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Germination of embryos from stratified and non-stratified seeds and growth of apple seedlings (*Malus domestica* Borkh cv. "Antonówka")

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Abstract

The germination of whole seeds, the seeds without coat and isolated embryos of apple cv. "Antonówka Zwykła" after 90 days of cold-stratification was compared with the germination of embryos isolated from non-stratified seeds. They were germinated under 16hrs during a day at temperature 25° C and 20° C during the night. It has been found that after 2 weeks whole stratified seeds germinated in 5 per cent, seeds without coat in 25 per cent and isolated embryos in 98 per cent. Isolated embryos from non-stratified seeds, after 2 weeks, germinated in the range from 75 to 88 per cent. The results indicate the similar germination ability of embryos isolated from non-stratified seeds. The seedling populations obtained from embryos stratified and non-stratified seeds were fully comparable and they evaluated: 1) a wide range of individual differences within population, 2) a similar number of seedlings in each class of shoot length, 3) a similar morphological habitus in each class of shoot length, 4) a similar fresh leaf weight and whole plant increment.

INTRODUCTION

Our earlier results (Czerski and Jankowska 1977), showing the lack of embryonal dormancy of seeds of apple-tree cv. "Antonówka" were in contradiction with the observations of the other authors (Nikolaeva 1967, Maciejewska and Maleszewski 1976, Lewak and Rudnicki 1977, Wyzińska and Lewak 1978). It has been found that high percentage (60-85) of embryos isolated from non-stratified seeds germinated in 10 days culture, and the seedlings obtained, were fully comparable to those derived from embryos after 90 days of cold-stratification of seeds.

MATERIAL AND METHODS

Experiments were carried out in September 1978 and July 1979, with the seeds of apple cv. "Antonówka Zwykła" harvested in 1977 and 1978. The seeds were received from the Garden Plant Culture Kurów, and stored in the linen bags under laboratory conditions. The seeds were stratified at 4° C, during 90 days in perforated polyethylene bags, containing 1 kg of roasted sand, of 60 per cent humidity. The germination was carried out on 10 cm diametre Petri dishes. To each dish thirty stratified, or non-stratified seeds, or suitable prepared embryos were placed, on filter paper moistened with 5 ml of distilled water. They were placed in light at 16 hour photoperiod. The light source was 40 W fluorescent lamps Flora and Daylight (1 : 1), light intensity was 10 Klux. The temperature changed from 25° C in the light to 20° C in darkness. The seeds or embryos were defined as germinated if they had at least the 3 mm long radicle. Only stratified embryos obtained from 90 days stratified seeds, showing none external symptoms of germination were examined.

The elongation growth of shoots of 14-weeks old seedlings obtained from stratified and non-stratified seeds, and fresh weight of leaves and whole seedlings were compared. Seedlings were growing on the mineral medium No 11 according to Bentley (1959) in 200 liters hydroponic containers.

In calculation of the seedlings and leaf weight, the standard deviation of the arythmetic mean was taken into account.

RESULTS AND DISCUSSION

Figure 1 presents the germination of embryos isolated from seeds harvested in 1978. On the 10th day of the culture 86 per cent embryos from stratified and 61 per cent from non-stratified seeds germinated. On the 14th day the difference between amount of germinated embryos from stratified and non-stratified seeds diminished to 11 per cent, and on the 20th day it did not exceded 1 per cent. It was observed a similar course of germination of embryos from non-stratified seeds, harvested in 1977 (Fig. 2).

Taking into consideration the opinion of Lewak and Rudnicki (1977) that the germination ability is the crucial criterion of the depth of embryonal dormancy, we did not confirm their results, but present data agree with our earlier observation (Czerski and Jankowska 1977). It is evident that the present results also deny the occurence of embryonal dormancy of seeds of apple cv. "Antonówka". Moreover the data of our experiments did not confirm the distinct differences in

