Literature Cited

1. Huang Chengjui. 1959. A preliminary study of rue plants in China (Part Three). Journal of Botanical Classification (Zhiwu fenlei xueboa), 8(1):69-124.
2. Hume, H. H. 1957. Citrus Fruits. pp. 90-95. The MacMillan Co.
3. Ye Jingyuan. 1960. Selected Works from Chinese Agricultural Science Heritage—Citrus. pp. 26-35, 71-82. Agricultural Publishing House.
4. Swingle, W. T. 1967. The Botany of Citrus and Its Wild Relatives. pp. 190-430 *in* The Citrus Industry (Rev. Reece, P. C., 1967).
5. Wan Chairong and others. 1980. A preliminary report on the experiment of short-stalked citrus. Chinese Citrus (Zhongguo ganju), 1980 (4):6-9.
6. Ye Yinmin. 198 . A study on citrus pollen pattern. Chinese Agricultural Science (Zhongguo nongye kexue) (5):62-66.

Citrus Clonal Selection, Progeny Testing and In Vitro Propagation

WEN-CAI ZHANG[1]

China is a citrus genome "reservoir" of the world. The history of citriculture has been recorded over 4,000 years, with abundant cultivar resources. Excavations dating back over 2,500 years ago, revealed citrus seeds and rind of *Citrus junos*. Han Yien-chih wrote the first citrus monograph "Chu Loh" dating back to 1178, recorded 27 superior cultivars in Zhejiang Province. Numerous original citrus species were found in southern provinces such as *C. honghoensis* YL DL, *C. medica muliensis* WYD, *C. ichangensis* Swingle, *C. junos* Sieb. ex Tanaka, and *Fortunella hindsii* Swingle.

There are three groups of citrus cultivars widely grown in China, i.e. *C. reticulata* Blanco, *C. sinensis* (L.) Osbeck, and *C. grandis* Osbeck. Owing to the frequent natural crossing and the phenomenon of nucellar embryony, numerous new cultivars and mutant were constantly formed. Recent clonal selection work revealed many nucellar mutants, but sports and chimera mutations. In clonal selection studies, it was shown that there are frequent bud mutations found in the vegetative shoots sprouted from the parenchymatous regenerations. This phenomenon was also observed in callus culture of the diploid cultivars which developed tetraploids, triploids and haploids. This indicated that the mechanism of citrus mutation originated from the inner parenchymatous cell layers.

Methods of citrus clonal selection were carried out in Huazhong Agricultural College according to the following procedures; determining the objectives of clonal selection, discussing the technical standards, selecting clonal lines, laboratory quality analysis, progeny testing of clonal selections, and propagation of the superior mutants. Technical quality standards for oranges, mandarins, 'Ponkan' and tangerines were discussed. Using *C. unshiu* as interstocks for topworking for all clonal cultivars proved compatible. The complete procedures for progeny test, selection, topworking and registration for distribution can shorten the clonal selection program to about 3 years. This technique is also used for testing nucellar selections and hybrid progenies.

In vitro propagation of citrus clonal selections was conducted by two tis-

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sue culture methods: i.e. shoot tip culture and shoot tip grafting. Shoot tip culture, using MS culture medium plus modified organic nutrients was successful. Shoot tip grafting using 0.14-0.18 mm shoot tips grafted onto virus-free citrange seedlings, and grafted under a microscope gave about 55% living plants. These grafted seedlings were later transplanted into sterilized compost soil.

Progenies were tested for cold resistance by physiological and biochemical methods for screening hardy selections. Amount of bound water, sugar content, peroxidase and electrolyte conductivity as indices for cold resistance are discussed.

I. China is a citrus genome "reservoir" of the world.

Abundant citrus varietal resources exist in China. Many wild and semiwild citrus species survived through the Quaternary glaciation period (7). *Citrus aurantium* L., *C. sinensis*, *C. medica* and many cultivars of *C. reticulata* were isolated and distributed from southwestern China eastward to Yangtze region and south China. As early as Yu-Sha dynasty (2286-1158 B.C.), *C. reticulata* and *C. junos* Sieb. ex Tanaka were recorded. Rind of *C. junos* was found in the tomb in recent excavations of a Jianglin Lord tomb buried about 600 B.C. Seeds of *C. sinensis* and *C. reticulata* were also found in Changsha tomb buried about 450 B.C. as shown in Figs. 1 and 2.

Numerous ancient writings described citriculture in China: Er-Ya (1000 B.C.), Chou-Li (1100-249 B.C.), Liu Sze Chungchu (237 B.C.) and Chu Zhong (343-290 B.C.). All recorded citrus cultivars and their cultural methods. About the period of Chuon-Kwoh (475-221 B.C.), many large citrus plantations were found in Sichuan and Hubei provinces (15).

Chu Loh, written by Han Yien-Chih in 1178, recorded 27 superior citrus cultivars in Zhejiang Province, and stated that citriculture was popular in Suzhou, Jiangsu, Jinzhou, Hubei, Wenzhou, and Zhejiang provinces.

