EFFECTS OF SOME INTERNAL AND EXTERNAL FACTORS ON THE EMBRYO AND SEEDLING DEVELOPMENT OF THE CHERRY

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SUMMARY

(1) It is shown that the correlation between earliness and seed viability, which is known to exist in American cherries, also occurs in European cherry varieties.
(2) In early varieties, which need 40 to 60 days to ripen their fruit, embryo culture proved to be an effective means of increasing germination. In late varieties, showing germination percentages of 50 or more after normal stratification, application of the embryo method is of no advantage.
(3) In artificial culture, excised embryos will germinate without after-ripening. Therefore, in all varieties a shortening of the breeding cycle by one year may be expected if this method is applied. It is still too early to draw definite conclusions from our experiments, but experiences of other authors on this point are promising. It must be kept in mind that the use of the embryo method for this purpose, probably means that germination will remain below 50 per cent.
(4) The results of the embryo method are sometimes impaired by abnormal growth of seedlings derived from embryo cultures. Crippled seedlings show but very little growth, and are often so weak that a number of them die during cold treatment given after the first growing period. The unfavourable effect of this disorder may be eliminated to a large extent, by long-day treatment with light of adequate intensity.

INTRODUCTION

In plant breeding in general, an increasing use is being made of the artificial culture of embryos. In cherry breeding too, this technique has been used by several authors as a means of controlling germination.

At the request of and in collaboration with Ir. C. J. GERRITSEN, who is in charge of cherry breeding at our Institute, a series of experiments was started in 1951 to investigate the possibility of improving the breeding technique by the use of embryo cultures. Two considerations have mainly determined the general direction of this investigation.

(1) The seed of early cherry varieties does not germinate if stratified and sown in the usual way. Therefore, in crosses carried out with the object of producing new early varieties the mother variety has to be a late one. As by means of embryo culture viable seedlings may also be obtained from the seed of early varieties, this method makes it possible to carry out crossings between early varieties on a practical scale, and this means that breeding for earliness may be performed more effectively.
(2) The average age at which a cherry seedling flowers and bears fruit for the first time is 6 years. To breeders a reduction of this juvenile period would mean a saving of space as well as time. As excised embryos germinate without after-ripening, the

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breeding cycle may be shortened by one year if the embryo technique is practised. LAMMERTS [5] obtained earlier flowering of peaches by this method.

In the following report we shall describe some observations on the relation between seed viability and earliness, on embryo development in artificial culture, and the growth of seedlings derived from embryo cultures. In addition some improvements of the embryo method will be suggested.

Unless otherwise stated, our experiments have been carried out with the sweet varieties: Früheste der Mark (early), Early Rivers (early), Bastaarddikke (late), and with the sour varieties: Meikers (early) and Morel (late).

RELATION BETWEEN EARLINESS AND SEED VIABILITY

In his studies on fruit and seed development of American sweet and sour cherries, TUKEY [6, 7] has shown that between full bloom and fruit maturity 3 successive developmental stages may be distinguished: stage I – rapid growth of the pericarp; stage II – reduced pericarp growth and accelerated growth of nucellus, endosperm and embryo; stage III – renewed growth of the pericarp.