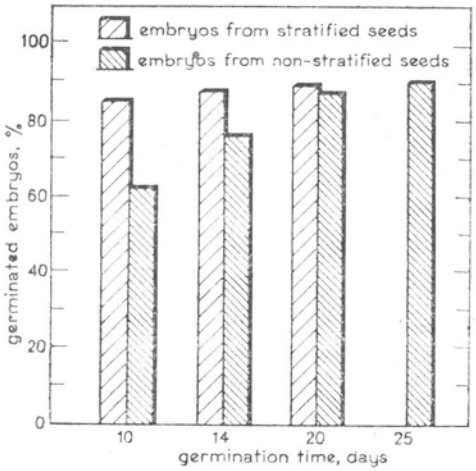

Fig. 1. Germination, at 25° C (day) and 20° C (night), of embryos isolated from stratified and non-stratified seeds harvested in 1978 (mean from 360 non-stratified and 390 stratified embryos). The seeds were stratified at 4° C for 90 days. Experiment was performed in 1979

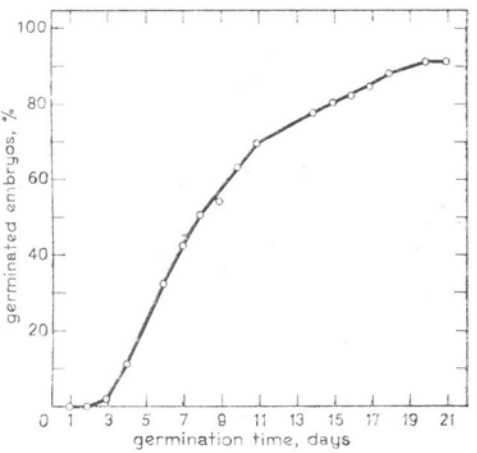

Fig. 2. Course of germination, at 25° C (day) and 20° C (night), of embryos from non-stratified seeds harvested in 1977 (mean from 150 embryos). Experiment was performed in 1978

dynamics of germination of embryos isolated from stratified and non-stratified seeds as reported by Lewak and Rudnicki (1977). We suppose, that described by these authors very rapid germination of embryos from stratified seeds, reaching 95-100 per cent on the 3rd day of the culture, may occur only when seeds already were in advanced stage of the germination. As an advanced stage of the germination we

consider not only the appearance of radicle but also the stage when under the pressure of elongation radicle the seed coat is just torn. Therefore, for our investigations on germination of stratified embryos, only intact seeds were selected as mentioned in Methods. The described procedure allows to obtain fully reproducible results. On the contrary when these authors used for germination seeds in different stage of embryo development, they obtained not reproducible results. For example Wyzińska and Lewak (1978) noted on the 3rd day of the culture 95 per cent germinated embryos from stratified seeds harvested in 1975, but only 30 per cent from harvest 1974. Such a different germination of stratified embryos could not be caused by qualitative differences in seed material harvested in different years. Namely, Maciejewska (1979) in the experiments on the germination of stratified embryos from the same batch of seeds harvested in 1974 and stored during several years, noted, after 3 days of the culture, the germination as large as 91 per cent. It should be stressed here that these seeds were stored under laboratory conditions in air-dry state at temperature not lower than 20° C. We suppose, that such great differences in germination rate, reported by mentioned authors, were due to not exact selection of the stratified seeds, from which the embryos were isolated. It can be assumed that these authors, for examinations of germination took the stratified seeds already germinated. It is known, that the population of seeds in the last period of stratification (90 days) differ in their physiological state, and there are: intact seeds, seeds with torn coat, seeds with different length of radicle, and even the seedlings several cm long. It is evident, that without proper selection of stratified seeds from the population consisting of seeds on different stage of development, the rate of germination of isolated embryos ought to be extremely different and accidental. Thus the differences in germination of stratified embryos could be explained on the basis their different degree of advancement of germination process, as mentioned above. However it is not understandable why the results on germination of non-stratified embryos, reported by Czerski and Jankowska (1977) and by Lewak and Rudnicki (1977) were different, although all authors were using the seeds from the same bag.

Figure 3 illustrates the state of dormancy of apple seeds harvested in 1978. It was found, that 10 per cent seeds germinated already on the 50th day of stratification, and 50 per cent on the last, 90th day. These results indicate a very wide differences in the state of dormancy of apple seeds. It was also reported by Suszka (1979).