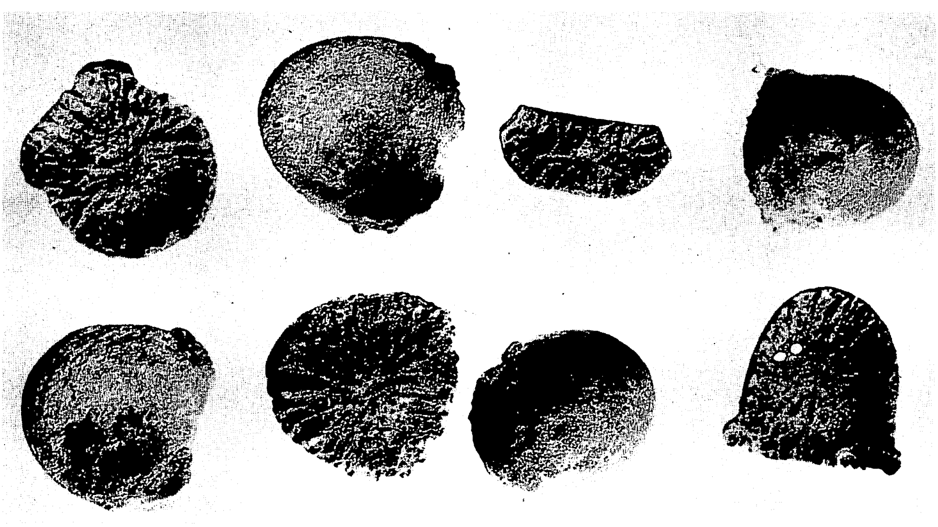

Fig. 1. Rind of *C. junos* Sieb. ex Tan. from Jianglin tomb (about 600 B.C.).

Fig. 2. Seeds of *C. sinensis* Osb. from Changsha tomb (about 450 B.C.).

Szu Wen (121) and Guang (502-551) recorded mandarins, tangerines, oranges and *C. junos* were planted in different climatic regions, and stated that there were 21 cultivars of mandarins planted (15).

There are many original species of citrus found in provinces south of Yangtze. Prof. Tanaka said that China had no wild citrus species, but since 1949, many wild citrus species have been discovered in China. Five are listed below with descriptions.

1) *C. honghoensis* YLDL, found in Honghoe County, Yunnan Province in 1978 (11); evergreen tree, single leaf blade with large wing as long as 12-18 cm, 2-3 times longer than the leaf blade; inflorescence with purple flower buds and white flowers; fruit large, sub-globular, with diameter of about 12 cm; and yellow rind, 1.5 cm thick

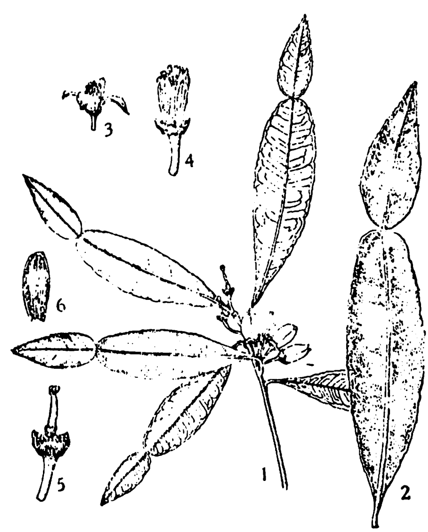

Fig. 3. *C. honghoensis* YLDL (T. C. Yu).

with protruded oil glands on the flavedo (Fig. 3).

2) *C. medica muliensis* WDY: Discovered in 1980 in Muli County, Sichuan Province by Yang Sze-Chin and Dan Li-Ming. Old existing tree specimens are over 100 years old, indigenous in that area of 1,200 m altitude (9) (Fig. 4).

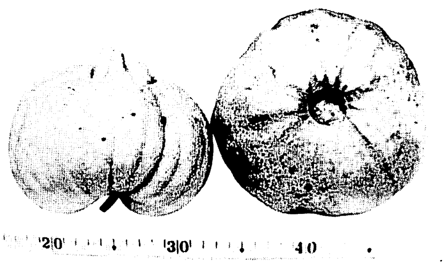

Fig. 4. *C. medica muliensis* WDY (S. C. Yang).

3) *C. ichangensis* Swingle: There are 7 types found in Hubei, Sichuan, Guizhou, Yunnan, and Hunan provinces since Prof. Walter Swingle discovered it in Ichang, Hubei. They are distributed in altitudes as high as 1,700-2,400 m and are cold resistant. They are used as a compatible dwarf rootstock for sweet oranges at Szechwan Fruit Research Institute. There are large trees over 100 years old which are 3.5 m high. According to the shape of fruit, three types were observed: oblong, round and flat. The oblong fruit type has solitary clustered, white flowers; fruit weight, 16-30 g; rind, lemon yellow; oil glands, protruded; sections, 5-7; bitter sour; seeds, few and large; chalaza brown; cotyledon white; monoembryonic; ripening in October. Round fruit has white solitary flower buds; purple-tinged flowers; sunken oil glands; 5-7 sections; is bitter sour; fruit average weight, 28 g; seeds, 4-8, round; chalaza large, brown, cotyledon, milky white; monoembryonic; ripening in October.