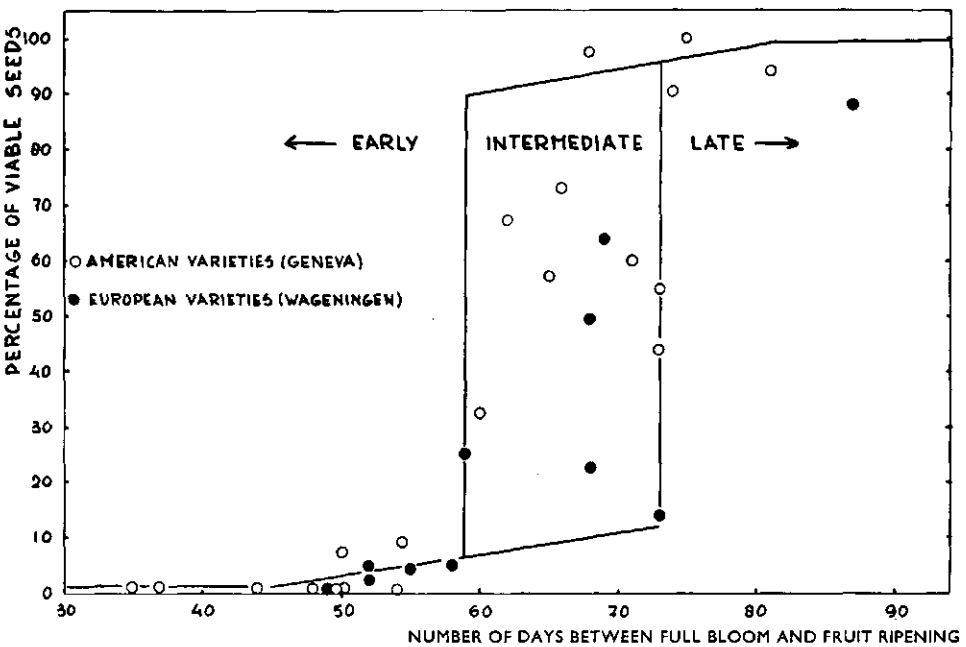

FIG. 1

The difference between a late and an early variety lies mainly in the duration of stage II. In a late variety the embryo is full-grown before the renewed pericarp growth of stage III begins. In an early variety embryo development is not yet completed at the beginning of stage III. A progressive disintegration of nucellus and endosperm may be

observed at this time, and the development of the embryo comes to a standstill. These under-developed embryos remain alive for some time, but they die during the after-ripening period. If removed from the fruits and cultured artificially, such immature embryos can often be made to develop into normal seedlings.
On the basis of the relation between ripening period and seed viability, TUKEY was able to divide American cherry varieties into 3 groups. From data collected by TUKEY at Geneva, New York, [6] the following characteristics of these 3 groups for the sweet cherry (*Prunus avium*) may be given:

a) Early varieties, ripening period shorter than 60 days; germination percentage very low or zero.
b) Mid-season varieties, ripening period from 60–75 days, germination percentage widely varying.
c) Late varieties, ripening period longer than 75 days, germination percentage practically 100.

In the graph of figure 1 this classification is shown, together with the data on 17 American sweet cherry varieties. For comparison, data on 11 European sweet cherries, which are commercially grown in the Netherlands, have been included in the graph. The information concerning the Dutch varieties was kindly supplied by Mr. C. J. GERRITSEN.
Figure 1 shows that the classification proposed by TUKEY applies equally well to the American and the Dutch varieties, which means that the same relation between earliness and seed viability exists in both groups of varieties. This led us to assume that the embryo technique developed by TUKEY for American cherries might be expected to produce similar results in our own varieties. In our work we have, therefore, followed the directions given by this author as much as possible.

MATERIAL AND METHODS

Unless otherwise stated, the embryos were prepared for culture as soon as the fruits had been harvested. The fleshy pericarp was removed, and the stones were washed and dried afterwards between sheets of filter paper. The stony pericarp was then cracked and the seeds soaked in tap water for some hours. After removal of seed coat and endosperm, the embryos were disinfected by immersing them for 5 minutes in a calcium hypochlorite solution prepared according to WILSON's formula [9]. In transferring the embryos from the disinfecting fluid to the cotton-plugged culture tubes containing 10 ml of agar medium, the regular microbiological technique was followed. The growing medium was prepared in the manner indicated by TUKEY [8] and sterilized in an autoclave. In addition to the necessary mineral salts and 0.7 % of agar, it contained 0.5 % of saccharose. The cultures were kept in a greenhouse at a constant temperature of 20 °C, and were shaded on sunny days.
When the embryos had produced roots and 3 true leaves, they were transplanted into a light soil on a greenhouse bench. The first week after transplanting the seedlings were covered with glass to prevent wilting. As soon as they resumed growth, they were potted and kept in the greenhouse at 20 °C.

GERMINATION OF EMBRYOS IN ARTIFICIAL CULTURE

Whether improvement of germination or shortening of the breeding cycle is intended, the value of the embryo method will be determined largely by its output of well-developed seedlings. Concerning the results obtained with embryo culture in cherries, the literature gives very little exact information. The germination percentages observed in our first few experiments, and those obtained by other authors [1, 4] are definitely lower than the figures reported for other stone fruits, such as peaches [5].

With the object of improving the germination of cherry embryos, we have studied the effect of a number of environmental and internal factors on the ultimate production of well-developed seedlings. The experimental results referring to the two successive phases, i.e. germination of embryos in the culture vessels and the growth of seedlings after they have been transplanted into soil, will be treated separately in this section and the next.

The outcome of the experiments dealing with embryo germination may be summarized as follows:

1. *Growing solution*

   Variations in the relative amounts of mineral salts, in the total salt concentration, and in the pH of the growing solution, proved to have no effect on embryo germination. The same was true for the addition of micro-nutrients, and of the vitamins thiamin (B_1), pyridoxin (B_6) or nicotinic acid (from the B_2-complex).

2. *Substrate*

   Replacement of agar as a substrate, by glasswool or filter paper, did not change the germination percentage significantly.

   Our experiences mentioned above are in agreement with those of TUKEY [8], who found that development of cherry embryos is rather insensitive to variations in the composition of the culture medium.

