FRuiTROP

Research and Methods

# The issues and prospects of varietal improvement for Mediterranean citrus growing

## Issues

Varietal selection is a major component in the establishing of sustainable, integrated farming systems. Indeed, the cultivars define the qualitative and quantitative potential of production and are an important element in strategies for integrated protection from pests and diseases. The context of Mediterranean citrus growing—subjected to strong biotic and abiotic constraints and responding to a fresh fruit market that is very demanding in terms of quality—clearly shows that varietal selection is a central issue for the development of citrus growing in the region. The undertaking of varietal creation and selection programmes seems all the more important for the major producer countries as the policy of protection of new varieties, which is fairly new for citrus, is becoming widespread. Possible exclusive rights to new, high-quality products should be a strong advantage in competition on the European market.

Mediterranean citrus orchards consist entirely of grafted plants. Rootstocks are generally propagated by the sowing of polyembryonic seeds. The cultivars are propagated by budding or double-grafting. The latter technique is commonly used in Spain and enables rapid rotation and thus better matching to the market. The clonal propagation of both stocks and varieties thus greatly simplifies varietal creation schemes since any élite individual can be multiplied identically, whatever its genetic structure. Furthermore, cultivating budded seedlings makes its possible to a certain extent to spread the genetic constraints between stock and cultivar. This is an important aspect in the light of the great climatic and ecological diversity observed in the cultivation area.

### Rootstocks

Adaptation to the different soil types and the pathogens that they contain is the main objective guiding the choice of stocks. Thus, tolerance to salt, adaptation to alkaline or acid soil are sought according to the soil type and in all cases resistance or tolerance of *Phytophthora* spp. and nematodes. The rootstock genotype makes it possible to modulate the behaviour of the stock/scion combination in numerous characters such as tolerance to tristeza and cold, and vigour and productivity. In addition to having an effect on harvest quantity, the rootstock has a certain effect on quality. It affects numerous factors of economic importance such as grade, peel thickness, juice content and fruit flavour.

The arrival of tristeza radically called into question the use of sour orange (*C. aurantium*) in the Mediterranean


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## Production and markets

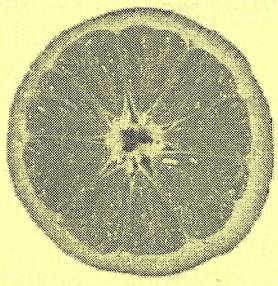
Citrus fruits were introduced in the Mediterranean area more than twenty centuries ago. It is thus a very important varietal diversification zone in which the various groups of orange and lemon cultivars in particular have been selected. Annual production totals some 18 million tonnes, with 80% going to the fresh fruit market. This market structure contrasts with that in the US where approximately 80% of production is used to supply the processing industry.

Mediterranean producers are thus devoting their efforts to the fresh fruit market. Orange remains the main crop with 8.02 Mt and 2.25 Mt for fresh sales and processing respectively. The Mediterranean area is also the main production region for easy peelers, and especially clementines that are greatly appreciated on the European market. Indeed, Mediterranean easy peeler production forms 81% of the fresh export fruit market and 40 % of the processing market. The Mediterranean area is also the main lemon/limes producer with almost 30% of world production.

The European market is the main export outlet for Mediterranean production. It is a very competitive market and increasingly demanding with regard to quality and some regions or producers' groups are establishing a label policy, thus differentiating products.

| In Mt | Citrus total | Oranges | Mandarins | Lemons/limes | Grapefruits |
|---|---|---|---|---|---|
| Production | 18.29 | 10.27 | 4.48 | 2.93 | 2.50 |
| Processing | 3.58 | 2.25 | 0.54 | 0.58 | 0.21 |

area, whereas it had been almost the only rootstock in the region. Most producer countries have thus undertaken to switch to stocks tolerant to the disease in their orchards. *Poncirus trifoliata* and certain hybrids of the latter with *Citrus* are resistant to tristeza and markedly tolerant to *Phytophthora* spp. and generally give oranges of mandarins of good quality. In contrast, they are very poorly adapted to saline or calcareous soils. Furthermore, *Poncirus* is very susceptive to exocortis (a viroid that can be spread by budding or pruning tools). Various rootstocks are known for their tolerance to ferric chlorosis associated with calcareous soils (*C. depressa, C. jambhiri, C. limonia,* etc.) or salinity (*C. limonia, C. reschni, C. macrophylla, C. amblycarpa*) but display susceptibility to other biotic constraints (tristeza, *Phytophthora,* etc.) or cause a decrease in the quality of sweet citrus, making them little usable in many production zones in the Mediterranean area. It thus seems that the creation of new rootstocks with tolerance to biotic (tristeza, *Phytophthora,* nematodes) and abiotic constraints (lime, salinity) while giving high quality oranges and easy peelers is a major objective for the region.



