EESTI NSV TEADUSTE AKADEEMIA TOIMETISED. XVIII KÖIDE
BIOLOOGIA. 1969, nr. 3

ИЗВЕСТИЯ АКАДЕМИИ НАУК ЭСТОНСКОЙ ССР. ТОМ XVIII
БИОЛОГИЯ. 1969, № 3

https://doi.org/10.3176/biol.1969.3.14

A. TIITS

STUDIES ON THE ETIOLOGY AND PATHOLOGY OF THE BLACK-CURRANT REVERSION

II. Preliminary observations on the growth of infected plant floral organs *in vitro*

A. TIITS. UURIMISI MUSTASÕSTRAREVERSIOONI ETIOLOOGIA JA PATOLOOGIA ALALT
II. Esialgseid tähelepanekuid nakatatud taime õisorganite kasvust *in vitro*

А. ТИЙТС. ИЗУЧЕНИЕ ЭТИОЛОГИИ И ПАТОЛОГИИ РЕВЕРСИИ ЧЕРНОЙ СМОРОДИНЫ
II. Предварительные наблюдения над ростом цветковых органов зараженных растений *in vitro*

It is well known that the reversion-diseased black-currant bushes carry reverted (mostly sterile or due to abnormality unfertilized) flowers a long time before dropping. The unfertilized normal flowers, however, drop immediately at the end of the flowering time. This fact gave rise to an idea to work with floral organ culture, to study this particular matter.

The first attempt to cultivate normal and reverted flower buds and flowers of *Ribes nigrum* L. *in vitro* was made at the Institute of Experimental Biology of the Academy of Sciences of the Estonian SSR in the spring of 1968. Later on, flower primordia were investigated there as well.

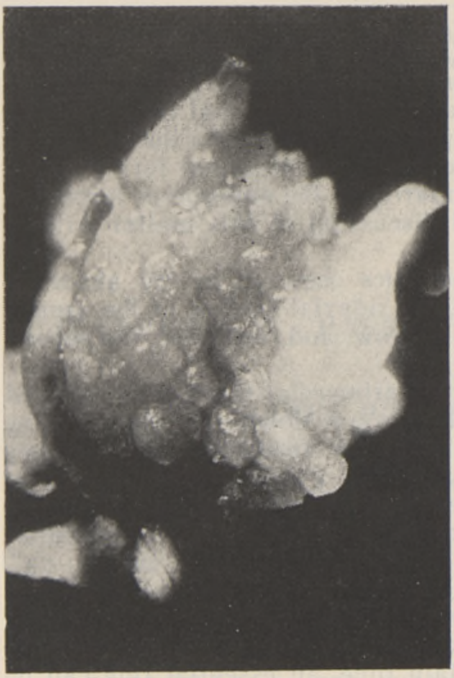
Methods

Flower buds and flowers sterilized in a solution of mercuric bichloride (in spring, 1968) or flower primordia (primordial racemes; in October-November, 1968) of healthy and reverted 'Goliath' and 'Boskoop Giant' black currant were placed on an agar medium in standard 100×20 cm Petri dishes. In writer's previous tests, black-currant callus grew more intensively on the Murashige's and Skoog's (1962) medium, whereas this medium was used in case of floral organ culture (only the media for the flower primordia culture contain hormones as follows: a) $NAA_{1.0} : K_{0.05} : GA_{2.0}$; b) $NAA_{0.0} : K_{0.05} : GA_{2.0}$; c) $NAA_{1.0} : K_{0.0} : GA_{2.0}$; d) $NAA_{1.0} : K_{0.05} : GA_{0.0}$; e) $NAA_{0.5} : K_{0.0} : GA_{0.0}$ *.

Results and discussion

Provisionally, the flower buds and flowers carried on agar medium behaved *in vitro* as it is common *in situ*: the unfertilized normal flowers died at the end of the flowering time, but the reverted ones stayed alive, and, after 2—3 weeks, their ovaries were swelled, as it is usual during

* Abbreviations: NAA, α-naphthalene acetic acid; K, kinetin; GA, gibberellic acid. The numbers indicate the concentrations of mg/l. The "a" ... "e" indicate the used media.