3. *Daylength*

   Embryo germination appeared to be independent of day length. This was established by comparing two sets of cultures, of which one received 8 hours of natural daylight, and the other a 16-hour day. This long day was given by prolonging the natural days of August until October with artificial light of 1500 lux from Philips HPL mercury vapour lamps.

4. *After-ripening*

   Embryos from early varieties showed hardly any response to after-ripening. In the case of late varieties, however, the germination percentage could be doubled by storing the culture vessels with excised embryos at a temperature of 5°C for 6 weeks. Fully-developed embryos are normally in a dormant condition, which is broken when they are exposed to a low temperature. Probably the immature embryos of the early varieties are checked in their development before they become dormant.

After-ripening may be considered an effective means of improving the germination of mature embryos, but the loss of time resulting from its application may be a disadvantage if a shortening of the breeding cycle is aimed at.

5. *Saccharose concentration*

Embryo germination proved to be affected not only by low temperature, but also by another external factor, i.e. by the sugar content of the growing solution.
It was already found by TUKEY [8] that the presence of sugar is essential for the development of very young embryos, but that fully-developed embryos grow better without it.

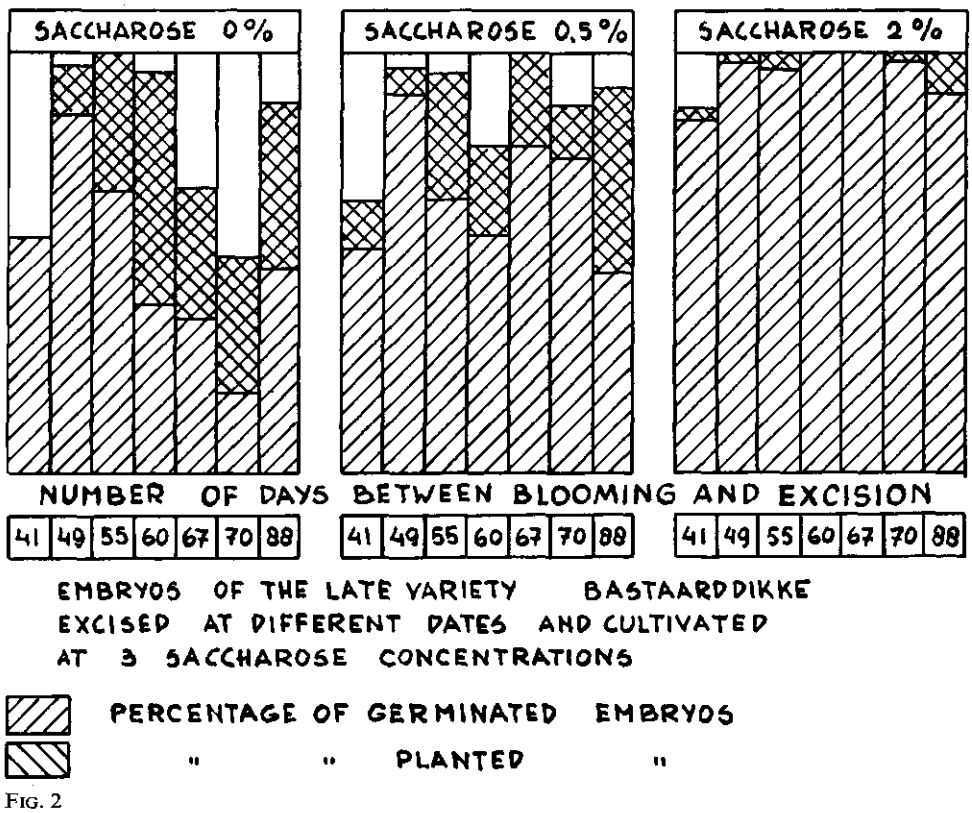

FIG. 2

From our experiments, it was established that saccharose stimulates and accelerates the early stages of germination in embryos of all ages. However, except in very young embryos, germination at its later stages is markedly slowed down or even arrested by saccharose. These two actions of saccharose are more pronounced when 2 % is used than with 0.5 %.
These facts suggest that also in cultures of mature embryos the addition of sugar may be advantageous if the harmful effect on the later stages of germination can be eliminated. In our opinion this might be achieved by treatment of freshly excised

embryos with a solution of saccharose, and subsequent cultivation on a sugar-free medium.

The effect of saccharose on embryo germination, described above, is illustrated by the graphs of figure 2. This figure gives the results of an experiment, in which embryos of the late sweet cherry variety Bastaarddikke were excised on seven different dates, and grown on media containing 0, 0.5 and 2 % saccharose respectively. Each lot consisted of 50 embryos.

6. *Age of the embryo*

The data given in figure 2 also demonstrate the relation existing between embryo germination and a combination of internal factors determined by the developmental stage of the embryo.