C. ichangensis types distributed in Ichang at altitude of 600-1,400 m. Two forms were discovered. Spindle-shaped types were found at 850-900 m altitude; early- and late-ripening forms; the flower is white with purple tinge; rind, yellow; weighing 26 g, 5-9 sections; no juice sacs; 27 seeds; oval shaped; cotyledon, white; monoembryonic; ripening in mid-October. Flat-shaped type were distributed at 800-1,300 m altitude, flower, purple color; fruit, small weighing 11 g; rind, yellow; oil glands, sunken; sections, 5; seeds, 12; cotyledon, white; monoembryonic (10) (Fig. 5).

4) *C. junos* Sieb. ex tan.: There are many types: large leafed, small leafed, round and narrow winged types, distributed along the middle Yangtze basin. The fruit forms are round and flat shaped; average weight of 90-100 g; rind, yellow; oil glands, few and flat; very fragrant; sections, 9-10; seeds about 40; flesh, juicy, fragrant; TSS, 15.4%; very acid; ripens in October; seeds, polyembryonic; average 7.3 embryos. 'Lohanchin' is probably

Fig. 5. *C. ichangensis* Swigle (S. C. Ting).

a hybrid of *C. junos*. It is used as rootstock for oranges and mandarins, and is cold and drought resistant. This is one of the very early species, existing over 3 thousand years (2).

5) *Fortunella* spp.: *Fortunella* was first recorded in Han Yien-Chih's monograph written in 1178. Robert Fortune brought it to England in 1812. Prof. Swingle named it as *Fortunella*. It was introduced to Japan in 1799 from Ningpo of Zhejiang Province. There are four species indigenous to China, i.e. *Fortunella hindsii* Swingle, *F. margarita* Swingle, *F. crassifolia Swingle,* and *F. obovata* Swingle. *F. hindsii* are grown wild in Zhejiang, Anhui, Jiangxi, Hubei, Hunan, Fujian, Guangdong, Guangxi, and Guizhou. Fruit are very small, 1-1.5 cm in diameter, round, 3-4 carpels, orange-red rind, acid sweet; seeds, oval, tetraploid, 2n = 36, polyembryonic (2).

II. **Mutation among citrus cultivars.**

1) Major cultivars of citrus in China: In a long period of citriculture in China, numerous citrus varieties were isolated and planted. At present, there are three large groups of citrus growing in the 14 southern provinces.

a) Sweet oranges (*Citrus sinensis*): Outstanding cultivars are 'Anlucheng,' 'Mingliucheng,' 'Xuegan,' 'Xiangshiucheng,' 'Huazhoucheng,' 'Xinhuicheng, 'Jincheng,' 'Xianfencheng,' 'Taoyihcheng,' 'Diahoncheng,' 'Bindoncheng,' 'Kailiangcheng,' navel orange, summer orange, etc.

b) Loose skinned oranges (*C. reticulata*): Major cultivars are 'Ponkan,' 'Tankan,' 'Wenchiumikan,' 'Erkan,' 'Siweikan,' 'Dahungkan,' 'Bentitsao,' 'Hungchih,' 'Hwanpikan,' 'Nanfengmichu,' 'Ziuchih,' 'Chuhungchih,' 'Tsaochih,' 'Mamchih,' 'Hsiangkan,' 'Sunchih,' etc.

c) Pummelos (*C. grandis*): Major cultivars are: 'Shatienyu,' 'Jinxiangyu,' 'Zaboyu,' 'Wentanyu,' 'Pingshanyou,' 'Liangshan yu,' 'Shiliuyu,' 'Changzhiuyu,' 'Sichiyu,' etc.

Frequent crossing and nucellar embryony caused a large amount of diversification among citrus cultivars. However, the frequency of clonal mutation among these old cultivars is low. Huazhong Agricultural College searched among 130,000 trees of *C. unshiu* and obtained only 5 early-ripening strains of 'Quoqing No. 1,' etc. From 1,200,000 trees of sweet oranges, only 4 superior strains were selected, such as 'Jincheng,' 'Xienfoncheng,' 'Tauyihcheng No. 8,' 'Huanglin Seedless,' etc. which are of superior quality (12, 14).

2) Modern clonal selection works:

a) Nucellar selection: As early as 1938, Zhang Wen-Cai, Hu Chang-chi, and Wu Chien-Chih started clonal selection work in Sichuan. From nucellar seedling trees, they obtained 'Jincheng No. 26,' and 'Xienfoncheng No. 20.' These 2 cultivars have been propagated and over 37,000,000 trees planted. They have high adaptability, good quality and are prolific. Owing to the nature of nucellar embryony, nucellar mutants were observed. Quxian in Zhejiang Province introduced 'Ponkan' from Guangdong about 400 years ago, obtained winter-hardy 'Ponkan' progenies. In 1959, Kikwei in Hubei Province introduced 'Ponkan' seeds from Quxian and obtained still more cold-resistant 'Ponkan' progenies. Huazhong Agricultural College planted 'Bentitsao' seeds from Zhejiang in 1957 and selected cold-resistant 'Bentitsao' strains which can stand −14°C in Wuhan. 'Liucheng' and 'Xinweicheng' seedlings planted in Liuchiu produced nucellar seedlings which bear larger fruit of good quality than the old cultivar. Yangcun Citrus Station in Guangdong selected seedless 'Ponkan' and 'Tankan' nucellar progenies. In Guangxi, a new strain of 'Guixia' was selected from 'Valencia Late' seedlings. Likewise, a new strain known as 'May Red' was selected from 'Valencia Late' seedlings in Sichuan.