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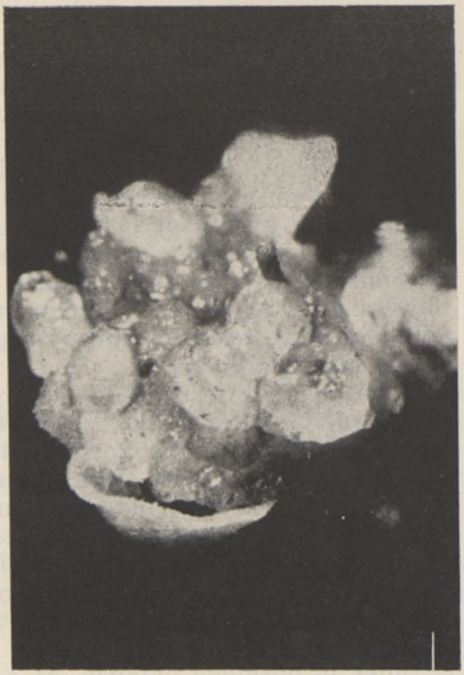

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Fig. 1 and 2. Ovaries of reverted black-currant flowers growing on Murashige-Skoog's medium, burst, the inside revealing numerous seed-like bodies.

the formation of berries. Later the ovary of the growing *in vitro* reverted flower burst, the inside revealing numerous seed-like bodies (see Figs). The seed-like formations grew at approximately the same speed as the seeds develop in normal berries *in situ*. Although the similarity was very great, a detailed examination indicated that the seeds growing *in vitro* did not have any embryos, containing endosperm tissue only. In addition to this fact, it can be mentioned that, according to the author's examination, many of the rarely developing berries of deeply reverted bushes have seeds without embryos as well.

It is known that a great concentration of auxins is very important for forming black-currant endosperm (Wright, 1956), and that giberellic acid is effective in inducing parthenocarpy in black currant (Zatykó, 1962). Obviously, the bearing of sterile reverted flowers a long time before the dropping and development of some flowers to berries is due to a greater concentration of these phytohormones than normal, and the particularly favourable nutrient condition furnished in media, in addition to it, can be a cause of the development of endosperm in all reverted flowers in culture. Because the endosperm of normal unfertilized flower did not grow in our study, it can be believed that unfertilized normal flowers need more auxin and as extra, supply of giberellic acid, in addition to the original Murashige-Skoog's medium. But it is possible that the mentioned phenomenon accompanies the reverted flowers only.

The discussed case of development of endosperm is a very interesting fact in connection with our previous knowledge of the plant reproduction physiology of angiosperms. According to complex cytological make-up with 3n nuclei of endosperm in normal organ, the origin of endosperm has commonly been assumed to be the result of a gametophytic union of a two female nuclei and one male (Eames, 1961). The above-mentioned fact is not in accordance with this assumption, and there might be another way yet. Perhaps, this is another characteristic reversional quality of the floral organs of diseased black-currant plants in addition to the previously discussed ones (Tiits, 1969). In this case, the exceptional formation of endosperm can be interpreted as a similarity to the endosperm formation occurring in gymnosperms. A detailed discussion of this matter will be possible after further investigations.

Some other changes of lesser importance occurred in the abnormal flowers growing *in vitro*. For example, the adaxial surface of the sepals-petals of some reverted flowers began to grow, and, due to it, the sepals-petals curled outwards.

There were differences in the growth of primordial racemes of reverted and normal bushes *in vitro* as well. The normal flower primordia grew inconsiderably on agar medium lacking auxin (medium "b"), or they remained in the initial state. A nearly normal growth was observed in medium "a". The reverted ones, on the contrary, grew better on medium "b", showing some decrease in the changes of the changed flower primordia. However, on Murashige-Skoog's original medium and on the "a", some tendency was observed to form a mass of callus.

Taking into consideration the above-mentioned facts, the changes in the flowers of reversion-diseased black-currant bushes can be due to changes in hormone balance. At a certain time, an essential role can be performed by an increase in auxin, known as hormone, which can direct the flowers to the female form (see Hughes, 1968) and, as we know, are characteristic of reverted black-currant flowers (Tiits, 1969). It is expected that further work with the culture of diseased floral organs of black currant *in vitro* on media of different concentrations of hormones might bring clarity in these problems.

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Received
March 11, 1969