This relation has some interesting aspects. Between the ages of 41 and 60 days, the output of transplantable seedlings rapidly reaches a maximum; then it drops and a second maximum is reached after 88 days, when the fruit is ripe. Our attempts to grow seedlings from embryos of 35 days were unsuccessful. The first rise in the curve beginning at 41 days may be ascribed to the progressive development of the embryos, which makes them more suitable for culture. It is known, that at the age of about 50 days, sweet cherry embryos attain their maximum length. Probably they enter the dormant condition shortly afterwards, and it seems plausible to assume that this causes the embryos of 60 days and older to germinate less readily. That a second maximum occurs at the moment of fruit maturity is quite remarkable and deserves further study.

Growth of seedlings obtained by embryo culture

The fact that seedlings obtained from artificially cultured embryos often show an abnormal and stunted growth is well known. In fruit crops this phenomenon has been frequently reported in connection with embryo culture [2, 3, 8]. In our cultures also, some of the seedlings were always affected.

These abnormal seedlings are characterized by broad and crinkled leaves, which often show white patches or streaks ('elephant ears'). The very short internodes give these plants a dwarfed appearance ('rosette plants'). They remain small, because the terminal growing point always aborts. The majority of such crippled seedlings recover by producing normal shoots from axillary buds after exposure to a temperature of 5°C for 6 weeks. Incomplete breaking of dormancy of the embryos is generally accepted as the cause of this abnormal growth. The following observations made in our experiments, however, render the correctness of this assumption doubtful.

We found that abnormal seedlings were not only produced by mature embryos but also by very young ones, which could hardly be supposed to be in a condition of dormancy. Sometimes abnormal leaves and abortion of the terminal meristem occurred in seedlings that had previously shown normal growth for a considerable time. In these cases internodal length remained normal. It also proved possible to produce seedlings with short internodes but with normal leaves and a functioning terminal growing point, by giving an 8-hour day to normal seedlings.

These facts suggest that under certain conditions the shortened internodes on the one hand and the leaf distortions, and abortion of the terminal bud on the other, may manifest themselves as separate phenomena, and that partial dormancy of the embryo cannot be the only cause of the crippled condition.

Our attempts to reduce the number of abnormal seedlings by long-day treatment had no success. Seedlings, which were given a 16-hour day by prolonging the natural day with light of 2000 Lux from Philips HO 2000 mercury vapour lamps, produced the same percentage of cripples as those receiving only the natural day length prevailing in the period between 1 September and 1 November.

Nevertheless, a long day proved to have a favourable effect on seedling production, because crippled seedlings recovered by the production of normal axillary shoots just as after cold treatment, and in normal seedlings termination of growth was postponed. Thus it appears that the arrested growth of abnormal seedlings is not inherent in the crippled condition. Probably it results from the onset of dormancy under the influence of shortening day length in autumn, before axillary buds can take over the function of the aborted terminal bud. FLEMION [2] could not prevent the production of 'rosette' plants from non-after-ripened peach embryos in autumn, by giving an artificial long day. However, the fact that such embryos produced normal seedlings in the longer days of spring is worth noticing.

Long-day treatment of seedlings, given from the moment they are transferred from the culture vessels to soil until they stop growing in November, is now part of the routine of our embryo technique. In this way larger and stronger plants are obtained that withstand the subsequent cold storage better than untreated seedlings.

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DISCUSSION

After-ripening period

According to the literature on this subject, an after-ripening period of 6 weeks is sufficient to break the dormancy of excised cherry embryos. In our experiments the embryos were always after-ripened at 5°C continuously for 6 weeks.

Mr. S. PAUNOVIĆ (Yugoslavia) records that if he kept seeds of cherries at a constant temperature of 5°C without any break, abnormal seedlings were produced next spring, but if cold storage at 5°C was interrupted by a period in which the temperature was 0.5°C, normal plants were obtained.
This was the same with peach and *Prunus mahaleb* (D. STANKOVIĆ, Yugoslavia).

The disinfection of the embryos
The embryos were always disinfected with a calcium hypochlorite solution as indicated by WILSON and TUKEY. In the first growth period only, the embryos planted out in the greenhouse showed abnormalities. Outdoors the growth was normal in the succeeding year. The oldest plants obtained by embryo culture are now 4 years old. (M. ZWINTZSCHER, Germany).
Dr. ZWINTZSCHER remarked, that the abnormalities obtained and which remain during further years, may be attributed to mutations induced by the disinfectant

Practical interest
Embryo-culture raises the number of plants obtained from seed of early varieties, but from late-ripening varieties the normal sowing gives more seedlings.
Although there are no flowering plants so far, one may may assume that it is possible to gain a year by embryo culture, since by this method seedlings are obtained in the year of seed harvest (H. KARNATZ, Germany).