

GENESIS OF JUICE SACS AND SUPPRESSION OF THE BITTER ESSENTIAL-OIL GENE IN DISTANT HYBRIDS OF THE RUE FAMILY (CITRUS)

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Cultivated citrus, not only in the Soviet subtropics—which constituted the northernmost zone of citrus cultivation—but also in many tropical and subtropical regions, are damaged by frost when cold air masses invade.

To obtain frost-resistant forms, cultivated citrus were crossed with the closely related genus *Poncirus*, which can withstand temperatures of -20 to -22 °C, as well as with their wild relatives, *Citrus* taxa of the *Archicitrus* group. However, this approach did not produce hybrids with edible fruit [4–6].

In our earlier work [2, 3], we showed the reason for the failure of distant hybridization: trifoliolate orange and wild citrus relatives possess dominant genes that suppress the flavor qualities of cultivated citrus. In particular, the bitter essential-oil gene (O) of trifoliolate orange and wild citrus relatives completely suppresses the non-bitter essential-oil gene (o) of cultivated citrus; moreover, the bitter-oil gene (O) and the kan-kan gene (K) are inherited together.

On this basis, we studied the formation and developmental characteristics of juice sacs in the genera *Poncirus*, *Citrus*, and *Fortunella*, and searched for taxa possessing epistatic genes with respect to genes of *Poncirus* and the *Papeda* section of the subgenus *Archicitrus*.

For studying juice-sac development, preparations were made from ovaries before pollination, 30, 45, and 60 days after pollination, and from mature juice sacs. The material included *P. trifoliata*; *F. margarita*, *F. japonica*, *F. ovata*, and *F. crassifolia*; *C. wilsonii*, *C. ichangensis*, *C. hystrix*; *C. sinensis* (Hamlin, Washington Navel, Turkish, Di Malta); *C. reticulata* (Unshiu, Shiwamikan, Clementine, Mikado); *C. limon* (Novogruzinsky, Villa Franca); and *C. grandis* (Azhakikan, Tengu, Natsumikan, Duncan, Foster, Marsh Seedless). Intergeneric and interspecific hybrids included *Citrumquat* (F.

margarita $\times$ P. trifoliata), Citrumelo (P. trifoliata $\times$ C. paradisi), Orangequat (C. sinensis $\times$ F. japonica), Citrange (C. sinensis $\times$ P. trifoliata), Citremon (C. limon $\times$ P. trifoliata), Citrandarin (C. reticulata $\times$ P. trifoliata), Ichandarin (C. ichangensis $\times$ C. reticulata), Tangor (C. reticulata $\times$ C. sinensis), Tangelo (C. reticulata $\times$ C. paradisi), and Citrangequat ((P. trifoliata $\times$ C. sinensis) $\times$ Fortunella).

Essential oils located in the tissues of the juice sacs, as well as in the flavedo and albedo, were also studied. To prepare essential-oil smears from juice sacs, transverse sections were made and the oil was squeezed onto microscope slides. Glands in the flavedo were removed, cut in half, crushed on slides, stained, and covered with coverslips. The experiments were conducted in three stages: before the fruit yellowed, after yellowing, and after natural fruit drop. The glands were examined *in vivo*, both stained and unstained. Acetic solutions of alkanin, indophenol blue, and Sudan III were used as stains for essential oils.

For permanent preparations, material was fixed in acetic alcohol. Transverse and longitudinal sections of juice sacs 12–16 μ m thick were made. Preparations were stained with ruthenium, eosin, and Ehrlich's hematoxylin, and sections were mounted in Canada balsam.

The beginning of juice-sac formation is associated with the reduction division of the microspore. Immediately after the first division of the macrosporocyte, individual cells of the epidermal tissue lining the inner wall of the carpel begin vigorous division and, by the eight-nuclear stage of the embryo sac, form protuberances. The appearance of these protuberances is coordinated with macrospore development. When the reduction division fails to occur, juice-sac protuberances do not form.

An atypical course of meiosis does not adversely affect formation of the protuberances or their subsequent development. The cells making up a protuberance are small, with dense, finely vacuolated cytoplasm and large nuclei and nucleoli.

After the first division of the secondary embryo-sac nucleus, intensive cell division begins in the protuberances along with endosperm development. As a result, finger-like outgrowths are formed that project into the ovarian cavity.

During the finger stage, the cells remain small and have dense, finely vacuolated cytoplasm. After the

finger-like outgrowths reach the middle of the ovarian cavity, they begin to take on a club-shaped form. Subsequently, the upper part gradually narrows and the structures become spindle-shaped. At this stage the cells are larger, have less-dense cytoplasm and large vacuoles.

By the first division of the zygote, the pointed ends of the developing juice sacs come into contact with the vascular system of the ovules or, when ovules are absent, intertwine with one another. At this time, the epidermal cells of the juice sacs become covered by a mucilaginous layer.