The parallel course of germination curves of stratified and non-stratified seeds (Fig. 4) prove to a very similar germination pattern, but the distance between the both curves indicates unequal amounts of phy-

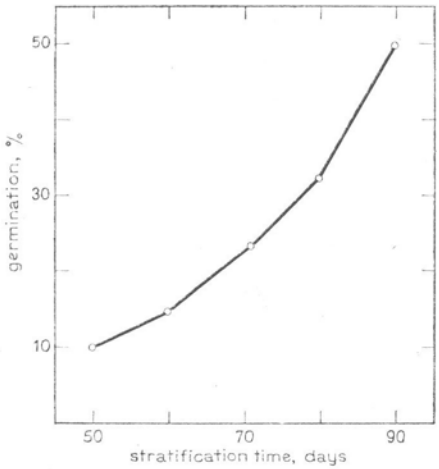

Fig. 3. Course of seed germination during stratification at 4° C. Seeds from 1978 harvest (mean from 1480 seeds). Experiment was performed in 1979

siologically active embryos in the seeds population, at the moment of starting the test for germination. An increase of growth activity of embryos from stratified seeds can be caused by the activation of enzymatic systems during the process of stratification (Lewak et al. 1975). Cytological and anatomical changes of embryos during seeds stratification (Dawidowicz-Grzegorzewska and Lewak 1977) also indicate their highly advanced development. It is evident therefore, that the comparison of embryos of non-stratified seeds after 12 hrs of imbibition, with the embryos from seeds after 90 days stratification (by these authors) is not acceptable for reasonable explanation of the difference in their rate of germination. So the conclusion that there are the essential differences between the germination dynamics of embryos from stratified and non-stratified seeds based on the first days of germination of embryos isolated from stratified seeds, without consideration of degree of their development is incorrect, for the same reasons as mentioned above. The earlier apperance of the other processes as chlorophyll synthesis and CO_2 assymilation in light of embryos isolated from stratified seeds (Maciejewska 1979) should not be compared with those from non-stratified ones.

It is known, in the gardening practice, that the apple seeds do not germinate at higher temperature. In spite of 90 days of stratification, also the whole intact seeds germinated poorly, as in 5 per cent after 14 days of culture (Fig. 4). Nikolaeva et al. (1960), Suszka (1967) and Kamiński (1971), poor germination of whole stratified seeds at higher temperature explain by the induction of the secondary dormancy of these seeds. Some increase of the embryos germination from strati-

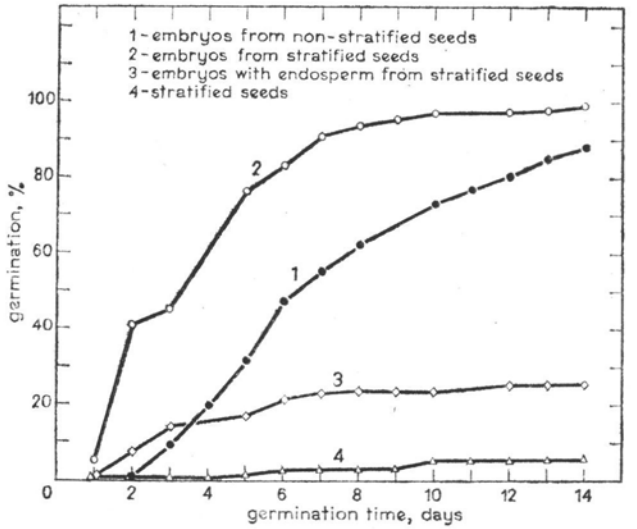

Fig. 4. Time course of germination of non-stratified seeds, embryos with and without endosperm and stratified seeds. In each experiment 150 seeds and 150 seeds without the coat, and 150 embryos isolated from stratified seeds and 240 embryos isolated from non-stratified seeds were used. The seeds were stratified at 4°C for 90 days. The seeds were harvested in 1978, and germination was tested in 1979

fied seeds can be obtained by removing of the seed coat, but it is still very little as compared to germination of the isolated embryos of these seeds. It is also seen (Fig. 3) the decissive role of endosperm as an inhibitory factor of seeds germination even after cold-stratification. Since the removing of this structure allows germination of embryos in 98 per cent as compared to only 25 per cent with an endosperm. The lack of growth of seedlings derived from non-stratified seeds is considerd as a second criterion of embryonal dormancy (Nikolaeva 1967, Lewak and Rudnicki 1977). Therefore in present study this criterion has been carefully examined. Percentage of plants in classes of shoot length, and the average of fresh weight of leaves and whole plants in each class, were determined. Populations consisted of 190 seedlings from embryos of non-stratified and 235 seedlings from stratified ones were tested. The seedlings from embryos of stratified and non-stratified seeds were divided into 22 classes, including shoot length in the range from 5 to 115 cm (Fig. 5). The habitus of the plants marked I-IV is shown on the photographs (Figs. 8 and 9). The results (Fig. 5) indicate the similar percentage of plants obtained from stratified and non-stratified seeds in all classes. The difference as large as 7 per cent, was noted in the class containing plants 40-45 cm length of shoot. Seven classes of plants up to 40 cm shoot length contained 60 per cent seedlings of