*Clementine, an ideotype for the European market*

## Varieties

Product quality is becoming the essential criterion for the fresh fruit market. The definition of organoleptic quality may vary according to consumer habits. In the West, it is determined by colour, aroma, juice content and the sugar:acid ratio. Considerable differences in appreciation nevertheless exist in the various European countries. Breeders must therefore try to develop a range of varieties that will match the diversity of these perceptions. The nutritional quality of the new varieties and their benefits for health should also guide breeding programmes. Seedlessness, easy peeling and regular skin contribute to the definition of fruit quality. In addition to seedlessness in new varieties, cultivars must be bred that are incapable of pollinating self-incompatible varieties, and especially clementine, which forms the bulk of Mediterranean easy peeler orchards. If this is not achieved, the producing clementine orchards next to the new varieties will produce fruits that contain numerous seeds.

Staggering production in time is also a very important objective in mandarin type easy peeler breeding programmes. The market is thus waiting in particular for late varieties. Disease resistance characters must also be sought, such as resistance to 'mal secco' (*Phoma tracheiphila*) in lemon and African citrus leaf spot that is threatening the Mediterranean area. The search for varieties resistant to Stubborn is also of definite interest for the southern Mediterranean area where high temperatures enhance expression of the disease.

The first rational citrus improvement programmes began in the United States at the end of the nineteenth century. They were aimed at giving orange trees better resistance to cold by means of hybridisation. Since then, improvement objectives have diversified strongly as a result of the appearance of many biotic constraints and the stricter quality requirements of markets. Today, varietal improvement

strategies use vegetative multiplication and are increasingly based on progress in biotechnologies. Spain, Italy, Israel and France are the countries most advanced in the use of these tools in the area. Turkey and Morocco have a long tradition of conventional improvement and are now following the same path.

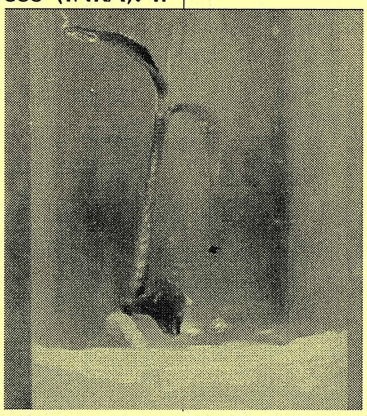
## The French programmes

In France, CIRAD and INRA have conducted for about a decade varietal creation programmes aimed at responding to the constraints of Mediterranean citrus growing and the expectations of the European market. Their work has been focused on the breeding of rootstocks tolerant to biotic and abiotic constraints and the diversification of easy peelers for the fresh fruit market.

### Improving rootstocks

Conventional citrus improvement is limited by biological constraints and the genetic structure of the species complex. Biotechnologies make it possible to circumvent some of these difficulties. Somatic hybridisation, consisting of the fusion of diploid protoplasts (cells) containing all the genetic information of each parent, has thus been developed successfully in citrus. The main advantage of this technique for rootstock improvement is that it can cumulate all the genes of both parents, whatever their heterozygosity level. It is thus hoped that all the dominant characters of the parents will be expressed in the somatic hybrid.

CIRAD has bred an allotetraploid somatic hybrid of *Poncirus trifoliata*, resistant to nematodes, *Phytophthora* and tristeza, and common mandarin, tolerant to abiotic constraints. On its own roots in the nursery, this hybrid displays interesting levels of tolerance to lime and salinity. Trials on grafted seedlings are in progress to validate this behaviour. Like *Poncirus*, FLHORAG1 is immune to tristeza and possesses a good level of tolerance to *Phytophthora citrophthora*. Agronomic evaluation for the Mediterranean area has begun in Corsica (SRA), the Lebanon (IRAL) and Morocco (INRA). If the results are positive, this rootstock could be made available to citrus growers in 6 or 7 years.



