislation of fruit tree certification, beginning in 2003, the Bahteem Citrus Center will distribute healthy budsticks of local Valencia and navel sweet orange varieties to selected nurseries who have joined the program on a voluntary basis. They have also improved the management of their nurseries, partially fulfilling agronomic and sanitary requirements.

In 1999, the total cultivated fruit tree area in Egypt was 420,000 ha. From this area 150,000 ha are currently under citrus, of which 140,000 ha are in production. The most important citrus cultivars are sweet orange (about 65%) followed by local mandarin (25%) and lime (10%). The average production fluctuates between 15-17 tons/ha (5), which is very low when compared to world production standards. The total citrus production in 1999 was 2,250,000 tons of which 250,000 tons (fob value about US $70 million) were exported. The bulk of the citrus production is consumed as fresh fruit, with a small proportion (less than 50,000 tons/annum) going into processing. Some 65,000 jobs are generated by the citrus industry.

Citrus is produced mainly in two largely homogenous regions; the Nile Delta (old lands) and in the newly reclaimed desert lands. Nearly 70% of the total citrus area is located in the Delta region. The cultivation practices applied in these two regions differ due to the site-specific conditions, soil types and the differing age of the trees. Most of the plantations in the old lands are over 40 yr old. Citrus in the new lands is generally as younger plantations, which have not as yet entered their maximum yield phase. The sandy conditions of the desert soils require cultivation techniques (e.g., fertilization, irrigation), which are different from those used in the Delta region with its heavy soils. In the Delta region the predominant rootstock is the sour orange, while in the desert area the preferred rootstock currently is Volkamer lemon.

Citrus nursery tree production and the problems caused by graft-transmissible pathogens. Several important graft-transmissible pathogens and diseases have been previously reported in Egypt, namely psorosis, stubborn, exocortis, cachexia, gummy bark, concave gum, impietratura and cristacortis (1, 2, 3, 4, 8, 17, 18). These diseases affect the productivity and longevity of citrus trees. The high degree of infection with several pathogens, especially the viroids, precludes the broader use of rootstocks other than sour orange and Volkamer lemon, which are tolerant to viroids.

Based on rough estimates, Egypt produces between two and three

million grafted citrus nursery trees per year. According to data from the Central Administration for Horticulture of the Ministry of Agriculture, the number of commercial citrus nurseries that were licensed in 2001 was 328. In many cases the origin, the variety and the sanitary status of the propagating materials are unknown and a high risk exists in the dissemination of graft-transmissible pathogens.

The absence of any sanitation and certification program, plus quarantine measures without biological indexing, increases the existing deterioration of the sanitary status of citrus. A possible introduction and spread of other very destructive diseases such as tristeza, huanglongbing (HLB) and witches' broom disease of lime could occur. These are diseases which can destroy or seriously debilitate an industry, as is currently happening in some neighbouring countries, and represents a serious threat not only for Egypt, but also for all citrus producing countries in the Mediterranean basin.

Citrus Improvement Project. A Citrus Improvement Project was established with the objective of contributing to the solution to these problems, within the framework of the Egyptian-German Citrus Cooperation.

This project started in October 1999 with a selection program of economically important, high yielding and good performing trees in different citrus growing areas in Egypt. The different steps during this selection process are presented in Fig. 1. Since the Egyptian indexing facilities are not yet fully operational, co-operation with the Istituto Agronomico Mediterraneo (IAM) in Valenzano, Italy has been started. The main support from IAM is in the fields of sanitation, biological indexing and training. The sanitized material should then serve as starting material in a future Egyptian certification program after re-evaluation of its horticultural performance in the field.

The main objective of this Egyptian–German Citrus Improvement Project is the establishment of a citrus certification program within a strict framework of regulations for the citrus nursery sector. This will substantially contribute to the increase of citrus productivity in Egypt. With the availability of healthy material, the potential and use of other rootstocks will be tested.