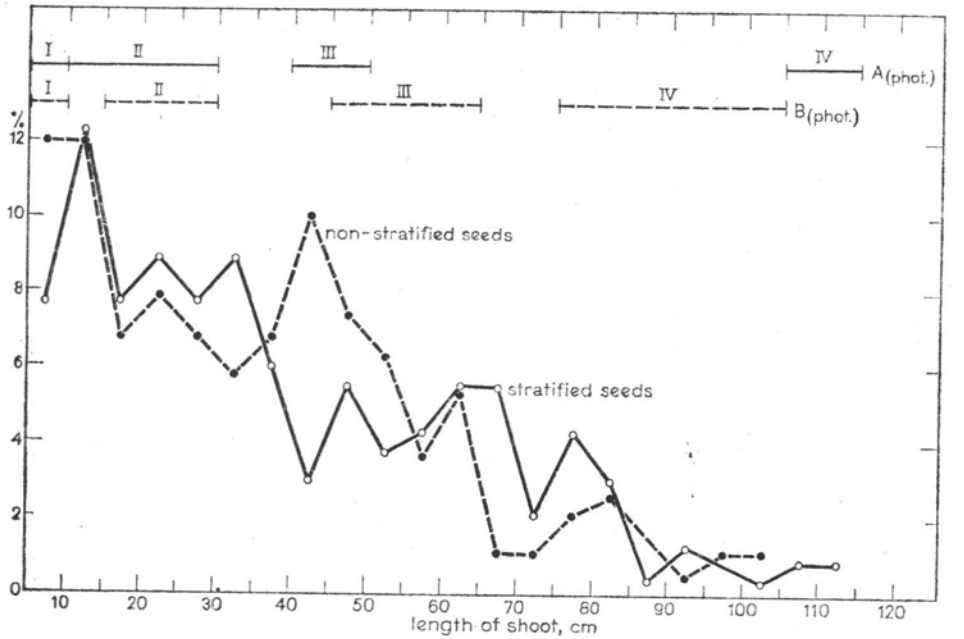

Fig. 5. Percentage of 14-week old seedlings derived from embryos stratified and non-stratified seeds, in the particular shoot length classes. Seeds were stratified at 4° C for 90 days