b) Bud sport selection: A. D. Shamel started citrus bud selection work more than 50 years ago. His outstanding achievements in searching bud sports among oranges, grapefruit and lemons are well known. From 'Washington' navel oranges, he obtained more productive, early-maturing clones of 'Robertson' and 'Thomson' navels. He also obtained more than 15 new strains of 'Valencia' oranges. It was observed that the navels, 'Valencia,' 'Jincheng,' 'Taoyihcheng,' 'Wenchiu' mikan, etc. are apt to produce bud mutants, which give an opportunity to select better clonal strains. From our experience, 'Taoyihcheng No. 8' has recently produced bud sport 'Taoyihcheng No. 139,' which has TSS of 15.5%, 'Jincheng' grown in Xinshan produced No. 101 bud sport which is almost seedless. Wang Shu-Chu in Ichange (10) selected 'Nihuncheng' from 'Jincheng No. 26,' which is almost seedless, with deep rind color. A seedless bud sport was found from a grafted orange tree in Ichang in 1974, a limb of which bears seedless fruit, now called 'Huanglin Seedless' orange. A new strain of navel orange was selected out from 'Robertson' navel, 'Navel No. 35,' which is prolific and of very good quality. Guangdong Fruit Research Institute selected a 'Tankan' which matures in late March. Yangcun Citrus Station in Guangdong obtained from grafted orange trees in 1973, late ripening orange strains of 'SW-15-8-18N' and 'SW-14-3-22,' which mature in late May. They also selected a sweet 'Ponkan' bud sport 'Ton-2-12-4' which has a sugar content 76% higher than the ordinary 'Jonkan' variety. Zhejiang Citrus Research Institute obtained a seedless strain of 'Bentitsao' tangerine No. 240 which has good canning qualities. Huazhong Agricultural College selected an extra-early-maturing mikan 'Quoqing No. 1' and 'Quoqing No. 4,' which ripened about the end of September (14).

During our process of clonal selection, in recent years, we have found that the adventitious sprouts originating from a regenerated old stump are more likely to produce bud sports. 'Taoyihcheng No. 8' originated from an old stump of a marcotted tree which is about 150 years old. Two adventitious sprouts produced fruit, one with sweet fruit and the other with sour fruit. Curiously, 'Lungjiang Orange No. 1466' is an old, sweet orange tree with three branches sprouted from an old stump. The central adventitious shoot bears sweet seedless fruits, the left branch bears seedless sour fruits, whereas the right one bears seedy sour fruit.

J. Janick (5), working on apple bud selection, suggested that the parenchymatous cells are composed of 3 layers of cells, L-1, L-2, L-3. Those originated from L-2 cells may produce hereditary mutants. Prof. M. Iwamasa (3, 4) in his cytological works, stated that the L-2 cells may produce nucellar mutants. Recently, we conducted cell culture with 'Jincheng' sweet orange, and found that the chromosomes in the inner layer of the callus cells can produce autopolyploid, tetraploid, triploid and monoploid mutants (Fig. 6).

c) Selection of chimera mutation: Chimera mutants are often found on citrus fruit. Fujian Agricultural College obtained 'Kaliangcheng,' with good quality which was produced from 'Liucheng' grafted on 'Fuchih' tangerine. This graft hybrid produces 5 types of fruit, red-fleshed orange type, red and yellow mixed orange type, yellow-fleshed orange type, chimeral tangerine type, and tangerine type. Under vegetative propagation, somatic segregation in fruit characters is often found (1).

Another graft hybrid of 'Huangyien Zaochih' (*C. reticulata* Blanco) grafted

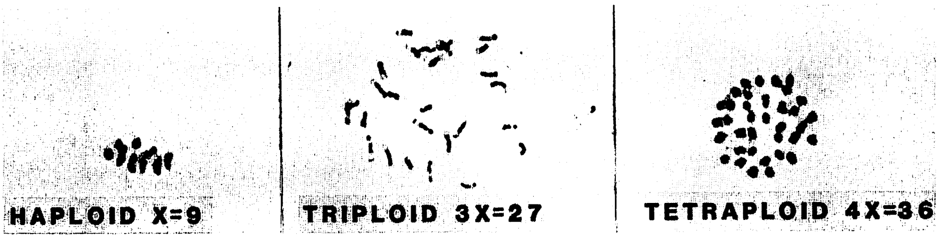

Fig. 6. Autopolyploids in 'Jincheng' sweet orange callus cells.

on 'Goutaocheng' (*C. aurantium* L.) known as 'Kiuhaochih,' was observed by researchers at Chekiang Agricultural University (2). Recently, an unshiu/Poncirus graft hybrid known as 'Zhimikan,' which constantly reveals somatic segregation, was found by the Hubei Fruit and Tea Research Institute.

