II.3 Isolation and Culture of *Citrus* Protoplasts

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1 General Account

1.1 Economic Importance of *Citrus* Cultivars

*Citrus* fruits rank second only to grapes in world fruit production. *Citrus* production was estimated in 1985 at a total of approximately 60 million metric tons (FAO 1985). Oranges comprise the most important *Citrus* product in world trade, followed by grapefruit, mandarins (which have recently increased, especially on the European market) and lemons. In addition to fresh fruits, large quantities of sweet orange, grapefruit and mandarin are processed into juice, concentrates, squashes and other products.

1.2 Distribution and Origin of *Citrus* Cultivars

The cultivars of *Citrus* are grown in tropical and subtropical climates, roughly covering a belt which circumvents the globe approximately between latitudes 40°N and 40°S. There are about 90 *Citrus*-producing countries, the most prominent among them being Brazil, USA, Mexico, Spain, Italy and China (FAO 1985).

The commonly cultivated citrus-type species belong to the genus *Citrus*. This genus also contains species of the subgenus *Papeda*, which do not bear edible fruits. In addition to *Citrus*, the subtribe Citrinae includes five other genera: *Eremocitrus*, *Poncirus*, *Fortunella*, *Microcitrus* and *Clymenia*. *Poncirus* is used as a rootstock for *Citrus* cultivars and the kumquat fruits (*Fortunella* spp.) have a limited market (Swingle and Reece 1967). Important *Citrus* cultivars are listed in Table 1.

The subtribe Citrinae ("true *Citrus* fruit trees") is native to a large Asiatic area from the Himalayan foothills in India to North-Central China, extending to Burma, Thailand and Indonesia in the South and to the Philippines, North-East Australia, New Guinea and New Caledonia in the South-East. The centers of origin of the main commercial *Citrus* cultivars were not defined with certainty because of probable hybridization in ancient times; but, using evidence from old

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Table 1. Important cultivars of *Citrus* and their affiliation with trivial and true species

<table>
<thead>
<tr>
<th>Common name</th>
<th>Species</th>
<th>Examples of cultivars</th>
</tr>
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<tbody>
<tr>
<td>Orange</td>
<td><em>Citrus sinensis</em> (L.) Osbeck</td>
<td>Shamouti, Valencia, Washington Navle, Hamlin, Trovita</td>
</tr>
<tr>
<td>Grapefruit</td>
<td><em>Citrus paradisi</em> Macf.</td>
<td>Marsh-seedless, Duncan, Foster, Thompson, Ruby</td>
</tr>
<tr>
<td>Mandarin</td>
<td><em>Citrus reticulata</em> Blanco&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Dancy, Nagpur, Wilking, Satsuma, Clemantine</td>
</tr>
<tr>
<td>Lemon</td>
<td><em>Citrus limon</em> (L.) Burm.</td>
<td>Villafranca, Eureka, Lisbon</td>
</tr>
<tr>
<td>Citron</td>
<td><em>Citrus medica</em> L.&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Corsican, Diamonte, Earle</td>
</tr>
<tr>
<td>Lime</td>
<td><em>Citrus aurantifolia</em> (Christm.) Swing.</td>
<td>Everglade, Mexican</td>
</tr>
<tr>
<td>Pummelo</td>
<td><em>Citrus grandis</em> (L.) Osbeck&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Kao-Phuang, Kao Panne</td>
</tr>
</tbody>
</table>

<sup>a</sup> True species according to Barrett and Rhodes (1976).

Chinese writings Cooper and Chapot (1977) support the contention that almost all presently known *Citrus* cultivars originated in China. This contention is also in accord with our own recent study on the chloroplast genomes of *Citrus* cultivars (Green et al. 1986).

1.3 Reproduction Mechanisms and their Bearing on Breeding

The reproduction of *Citrus* cultivars is inflicted with mechanisms which complicate genetic studies and breeding. These mechanisms include nucellar embryogeny (Cameron and Frost 1968; Frost and Soost 1968), heterozygosity, self-incompatibility (Soost 1964), sterility (Iwamasa 1966) and an extended juvenile period. While it is relatively easy to breed, by sexual hybridizations, new mandarin types (some of the mandarin types are mono-embryonic), it is rather difficult to improve, by cross-breeding, the polyembryonic cultivars of sweet orange, grapefruit and lemon. Because of these obstacles, *Citrus* breeders lack essential information on the mode of inheritance of desirable traits (Vardi and Spiegel-Roy 1978).

1.4 Relevance of Cell Manipulation to *Citrus* Improvement

There is an urgent need to introduce disease resistance and stress resistance (e.g. salinity, drought) into *Citrus* cultivars and rootstocks. Only a few studies have been devoted to the use of mutagenesis to improve *Citrus* cultivars (e.g. Hensz 1971), and none of these was aimed at improving rootstocks.

Sources for disease- and stress resistance are known to be harboured in *Citrus* relatives, but sexual hybridization between the latter and rootstocks or cultivars is difficult (Barrett 1977). Thus cell-manipulation methodologies, and especially protoplast manipulation, should be of great relevance for *Citrus* breeding. The latter approach will be useful if functional *Citrus* trees can be derived from isolated protoplasts.