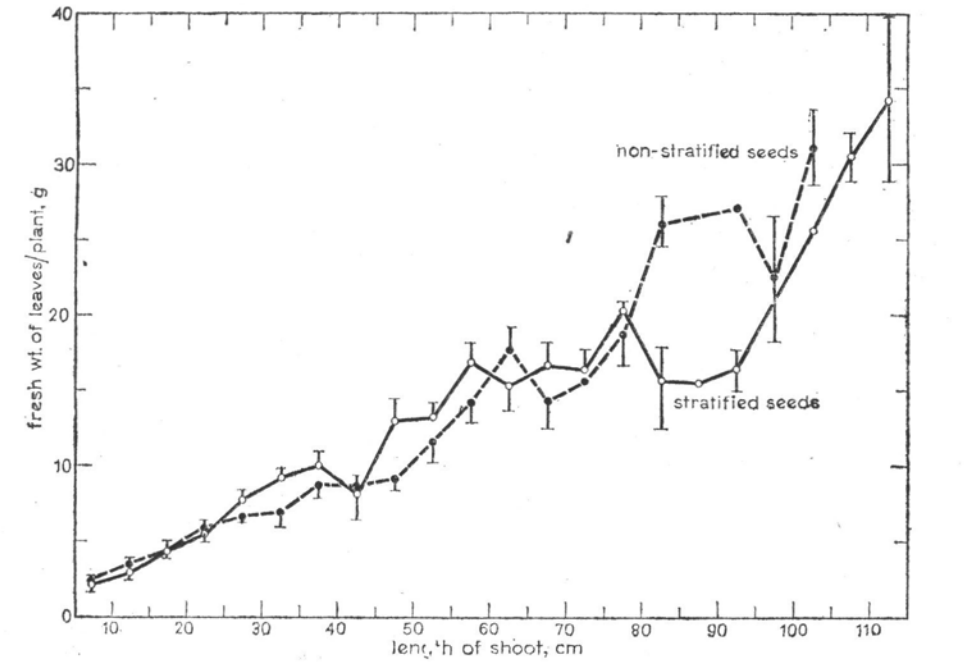

Fig. 6. Fresh weight of leaves of 14-week old seedlings derived from embryos of stratified and non-stratified seeds (mean for seedlings in the given class)

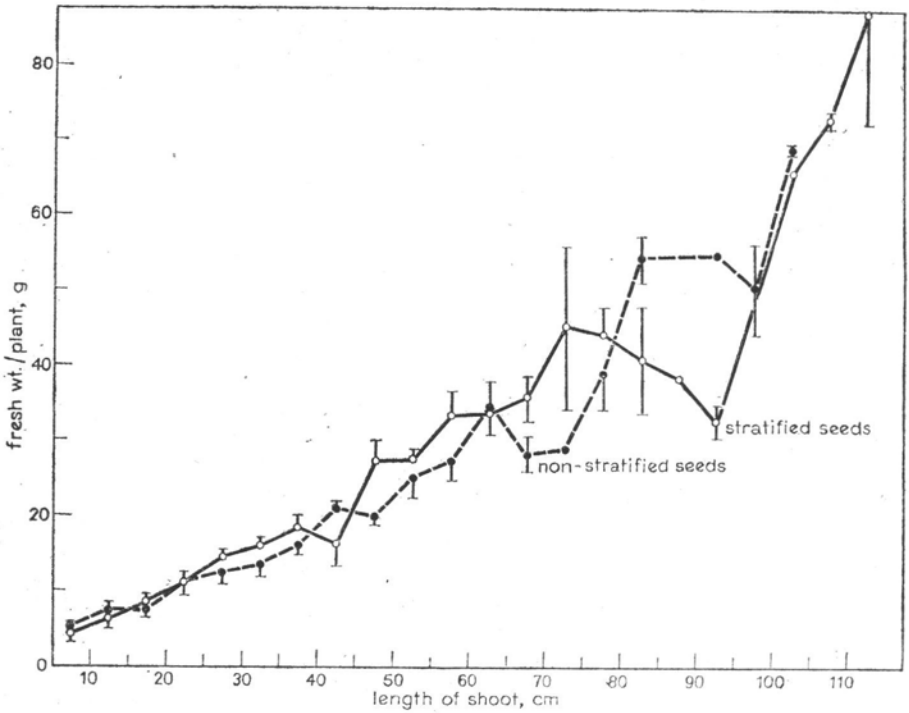

Fig. 7. Fresh weight of 14-week old seedlings derived from embryos of stratified and non-stratified seeds (mean for seedlings in the given class)

population, and the next fifteen classes contained remaining 40 per cent seedlings. A relatively numerous group of seedlings 5-10 cm high representing 7.7 per cent plants from stratified and 12 per cent from non-stratified seeds exhibited lack of growth and formation dwarf forms (Fig. 8, photo IA and IB). It was also noticed the wide differences in shoot elongation and fresh weight increment. The data presented in Figs. 6 and 7 show that the fresh weight of whole stratified plant as well as the leaf was 16-18 fold lower for the class 5-10 cm shoot length as compared to 110-115 cm. Similarily the seedlings from embryos of non-stratified seeds exhibited the great differences in these characteristics. The fresh weight of leaves and whole seedlings with shoot 5-10 cm long was 13-fold lower as compared to the seedlings with shoot length 100-115 cm.