In fully developed juice sacs, three tissues can be distinguished: epidermal, mesophyll, and intercalary tissue. The cells of the latter are fairly large, with enormous vacuoles and nuclei positioned against the cell wall. Their cytoplasm is less dense. Structurally they are identical to the cells of carpel hairs. Since both hairs and juice sacs originate from epidermal cells, it can be assumed that juice sacs develop from hair-forming cells.

Compared with the intercalary tissue, mesophyll cells are smaller but richer in assimilates; from the second half of August through September they accumulate very large quantities of inulin.

In cultivated citrus, development of the juice sacs ends at the stage of tissue differentiation. In wild *C. wilsonii*, *C. hystrix*, *C. tangelos* and others, as well as in *Poncirus*, the intercalary tissue transforms into an essential-oil gland.

Thus, among the rue-family citrus relatives there are two types with respect to fruit-pulp formation: one forms pulp containing essential-oil glands, while the other forms pulp without them. Fruits are edible only in the second type, i.e. in taxa that do not develop glands in the fruit pulp. Forms also occur in which glands develop in the juice sacs but the oil does not have the bitter taste that damages fruit quality [1]. One such form is the Pear-shaped Shaddock found in Adjara. It is probably a hybrid between taxa of *Archicitrus* and *Metacitrus*; apparently one parent possesses a suppressing property with respect to bitterness of the essential oil.

Histological study showed that the double intergeneric hybrids *P. trifoliata* × *C. sinensis*; *C. sinensis* × *P. trifoliata*; *C. tangerina* (Clementine) × *P. trifoliata*; *C. unshiu* × *P. trifoliata*; *C. limon* × *P. trifoliata*; *C. limonia* × *P. trifoliata*; *C. paradisi* × *P. trifoliata*; *C. maxima* × *P. trifoliata*; *C. medica* × *P. trifoliata*;

C. aurantium × *P. trifoliata*; *C. aurantifolia* × *P. trifoliata*; and *C. myrtifolia* × *P. trifoliata*, like trifoliolate orange itself, form glands in their juice sacs in which bitter essential oil accumulates.

In the triple hybrid Citrangequat ((*C. sinensis* × *P. trifoliata*) × *Fortunella*), glands do not develop and its fruits are edible. In the hybrid Limequat (*C. lime* × *Fortunella*) × *P. trifoliata*, although glands do form, they accumulate non-bitter essential oil.

The quadruple and quintuple hybrids [(*C. sinensis* × *P. trifoliata*) × (*C. reticulata* × *Fortunella*)] and [(*C. sinensis* × *P. trifoliata*) × (*C. reticulata* × *Fortunella*)] × *C. Dai-Dai* do not form glands in their juice sacs. Thus, in the presence of the kan-kan gene, the bitter essential-oil gene of trifoliolate orange can be completely or partially suppressed.

Interesting results were obtained from our digenomic hybrids, particularly *P. trifoliata* × *F. japonica*, *C. wilsonii* × *C. kinokuni*, and *C. ponensis* × *C. wilsonii*, which do not develop essential-oil glands in their juice sacs. Their formation is suppressed in both integrative and introgressive hybridization by taxa of *Fortunella* and by some small-fruited mandarins.

Sometimes the ability to form glands containing bitter essential oil appears in hybrids even though their parental forms lack it. For example, *C. junos* and *C. ichangensis*, introduced into the Soviet subtropics from America, do not form essential-oil glands in their juice sacs, but this property is restored when they are hybridized with cultivated taxa of *Citrus*. The hybrids *C. unshiu* × *C. junos* (506) and *C. unshiu* × *C. ichangensis* (508) form glands containing bitter essential oil in their juice sacs.

All this allows us to suppose that cultivated citrus arose through introgressive hybridization, as a result of suppression of gland formation characteristic of archaic citrus. The facts presented indicate that, during the evolution and formation of cultivated citrus, introgression, alongside mutation, played an important role, with genes of *Poncirus* and *Fortunella* repeatedly entering the genus *Citrus*.

Summarizing the material presented, the ability of members of the *Papeda* section of the subgenus *Archicitrus* to form glands in their juice sacs is suppressed by taxa of *Fortunella* and by some small-fruited forms of *C. reticulata*.

CONCLUSIONS

1. *P. trifoliata* and species of the Papeda section of the subgenus *Archicitrus* form essential-oil glands in their juice sacs, whereas these glands do not form in taxa of the subgenus *Metacitrus*.
2. Taxa of the Papeda section of the subgenus *Archicitrus*, as well as *Poncirus*, develop essential-oil glands both in the flavedo and in the juice sacs, where bitter essential oil accumulates. This property is inherited in hybridization, making their fruits unsuitable for fresh consumption.
3. Genes of *Fortunella* possess a suppressing effect with respect to genes of *Poncirus*. In hybrids between *Fortunella* and Citrange, essential-oil glands do not form in the juice sacs; in other hybrids, although glands develop, they accumulate non-bitter essential oil.
4. Formation of essential-oil glands in representatives of the Papeda section can, in addition to taxa of *Fortunella*, also be suppressed by certain cultivated citrus taxa, including *C. kinokuni* and *C. ponensis*.