Improving the quarantine infrastructure and indexing facilities will permit to import at low risk, budsticks from other certification programs. The future creation of infrastructure conditions and training for a shoot-tip grafting unit will further support the future quarantine and certification program.

Preliminary data on field selection of candidate trees. Local citrus trees were selected in 1999 for their general appearance and showing characteristics typical of the cultivars or rootstocks (fruit size, color, organoleptic characteristics, bearing, and tree size). They were also observed for the absence of disease symptoms (leaf mottling, mosaic, chlorosis, malformations, enations, gumming, concavities, bark scaling, pitting, galls in the trunk or branches and bud-union abnormalities). Regarding gummy bark disease, since no indexing procedure is available (4), all sweet orange trees were examined by removing a piece of bark at the bud union area and looking for gumming in the bark and pitting in the wood of the scion (18).

Observations were carried out on fruit characteristics during the bearing season and for the presence of leaf symptoms of pathogens during the spring and late summer flushes of growth over a 2-yr period. One to 10 trees per orchard were observed and one to two trees per variety were selected.

Ultimately, 56 trees were collected consisting of 14 varieties, mostly navel and Valencia sweet

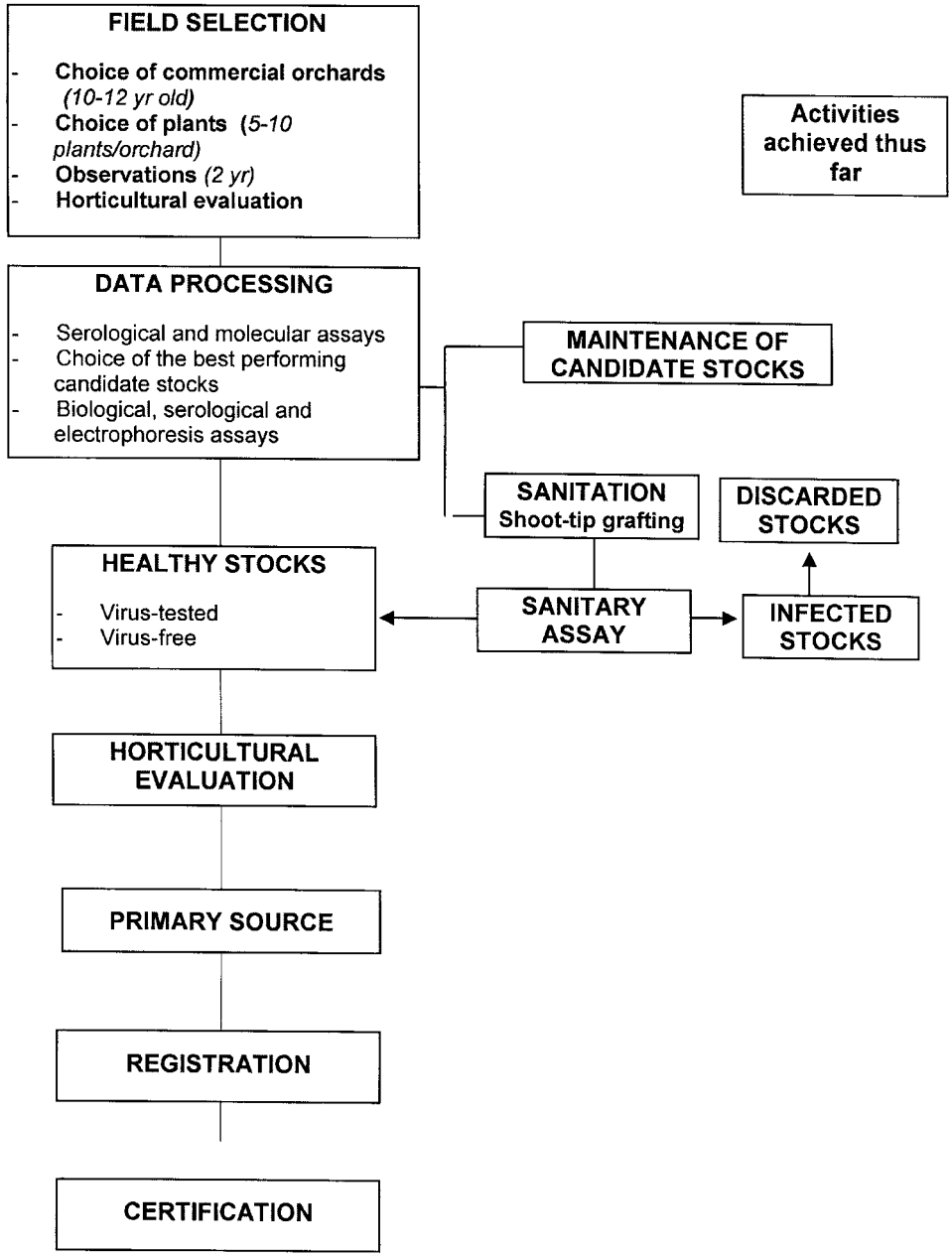

Fig. 1. Scheme for the Egyptian citrus certification program.

oranges. Other selections were a Ponkan mandarin, a Minneola tangelo, a Star Ruby grapefruit, a Tahiti lime and selections of the two most utilized rootstocks, sour orange and Volkamer lemon.

Preliminary data on indexing of candidate trees. Four bud sticks (10-20 cm long) with leaves attached were individually collected from the quadrants (north, south, east and west) of each candidate tree. These were washed of sand and dust and stored at 4°C in plastic bags with moist tissue paper enclosed. Buds were grafted onto sour orange seed-