Thus the results show an entire similarity in all examined characteristics of embryos and seedlings, obtained from stratified as well as non-stratified seeds. It present an additional evidence and also confirm

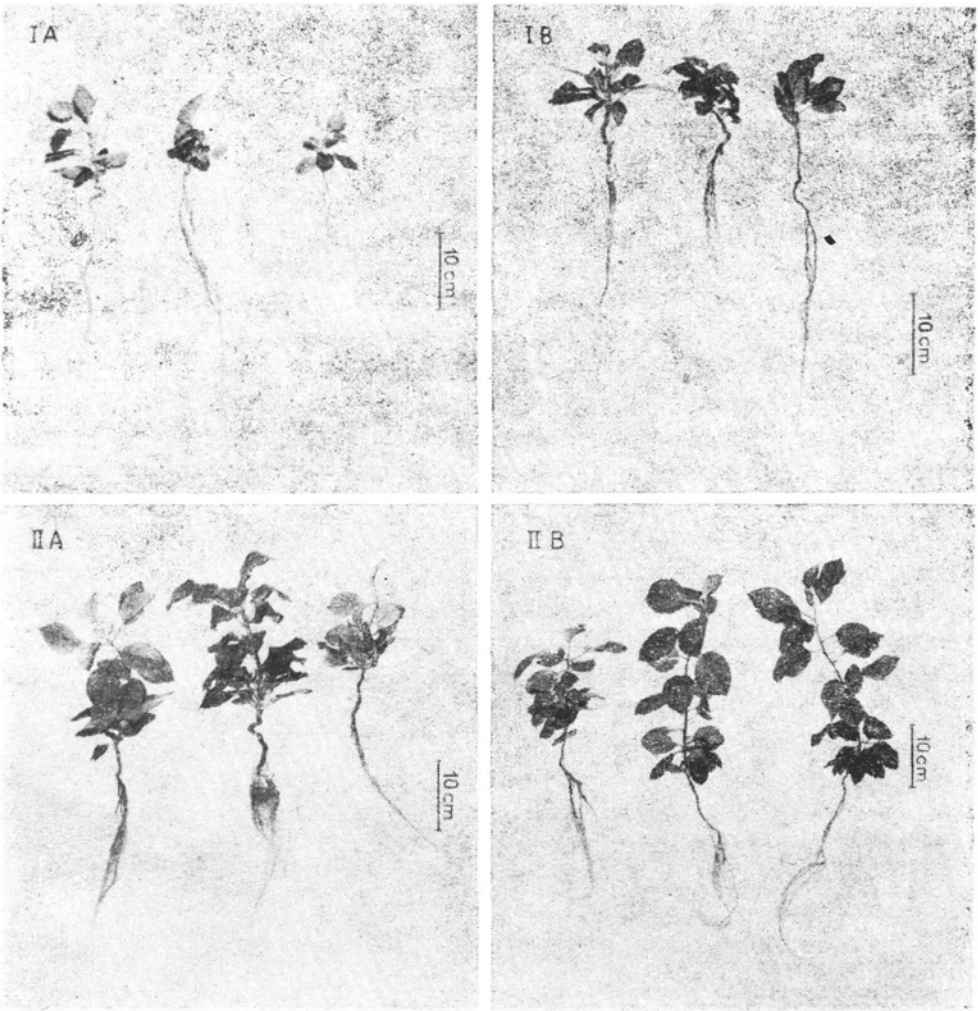

Fig. 8. Habitus of 14-week old seedlings obtained from embryos of stratified (IA and IIA) and non-stratified (IB and IIB) seeds. I and II — shoot lenght classes (see Fig. 5).

our earlier conclusions that seeds of apple cv. "Antonówka Zwykła" lack the embryonal dormancy.

According to S u s z k a (1979) the normal seedlings derived from dormant seeds of fruit trees can only be obtained after full germination of seeds during stratification. To test the validity of Suszka's assumption the next experiments were carried out and the results will be presented in the forthcoming paper.