III. Methods of clonal selection.

Historical citrus selection work was performed by our ancestors for thousands of years by tasting, selection, isolation, propagation and cultivation. Numerous superior cultivars were thus obtained. In 1956, Late Premier Chou En-Lai conducted the "Twelve Years' Scientific and Technological Development Program" which suggested a fruit improvement program of investigation, preservation and utilization of fruit cultivar resources in China. During 1958-1959, a nationwide survey of citrus cultivar resources was enacted in 14 provinces, except Taiwan, and numerous superior selections were obtained. Since then, the procedures of citrus clonal selection have been conducted as follows:

1) Determining the objectives of clonal selection. The objectives of selection were determined according to the growth and fruiting characteristics of the major local cultivars. According to the date of ripening, early- and late-ripening clones are to be identified. The superior quality selection, seedlessness, high TSS and low acid, with oval fruit form, is the objective. There is a close correlation between the oval shape of fruit and seedlessness. According to the objective for higher adaptability, we are focusing on prolific and productive annual bearing, relatively cold resistant, and with good keeping quality. Our overall objective for selection is to preserve good merits and improve defects of the existing cultivars in the region.

2) Determining the quality standards of selection. Popular quality standards of citrus cultivars claimed by the growers in China are good taste, good appearance, good keeping quality, high productivity, and wider adaptability. Cold resistance is an important standard for regions in central and east China. The standards for fruit judging were used on a percentage basis. Sampling of fruit was from outer branches, 2 fruit each from east, west, south and north sides and top of the tree. Fruit samples must be taken from healthy, bearing trees for the quality analysis in the laboratory.

Special emphasis was on early- and late-maturing mutants (12, 13). The standards used for different species of citrus are listed in Table 1.

3) Training course for mass selection. Commune technicians and cadres in the citrus-producing regions were organized for the mass selection work and a training course was given to discuss objectives, standards, and techniques for the clonal selection in the region.

Table 1. Standards for citrus clonal selection.

Character	C. unshiu (%)	C. sinensis (%)	C. tangerina (%)
Productivity	15	15	20
Cold resistant	15	5	15
Ripening stage	5	—	—
Fruit size (gram)	5	5	5
Form index (H/W)	—	5	—
Fruit appearance	5	7	5
Rind thickness (3 mm)	5	5	5
Edible portion (%)	5	5	5
Melting pulp	5	—	5
TSS (12% over)	15	10	15
Acid (1.5-0.8%)	10	10	10
Taste (5 persons)	15	15	15
Seedlessness	—	10	—
Juice content (%)	—	8 (above 45%)	—

4) Mass selection and laboratory analysis. General survey and selection of the mutants in the citrus region must have support from the local inhabitants working together with the technicians in order to shorten the process of selection. Local inhabitants are usually acquainted with the fruit characters of almost every tree. For instance, in 1975, a coordinated selection group was organized in Ichang District for the orange clonal selection. A total of 1,221 persons joined, consisting of 30 communes and 362 citrus-producing groups. During 1 month, 163 superior trees were selected from 1,205,112 orange trees. This shows the superiority of the collaboration of technicians with the local commune members (14, 15).

5) Progeny tests for clonal selection. The usual process of clonal selection requires 4-5 years' performance observation for the selected tree. Another 4-5 years of observation are required to record the performance of grafted progenies before distribution. Thus, a new clonal line requires at least 10-15 years before propagation and planting by growers. Owing to the urgent need for superior cultivars of citrus for the expanding production in the country, the program of clonal selection must be hastened. Several years of experiments show that by topgrafting selected bud mutants onto 8- to 9-year-old bearing mikan shoots in October-November or April-May, one bud can bear 5 to 6 fruit in the third year. This fruit can be compared with that of selected original parent tree for its characteristic performance, such as seedlessness, early maturity, prolificness, size and quality. If these superior characters can be transmitted to their grafted offspring, they can be propagated for variety trials. Thus, first-year selection, second-year grafting, and third-year registration and propagation allow the entire program to be shortened to 3 to 4 years. This program has been carried out in the Huazhong Agricultural College experimental citrus grove for many years; 50-100 selected clones are tested every year (Fig. 7).

We have addressed the question of rootstock influence in this progeny test. Our experience is that if these important characters, such as seedlessness, early maturity, fruit size and quality, are inherited, other morphological variations can be ignored. According to our 1975-1976 progeny tests for unshiu mikan and orange selections, these important characters can be transmitted to grafted progenies (14) (Table 2).

Citrus unshiu used as interstocks showed a high degree of compatibility with all citrus species. Usually the scion produced flowers and fruit in the next year. All superior clonal selections tested and registered are listed in Table 3.

VI. In vitro propagation of clonal selection.

Recent expansion of citrus areas in 14 southern provinces requires a large

Fig. 7. Topworking clonal selections on *Citrus unshiu* for progeny tests. (NOTE: Arrow shows grafting point.)

amount of superior citrus nursery stocks for planting. Clonal selections, after county, provincial and national registration, were used to establish parent gardens and budwood plantings.

Since 1980, the Citrus Research Institute established by the Ministry of Agriculture, Animal Husbandry and Fishery, in Huazhong Agricultural College, has undertaken the in vitro propagation of virus-free clonal selections for distribution. Two methods have been used: shoot tip culture and shoot tip grafting.