*Saving triploid embryos*

### Improving cultivars

The main objective is the diversification of the range of seedless easy peelers using criteria of high quality with a lengthening of the production period and avoiding the risk of cross-pollination with clementine. The search for sterility has led the French teams to concentrating their varietal selection programmes on the breeding of triploid cultivars. Indeed, plants with three sets of chromosomes instead of two (diploids) are known for their high level of sterility in both ovules and pollen. This is the way in which seedless water-melon is produced, for example. Several natural triploid varieties exist among the citrus, and especially the large green lime (Tahiti lime), with a very high level of sterility. Various biotechnology-based strategies have been developed by the French teams in order to obtain triploid hybrids.

The first strategy consists of selecting spontaneous triploidisation events. Small pips containing triploid embryos form regularly after the hybridisation of diploid varieties. Saved *in vitro* and selected using flow cytometry, which enables rapid evaluation of the ploidy

### Strong constraints

Mediterranean citrus growing is subjected to strong abiotic constraints, especially in the southern part of the area. Salinity is a major problem in the Mediterranean area, affecting nearly 150,000 km² and worsening as a result of poor irrigation practices. Alkaline pH levels resulting from calcareous soils are another serious constraint for citrus. They cause ferric chlorosis that is particularly marked in semi-arid regions where the irrigation water has a high bicarbonate content.

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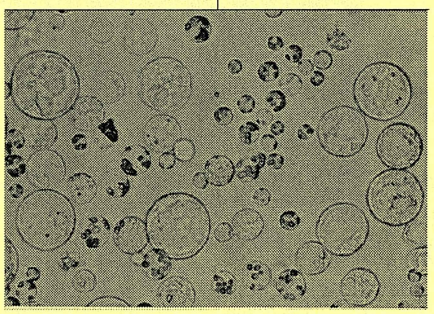
level, these embryos have been used to obtain numerous triploid hybrids of mandarins, of mandarin and orange (tangor) and mandarin and pomelo (tangelo). The first fruitings have confirmed the very low fertility of these hybrids and fruits with interesting pomological and organoleptic characteristics have been obtained.

The second strategy consists of crossing diploid and tetraploid varieties. As most citrus genetic resources consist of diploids, the first phase of the programme consisted of the creation of a pool of tetraploid parents. These are bred by somatic hybridisation or selected from nucellar seeds, a few percent of which give tetraploid seedlings. Several tens of allotetraploid and autotetraploid hybrids have been obtained. The first flowering of some of these this year has made it possible to begin the second phase of this strategy by pollinating diploid varieties.



*Protoplasts before fusion*

Finally, a method for diploid and haploid protoplast fusion has been applied, enabling the direct synthesis of triploid hybrids while conserving the entire genetic make-up of the preselected diploid cultivars. It is thus hoped that the creation of varieties with predefined characteristics can be guided more effectively.

After preliminary selection according to qualitative aspects, the best hybrids resulting from these different strategies will be subjected to varietal trials for evaluation of their agronomic potential. As the juvenile phase lasts for 5 to 6 years, a period of at least 13 or 14 years is needed between hybridisation and the extension of a new variety. At the international level, the strategies and methodology developed by the French teams mean that they can establish collaboration with partners in Morocco, California, Chile and Vietnam who face the same set of problems ■

**Patrick Ollitrault, Cirad-flhor**
**patrick.ollitrault@cirad.fr**

## Pests and diseases

The Mediterranean area is still spared today by several major bacterial diseases of citrus such as Huanglongbing, which causes considerable damage in Asia, citrus variegated chlorosis, which is spreading in America from Brazil, and citrus canker, which is scattered all over the world. However, most virus diseases are found and especially tristeza, which is leading to the abandoning of the traditional rootstock for citrus in the area—sour orange, whose combinations with orange are the most susceptible to the disease. Thus, the death in Spain since 1957 of more than 15 million trees grafted on sour orange has been attributed to tristeza. Soil pests and diseases such as *Phytophthora citrophthora* and *Phytophthora parasitica* and nematodes *(Tilenchulus semi-penetrans)* are present in the Mediterranean area.

Although chemical control is possible, it cannot be envisaged on a systematic basis for reasons of cost and environmental impact. It is therefore essential to use rootstocks that are tolerant to nematodes and to *Phytophthora* spp.

Finally, the threats formed by two diseases that are today at the frontiers of the Mediterranean area can be noted. Witches'-broom disease of lime, present in the Near East, is caused by a phytoplasma and mainly attacks sour citrus. Africa citrus leaf spot is caused by *Phaemularia angolensis*. It appeared in central Africa and is now present in Guinea and Senegal and is particularly virulent on orange and pomelo.