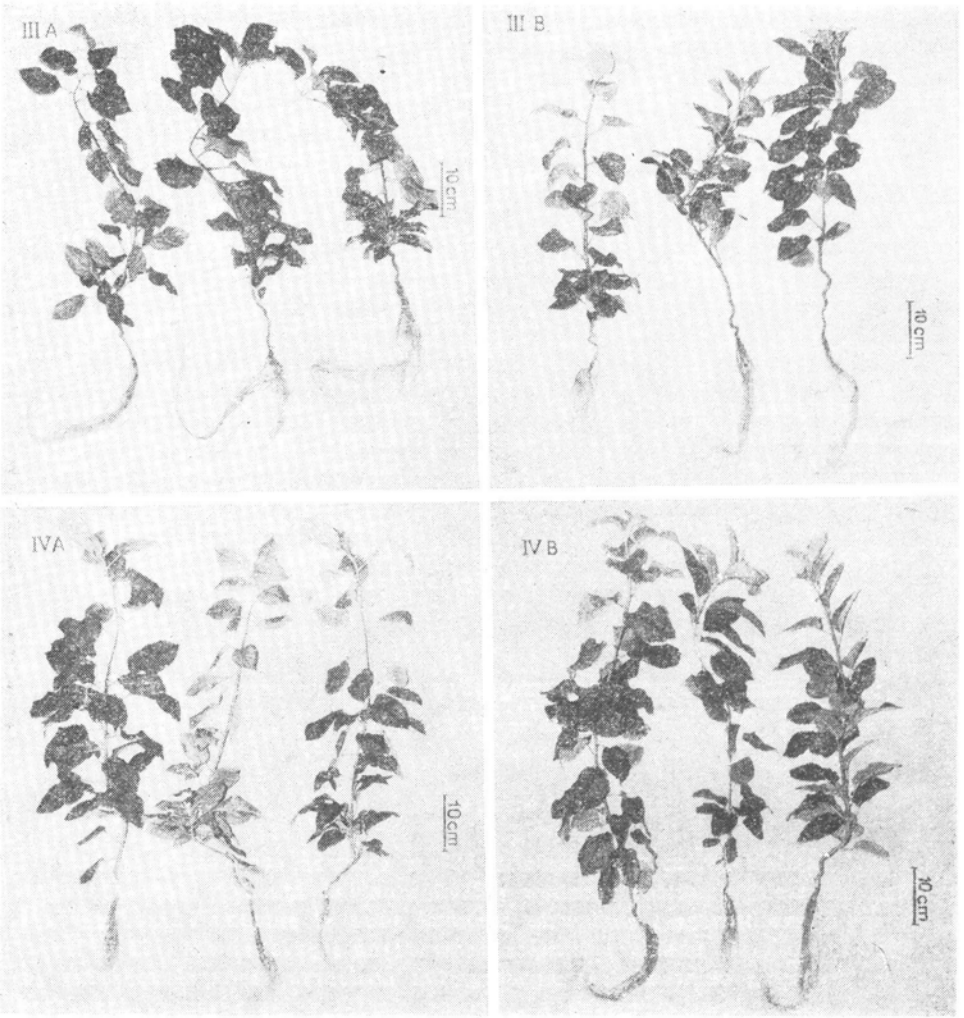

Fig. 9. Habitus of 14-week old seedlings obtained from embryos of stratified (IIIA and IVA) and non-stratified (IIIB and IVB) seeds. III and IV — shoot lenght classes (see Fig. 5)

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Kiełkowanie zarodków z stratyfikowanych i niestratyfikowanych nasion oraz wzrost siewek jabłoni (Malus domestica Borkh cv "Antonówka")

Streszczenie

Po 90-godzinnej stratyfikacji w 4° C, porównano kiełkowanie nasion w podwyższonej temperaturze i przy dostępie światła: zarodków niepozbawionych bielma oraz izolowanych zarodków, z kiełkowaniem zarodków izolowanych z nasion nie poddanych działaniu chłodu. Nasiona nie wykazujące objawów kiełkowania, przeniesione do temperatury 25° C, skiełkowały w ciągu 14 dni hodowli zaledwie w 5%. Usunięcie łupiny nasiennej spowodowało skiełkowanie 25% zarodków. Dopiero po usunięciu łupiny nasiennej i bielma uzyskano skiełkowanie zarodków z nasion stratyfikowanych w 98%. Otrzymane wyniki świadczą o podobnej zdolności do kiełkowania zarodków izolowanych z nasion niestratyfikowanych jak i stratyfikowanych przez 90 dni. Populacje siewek otrzymane z zarodków z nasion stratyfikowanych i niestratyfikowanych są w pełni porównywalne, to jest wykazują: 1) duży zakres zróżnicowania osobniczego w obrębie populacji, 2) podobną liczebność siewek w poszczególnych przedziałach klasowych długości pędu, 3) jednakowy pokrój morfologiczny w obrębie przedziałów klasowych długości pędu, 4) podobny przyrost świeżej masy liści i całych roślin.