1) Shoot tip culture. Shoot tips less than 0.2 mm long from either greenhouse trees or culture plants, are passed through a series of disinfection, and cultured in MS medium to differentiate callus, then shoots and roots. Addition of 2,4-D at 1-1.5 mg/1, NAA 1.5 mg/1, KT 0.25 mg/1, and BA 0.5 mg/1 and Zeatin 0.5 mg/1 is satisfactory for differentiation, as shown in Fig. 9 of 'Jincheng Orange No. 139.' From shoot tip cultures, we found that clonal selections differ in their callus differentiation. Many clones do not readily differentiate roots. We employed a technique to graft shoots differentiated in culture media onto rootstocks grown in the greenhouse (Fig. 8).

Table 2. Progeny test for clonal selections on top-grafted trees (1975-1976).

Clonal selection	Date ripe	Wt. (g)	Form index H/D	Rind thickness (mm)	No. segments	No. seed	TSS (%)	Acid (%)	TSS/acid ratio	Edible part (%)
Quoqing #1, P*	28/9	130	0.80	2.3	10.0	0	9.0	0.76	11.80	82.9
Quoqing #1, G*	26/9	185	0.82	2.0	10.0	0	11.2	0.86	13.02	83.0
Quoqing #4, P	8/10	117	0.81	2.5	10.0	0	10.0	0.77	13.00	76.0
Quoqing #4, G	8/10	120	0.87	3.0	10.2	0	11.0	0.78	12.82	72.0
Huanglin seedless, P	10/11	173	0.98	3.0	11.5	0	12.0	0.98	12.50	79.0
Huanglin seedless, G	16/11	190	0.99	3.0	12.0	0	12.1	1.00	12.10	78.5
Taoyihcheng #8, P	10/11	135	0.96	2.5	12.0	6.1	12.4	0.40	31.00	77.0
Taoyihcheng #8, G	6/11	142	0.91	2.8	12.5	6.3	11.5	0.31	37.10	79.0

*P = parent tree; G = grafted tree.

Table 3. Registered and tested clonal selections of citrus in Hubei Province.

Clonal selection	Fruit wt (g)	Form index (H/D)	Rind thickness (mm)	Seed/ fruit	Edible part (%)	TSS (%)	Acid (%)	Taste
Citrus unshiu								
Quoqing #1	130	0.80	2.0	0	82.9	9.0	0.76	Sweet, melting, fragrant
Quoqing #4	117	0.81	2.5	0	76.0	11.2	0.86	Sweet, melting
Changyang 1-1	111	0.79	2.0	0	80.0	11.3	0.62	Sweet, melting, fragrant
Itu #27	86	0.82	1.8	0	83.1	12.0	0.88	Sweet, melting
Yaowan #32	125	0.83	3.1	0	75.0	13.0	0.96	Sweet, melting
Yaowan #16	130	0.76	2.3	0	79.0	12.1	1.27	Sweet acid, melting
Citrus sinensis								
Taoyihcheng #8	135	0.96	2.5	6.1	77.0	12.4	0.40	Sweet, crisp, fragrant
Taoyihcheng #139	151	0.95	2.8	4.0	79.0	15.5	0.29	Sweet, crisp, very fragrant
Huanglin seedless	173	0.98	3.0	0	79.0	12.0	0.96	Sweet, melting, fragrant
Jincheng Xinshan #101	175	1.08	2.6	1.1	70.7	12.0	1.03	Sweet, melting, fragrant
Jincheng #44	180	1.06	3.8	4.0	73.8	13.2	1.06	Sweet, melting, fragrant
Navel #35	185	1.03	3.4	0	76.0	12.5	1.00	Sweet, crisp, fragrant
C. ponkan #31	160	0.56	3.7	10.4	69.7	13.0	0.65	Sweet, melting

2) Shoot tip grafting. Virus and viroid infection of citrus in South China has devastated many citrus groves. Huanglungbien, a kind of mycoplasma disease has been found in Guangdong, Guangxi, and Fujian provinces.

Since 1980, we have succeeded in shoot tip grafting by using shoot tips of the clonal selections grafted in vitro on Troyer rootstocks. Troyer citrange seeds were treated at 50°C for 55 minutes, disinfected with 10% sodium hypochlorite solution for 15 minutes, and planted in MS media. They could be grafted about 2 weeks after sowing. Shoot tips of 0.4-0.18 mm were taken, disinfected in 2% Na-hypochlorite solution for 5 minutes, immediately grafted onto Troyer stocks under a dissecting microscope with "T" or side budding, and cultured in Heller holder under 1,500 lux inflorescent light at 25-30°C. About 55% "take" was obtained, as shown in Fig. 9. When the grafted plants had grown 2 leaves, they were transplanted out in

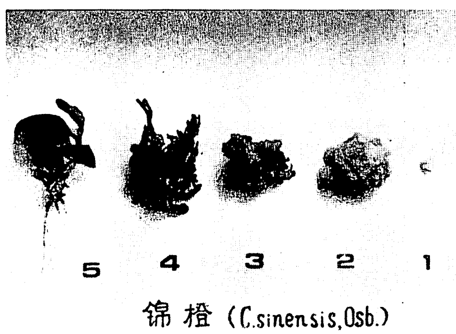

Fig. 8. Shoot tip culture of 'Jincheng No. 139.'

prepared compost. Ordinarily, they can flower and fruit 2 years after planting without a juvenile stage. Virus indexing was conducted by the ELISA technique and virus-sensitive indicator plants. These clonal selections were propagated under the polyethylene shed as shown in Fig. 10.