lings and maintained under protected conditions in an insect-proof screenhouse. These were to be the candidate sources as starting material for indexing and sanitation. All candidate sources were initially indexed for specific pathogens by serological methods using: (i) DTBIA for *Citrus tristeza virus* (CTV) (14); (ii) DAS-ELISA for CTV, *Citrus variegation virus* (CVV), Citrus vein enation virus (CVEV) and *Spiroplasma citri* (6); and (iii) TAS-ELISA for *Citrus psorosis virus* (CPsV) (11, 12). During 2000-2001, the candidate sources were indexed by graft-inoculation to four woody indicators (Madam Vinous sweet orange, Dweet tangor, Mexican lime and 861-S1 Etrog citron) (19).

Also, complementary molecular assays were done by PCR (7, 15) at IAM for the detection of phloem-restricted prokaryotes (*S. citri*, and the HLB bacteria "*Candidatus* Liberibacter asiaticus" and "*Candidatus* L. africanus") whereas citrus cachexia viroid (CCaVd) and *Citrus exocortis viroid* (CEVd) were detected by dot blot hybridization and sPAGE electrophoresis (10, 20). A summary of the results is presented in Table 1.

Seven candidate trees out of the original collection of 56 were found negative for all tested pathogens and 49 of the candidate trees were found infected by one or more of the following pathogens: CEVd, CCaVd, CPsV, psorosis and oak leaf pattern diseases. None of the seven apparently healthy trees has been distributed to nurseries at this stage because the biological indexing has been done using only four different indicators. A further three of the 56 trees were found infected with CVEV and one tree with CVV.

Preliminary data on sanitation and release of citrus healthy stocks. In 1999, as a first step, nine of the 56 candidate trees were submitted to pathogen clean-up in collaboration with IAM. These nine trees sources are marked with a "+" in Table 1 under the column "source". Following established protocols, shoot tips were grafted *in vitro* onto citrange rootstocks. After approximately 2 mo, plants were grafted *in vivo* onto 1-yr-old sour orange rootstocks (9) and grown in a greenhouse at 27°C. For elimination of CPsV in psorosis-infected candidate trees, shoot tips were collected from plants, which had been maintained in a heat chamber at 30-35°C for 20 days (16).