V. **Test for cold resistance.**

Frost injury occurs every 10 to 15 years in citrus regions of the middle and lower Yangtze provinces. Huazhong Agricultural College has conducted a series of investigations on the physiological and biochemical indices for cold resistance of clonal selection.

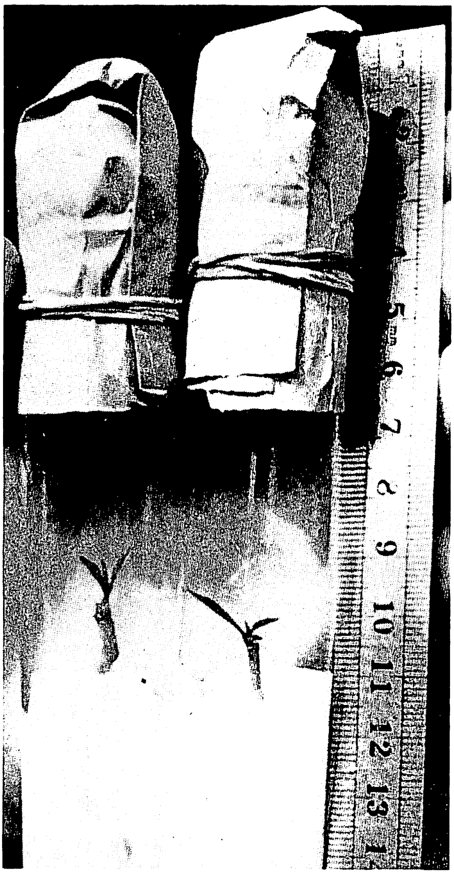

Fig. 9. Shoot tip grafting of virus-free clones.

Ji Zhongxiong (6) studied the biochemical and physiological indices in Huazhong Agricultural College from September 12 to March 12; there were significant differences in cold resistance among different cultivars of citrus species.

1) Cell water content in relation to cold resistance. The rate of reduction in total water, free water and bound water in leaves is closely related to the degree of cold resistance. Cold-resistant cultivars have fewer fluctuations in water content than the tender species. But the ratio of fluctuation of the bound/free water in the leaves is larger in cold-resistant cultivars than in the less resistant ones (Table 4).

It can be concluded that during the period of over-wintering, the value of bound water/free water over 1.0 is considered as the cold-resistant type, whereas the value below 1.0 is considered as the tender type. The order of cold resistance according to the value of bound water/free water may

Fig. 10. Virus-free clones propagated under polyethylene shed.

be listed as follows: *Citrus ichangensis*, 'Bentitsao' tangerine, *C. sinensis*, 'Suichuan' kumquat, 'Owari' mikan, 'Jinxiang' pummelo.

2) Sugar content in relation to cold resistance. During the cold winter period, sugar content in citrus leaves increases with the decrease in temperature. The fluctuation in hydrolyzable sugar is greater than that in reducing sugar. In cold-resistatnt cultivars, the content of reducing sugar is above 7.5%, and the hydrolyzable sugar above 10%, whereas in the tender cultivars, reducing sugar is below 7.5%, and the hydrolyzable sugar below 10%. Thus, the sugar content in the leaves may be regarded as an index for cold resistance (Table 6).

3) Peroxidase in relation to cold resistance. During the cold winter period, the fluctuation of peroxidase activity can be used as an index for cold resistance. In hardy cultivars, the fluctuation of peroxidase activity can be used as an index for cold resistance.

Table 6. Sugar content in leaves of different cultivars (1981).

Cultivar	Reducing sugar (%)	Hydrolyzed sugar (%)
Seedlings:		
C. ichangensis	8.64	14.43
Bentitsao	7.87	13.55
Sweet orange	6.96	8.68
Grafted trees:		
Suichuan kumquat	8.56	14.20
Owari mikan	7.86	13.71
Jinxiang pummelo	5.81	7.36

In hardy cultivars, the fluctuation of peroxidase activity is smaller below 16.00 units, whereas in tender cultivars, the peroxidase activity is often above 20.00 units, as shown in Table 7.

According to the fluctuation in peroxidase activity during the cold winter period, the degree of cold resistance

Table 4. Fluctuation of bound/free water (1981).

Cultivar	Cold resistance	Free water %	Free water Fluctuation	Bound water %	Bound water Fluctuation	Bound/free Value	Bound/free Fluctuation
Suichuan kumquat	Resistant	25.05-21.23	3.82	33.43-32.61	0.82	1.65-1.32	0.33
Suichuan kumquat	Resistant	25.05-21.23	3.82	33.41-32.61	0.82	1.65-1.32	0.33
Owari mikan	Less resistant	31.55-25.74	5.81	30.05-28.68	1.37	1.32-1.01	0.31
Jinxiang pummelo	Tender	49.95-40.98	8.06	14.22-11.05	3.17	0.29-0.23	0.06

Table 5. Amount of bound water and cold resistance (1981).

Cultivar	Bound water (%)	Ratio bound/free	Date of disbudding	Cold injury index	Winter hardiness
Seedlings:					
C. ichangensis	39.61	2.36:1	30/8	0	1
Bentitsao	30.04	1.14:1	14/9	17.50	2
Sweet orange	14.33	0.33:1	28/9	58.40	3
Grafted trees:					
Suichuan kumquat	33.43	1.51:1	16/9	1.00	1
Owari mikan	30.05	1.24:1	15/9	15.40	2
Jixiang pummelo	11.05	0.23:1	30/9	65.70	3

of different cultivars can be ranked as follows in descending order of hardiness: *C. ichangensis*, 'Suichuan' kumquat, 'Owari' mikan, 'Bentitsao' sweet orange, 'Jinxiang' pummelo. The peroxidase activity is influenced less in cold-resistant cultivars than in the tender cultivars. This suggests that the fluctuation in peroxidase activity is related to the physiological process of over-winter hardening. Greater fluctuation may be more sensitive to the temperature changes, and more susceptible to frost injury.

4) Electrolyte conductivity in relation to cold resistance. Qu, Shengxiang et al. (8), working on electrolyte conductivity of citrus shoots taken from November to the end of February in Huazhong Agricultural College, showed that spring shoots and leaves of different cultivars, after −13°C treatment for 12 hours, showed differences in the electrolyte conductivity.

Electrolyte conductivity applied to hybrid seedlings also showed a close relationship with the degree of cold resistance. When 'Owari' mikan female was crossed with 'Sichuan' tangerine male, the hybrid F_1 after 13°C treatment showed electrolyte conductivity intermediate between the 2 parents as shown in Table 9.

Literature Cited

1. Fujian College of Agriculture, Fruit Tree Breeding Section. 1978. A preliminary study on the character change in improved citrus orange. J. Genetics (Yichuan xuebao) 5(1):45-51.
2. Huazhong College of Agriculture, Yichange Campus. 1977. Citrus Production Technique and Scientific Experiment. Science Publishing House.
3. Iwamasa, Masao. 1976. Citrus Variety. Jinggang County Citrus Business Association.
4. Iwamasa, Masao. 1977. Problems related to citrus breeding. Agriculture and Horticulture 52(2):1547.
5. Janick, J., et al. 1975. Advance in fruit

Table 7. Peroxidase activity of citrus cultivars (1981).

Cultivar	Cold resistance	Peroxidase activity (mg/g/hr)	Fluctuation
Seedlings:			
C. ichangensis	Very resistant	40.18 - 29.87	10.31
Bentitsao	Resistant	38.12 - 22.94	15.18
Sweet orange	Tender	38.20 - 16.58	21.62
Grafted trees:			
Suichuan kumquat	Resistant	36.47 - 25.74	10.73
Owari mikan	Resistant	37.29 - 23.60	13.69
Jinxiang pummelo	Tender	37.54 - 12.54	25.00

Table 8. Electrolyte conductivity in relation to citrus cold resistance (1981).

Cultivar	Electrolyte conductivity of twigs (mohms)	Linear regression value b	Winter hardiness
Citrus ichangensis	236	—0.67	2
Poncirus trifoliata	227	1.82	1
Sichuan tangerine	397	—11.65	3
Bentitsao tangerine	412	—18.98	4
Suichuan kumquat	525	—23.70	5
Owari mikan	630	—26.82	6
Sweet orange	515	—30.95	7
Jinxiang pummelo	635	—31.18	8

Table 9. Electrolyte conductivity of citrus hybrid seedlings (1981).

Cultivars and their hybrids	Electrolyte conductivity after —13°C (%)	Regression b value	Winter hardiness
Owari mikan	50.81	—26.82	3
Owari mikan X Sichuan tangerine	47.74	—20.91	2
Sichuan tangerine	36.09	—11.65	1

breeding. pp. 11-13. Purdue University Press, U.S.A.

6. Ji, Zhongxiong. 1982. Physiological and biochemical target of citrus fighting cold character. J. of Huazhong College of Agriculture, Issue No. 4.
7. Li, Siguang. 1975. China's Fourth Glacier. Science Publishing House.
8. Qu, Shengxiang, and Ma Xiangtao. 1981. Method of conductivity and its application to the testing for cold hardiness in citrus. J. of Huazhong College of Agriculture, Issue No. 3.
9. Sichuan Xichang Region, Bureau of Agriculture. 1975. Fruit Tree Cultivation in Xichang.
10. Yichang County Bureau of Special Local Products. 1979. Yichang Citrus.
11. Yu, Dejun. 1979. Taxonomy of Chinese Fruit Trees. Agricultural Publishing House.
12. Zhang, Wen-Cai. 1939. Seed selection method and standard for the sweet orange trees in Jiangjin. New Report on Farming and Forestry (Nonglin xinbao) 16:26-28.
13. Zhang, Wen-Cai. 1975. Cultivating method for new citrus varieties. Hubei Province, Bureau of Agriculture, Cultivating Good Citrus Varieties in Hubei.
14. Zhang, Wen-Cai. 1977. Make great efforts to reach the peak of citrus production in the world. Hubei Agricultural Science (Hubei kexue) 12:24-27.
15. Zhang, Wen-Cai. 1981. Thirty years' achievements in citrus varietal improvement work in China. Proc. Intern. Citrus Congr. 1:51-55.

1985 APS MEETING

APS BUSINESS MEETING

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