After shoot-tip grafting, with preconditioning by heat, and future re-indexing assays for testing the major citrus diseases (13), those which pass as virus-free will be released. Currently, four plants have been released: two Valencias and one navel orange plus one sour orange rootstock. These will be ready to enter the citrus propagating material program in Egypt in 2003 as healthy stocks in a virus-tested category. The remaining five candidate sources, maintained on sour orange rootstocks under greenhouse conditions, are also being sanitized and will be fully re-indexed before being released to the program when available, probably in 2004.

All of these healthy stocks will be planted in the field in the Delta and in the desert regions and a continuous evaluation for trueness-to-type and fruit quality will begin. After 3-4 yr of evaluation, the healthy stocks will be declared primary sources, will be registered and utilized in the certification program.

Future organization of the certification activities. The Egyptian Ministry of Agriculture and Land Reclamation (MALR) is the responsible certifying authority. Among its different tasks will be the control and management of activities related to each certification step. Primarily it will control and release of the labels as well as regulating nursery activities. A Fruit Tree Certifying Agency will be created inside the MALR. A project of organization of citrus certification is presented in Table 2.

TABLE 1
SANITARY STATUS OF 56 EGYPTIAN CITRUS CANDIDATE TREES

		Indexing[1]						ELISA[2]				PCR[2]		Dot blot[2]		sPAGE
Selection No.	Variety[1]	Source	M.V	D.T	M.L	Et.	CVV	CTV	CPsV	CVEV	*S. citri*	*S. citri*	HLB	CCaVd	CEVd	Viroids
1	Balady	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—
2	Ponkan	+	-	+	—	—	—	—	—	—	—	—	—	—	—	—
3	N. Valencia	+	+	+	—	+	—	—	—	—	—	—	—	—	—	—
4	N. Valencia	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
5	N. Valencia	+	+	+	—	—	—	—	+	—	—	—	—	+	—	+
6	W. navel	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
7	W. navel	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
8	Balady	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	W. navel	—	+	+	—	—	—	—	+	—	—	—	—	+	—	+
10	W. navel	+	—	+	—	—	—	—	+	—	—	—	—	—	—	—
11	W. navel	—	+	+	—	+	—	—	+	—	—	—	—	—	+	+
12	W. navel	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—
13	W. navel	—	+	+	—	—	—	—	+	—	—	—	—	—	—	—
14	W. navel	—	—	—	—	+	—	—	—	—	—	—	—	—	+	+
15	W. navel	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16	W. navel	—	—	—	—	+	—	—	—		—	—	—	—	+	+
17	W. navel	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+
18	Succari	—	—	—	—	+	—	—	+	—	—	—	—	—	+	+
19	Succari	—	+	+	—	—	—	—	+	—	—	—	—	—	—	—
20	W. navel	—	+	+	—	—	—	—	—	—	—	—	—	—	—	—
21	W. navel	—	—	+	—	—	—	—	—	—	—	—	—	+	—	+
22	W. navel	—	—	—	—	+	—	—	—	—	—	—	—	+	+	+
23	W. navel	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
24	Valencia	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
25	Valencia	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+

[1]LL = Lane Late, N = nucellar, E = Etrog citron, W = Washington, C = Cutter, DT = Dweet tangor, G = Gilette, O = Olinda, MV = Madam Vinous, ML = Mexican lime.
[2]CVV = *Citrus variegation virus*, CPsV = *Citrus psorosis virus*, CVEV = Citrus vein enation virus, CEVd = *Citrus exocortis viroid*, CCaVd = Citrus cachexia viroid, CTV = *Citrus tristeza virus*, HLB = huanglongbing.

TABLE 1 (CONTINUED)
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Selection No.	Variety[1]	Source	M.V	D.T	M.L	Et.	CVV	CTV	CPsV	CVEV	*S. citri*	*S. citri*	HLB	CCaVd	CEVd	Viroids
26	Valencia	—	—	+		—	—	—	—	—	—	—	—	—	—	—
27	Valencia	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
28	Valencia	—	—	+	—	+	—	—	—	—	—	—	—	—	+	+
29	Valencia	—	—	+	—	+	—	—	—	—	—	—	—	+	+	+
30	Valencia	—	—	+	—	+	—	—	—	—	—	—	—	—	+	+
31	Valencia	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
32	Valencia	—	—	—	—	+	—	—	—	—	—	—	—	+	+	+
33	Valencia	—	—	—	—	+	—	—	—	—	—	—	—	+	+	+
34	Valencia	+	+	+	—	+	—	—	+	+	—	—	—	—	+	+
35	G.W. navel	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+
36	G.W. navel	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+
37	C.N.Valencia	—	+	+	—	—	—	—	—	+	—	—	—	—	—	—
38	C.N.Valencia	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
39	O.N.Valencia	—	—	+	—	+	—	—	—	—	—	—	—	+	+	+
40	O.N.Valencia	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
41	Star Ruby	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
42	Star Ruby	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
43	L.L W. navel	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
44	L.L.W. navel	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+
45	L.L.W. navel	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
46	Tahitian	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+
47	Minneola	—	—	+	—	+	+	—	—	—	—	—	—	—	—	—
48	Dancy	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
49	W. navel	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
50	Thornless sour	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
51	Spanish sour	+	—	+	—	—	—	—	+	—	—	—	—	—	—	—

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Selection No.	Variety[1]	Source	M.V	D.T	M.L	Et.	CVV	CTV	CPsV	CVEV	*S. citri*	*S. citri*	HLB	CCaVd	CEVd	Viroids
52	Brazilian sour	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
53	Sweet sour	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
54	Balady sour	+	+	—	—	—	—	—	+	—	—	—	—	—	—	—
55	Volkamer	—	—	—	—	+	—	—	—	—	—	—	—	—	—	+
56	Volkamer	—	—	—	—	+	—	—	—	—	—	—	—	—	—	+

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TABLE 2
ORGANIZATION OF THE FUTURE CITRUS CERTIFICATION ACTIVITIES IN EGYPT

Phases	Material category	Controls		Growing conditions	Responsible agencies
		Trueness to type	Sanitary		
Conservation for pre-multiplication	Pre-basic	Phenologic	Test	Screenhouse	Public Agencies (Bahteem Citrus Center)
Pre-multiplication	Basic	Phenologic	Test	Screenhouse	Public Agencies (Bahteem Citrus Center)
Multiplication	Certified	Phenologic	Test	Screenhouse	Nurserymen's Association
Nursery Propagation	Certified	Biometric characteristic		Net tunnel	Nurseries

The Bahteem Citrus Center will produce, under screenhouse conditions, the mother trees (basic category material) that will be distributed to some key nurseries or nursery associations for the creation of their own increase blocks. A nursery and increase block control system will be developed. Responsible personnel will be trained in these activities and a label will be attached to all grafted nursery trees fulfilling certification requirements.

The legislative and regulatory framework of the technical protocols and rules for the propagation of citrus certified materials should be issued during 2003-2004.

The pre-basic trees will be indexed using standard biological indicators (19). The diseases which will be specifically tested for and will be formally included in the legal regulations (13) are: Crinkly leaf, leaf rugose, infectious variegation, concave gum, cristacortis, impietratura, psorosis, tristeza, tatter leaf, stubborn and the various viroids.

The establishment of a certification program is of paramount importance, but the needed legislation has not as yet been approved. The biological indexing is in the way of being established. It is clear that a certification program will not be in operation for many years. However, the work underway will contribute to improve the quality of the citrus material that will be used by some nurseries. This is a beginning and a first step to materially improving the citrus in Egypt. The certification program, which will start on a voluntary basis, will later be made mandatory.

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