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Morphological Aspects of Apple Seedling Early Development in Relation to Embryonal Dormancy

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Abstract. The growth of root, hypocotyl and cotyledons was measured in apple seedlings cultured for nine days from embryos isolated from seeds after various periods of after-ripening. Greening of the cotyledons was taken into account and the chlorophyll content determined as well.

Embryonal dormancy results in anomalies already appearing in the early stage of development. They are manifested by differentiated greening and uneven growth of cotyledons, as well as by hypotrophy of root or hypocotyl. The frequency of those anomalies in the population decreases during the removal of dormancy, and therefore it may be accepted as the criterion of its depth.

The mature apple seeds are in a state of deep dormancy which is removed by three months cold treatment (stratification). Embryos isolated from completely or partly dormant seeds are able to germinate, however, their germination is slow and results in many developmental anomalies (Flemion 1934, 1956, Nikolaeva 1967, Côme 1970, Paillard 1974). The best known are: dwarfism of seedlings (Flemion 1934, 1956), asymmetric growth of cotyledons (Côme 1970, Paillard 1974), perturbations in the appearance of photosynthetic apparatus (Maciejewska *et al.* 1974) or different character of metabolic activities (Lewak *et al.* 1975, Rychter *et al.* 1970, Tissaoui and Côme 1975). Although those anomalies regarding completely dormant seeds have been reported frequently and in detail, no attempt has been made to describe their frequency in partly dormant seeds isolated before the end of stratification.

The ability to germinate and the temperature range in which germination occurs are commonly accepted as criteria of the depth of dormancy (Vegis 1964). Therefore supplementing of those criteria with morphologic data concerning the early development of seedlings was also the aim of the present work.

Material and Methods

Observations and experiments were done on apple (*Malus domestica* Borb, cv. Antonówka) seedlings cultured from embryos isolated from seeds of 1974 and partly 1975 collections, obtained from the Experimental Station (ZNOS) at Kurów.

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The stratification was carried out at 4 °C as described previously (LEWAK *et al.* 1975).

Embryos isolated from seeds after various periods of stratification were grown on Petri dishes (diameter 9 cm) on paper discs moistened with 5 ml of distilled water. Lots of 30 embryos per dish were grown in 12 h day cycle at day temperature 22 °C and night temperature 20 °C. The total number of embryos per experiment varied from 90 to 480.

The following observations were made after 3, 6 and 9 d of culture of embryos isolated from seeds of 1974 collection:

I) percentage of germination;
II) area of greening of lower and upper cotyledons;
III) ratio between the surfaces of lower and upper cotyledons;
IV) changes in the length of the main roots;
V) changes in the length of hypocotyls.

Observations I and II were repeated on seedlings grown from embryos of 1975 collection. Changes in the total chlorophyll level were estimated in the cotyledons of seedlings cultured for 9 d.

Chlorophyll was assayed in the acetone extract from homogenized cotyledons according to SHLYK (1968), and the total amount of chlorophyll was expressed in μg, each result being the mean from triplicates.

The embryos with the root length exceeding 3 mm were considered as germinating.

The area of greening and the ratio between surfaces of cotyledons were evaluated visually.

The length of the main roots and hypocotyls was measured with 1 mm accuracy, and the results were submitted to statistical analysis.

The results of observations were usually expressed as the percent contributions of the particular seedlings in the total sample.

Results

1. Germination of Isolated Embryos

Data presented in Fig. 1 indicate the after-ripening of seeds from 1975 to occur more rapidly than that of seeds collected in 1974. Therefore the dormancy of 1975 seeds was less deep than that of the 1974 ones.

2. Changes in the Morphology of Cotyledons

2.1. The area of greening and the size of cotyledons.

Regarding the area of greening of the cotyledons, 4 types of seedlings were classified among the population grown from embryos isolated after various periods of stratification (Fig. 2).

Regarding the size ratio between both cotyledons 3 types of seedlings were classified in the population grown from embryos after various periods of stratification (Fig. 3).

Data presented in Fig. 4 indicate the distribution of the particular types of seedlings in 9-d old population to be related to the period of seed stratification.

In population cultured from dormant or shortly stratified seeds, seedlings with incomplete greening of both cotyledons were frequent (Fig. 4A). A shift towards the occurrence of seedlings with both fully green cotyledons — *i.e.* normally developing seedlings — appeared during the removal of dormancy. The suggestion of less deep dormancy of the seeds collected in 1975, mentioned previously in connection with the ability to germinate, was also

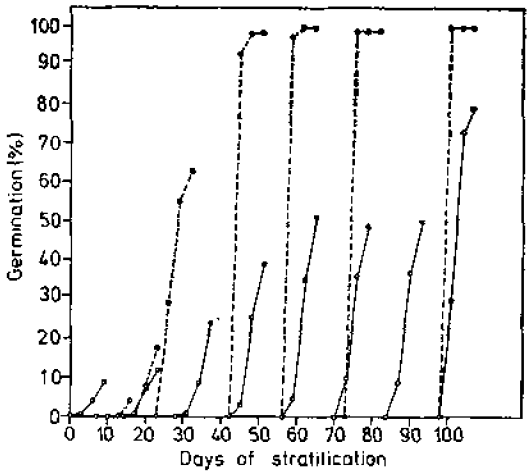

Fig. 1. Percentage of germination of apple embryos cultured for 3, 6 and 9 d; harvest (———) 1974, (— — —) 1975.

confirmed by the quicker complete greening of both cotyledons in the seedlings cultured from seeds collected in 1975.

Data concerning changes in the ratio of size between lower and upper cotyledons presented in Fig. 4B indicate the occurrence of c^1 type — overgrown lower cotyledon — decreasing during the stratification. In the seedlings with overgrown lower cotyledons usually only this one is green.

The morphological differentiation of seedlings after 9 d culture of embryos isolated after various periods of stratification results from gradual changes appearing along the culture. The trend of changes in greening and in the size ratio of cotyledons during the culture is the same, independently of the depth of embryonal dormancy. Changes during the culture of embryos isolated from seeds collected in 1974 and stratified for 56 d are presented as an example (Fig. 5).

2.2. Chlorophyll level in cotyledons.

Data from Fig. 4A and 4B implies that the embryonal dormancy influences the greening of cotyledons to a larger extent than it does its differentiation in size. Therefore the obtaining of more precise data obtained by measuring the chlorophyll content in the cotyledons of all types of seedlings was assumed to be purposeful.

Fig. 6 shows the amount of chlorophyll in cotyledons of 9 d old seedlings cultured from embryos isolated from seeds after various periods of stratification. The amount of chlorophyll was assayed in cotyledons of seedlings type b, c and d separately.

In seedlings with both cotyledons completely green (type d) the amount of chlorophyll increases gradually in the first period of stratification, reaching the maximum before all the embryos are able to germinate (Fig. 1) and previously to complete greening of both cotyledons in all the seedlings (Fig. 2, 1975). The total amount of chlorophyll per cotyledon in the seedlings of b and c types is much lower and does not increase along the removal of dormancy, in contrast to the d type. The chlorophyll level in the cotyledons is the same irrespectively of the embryos being isolated after longer periods of stratification. It may therefore be concluded that this period of removal of apple seed dormancy leads to full development of the photosynthetic apparatus under the condition of culture.

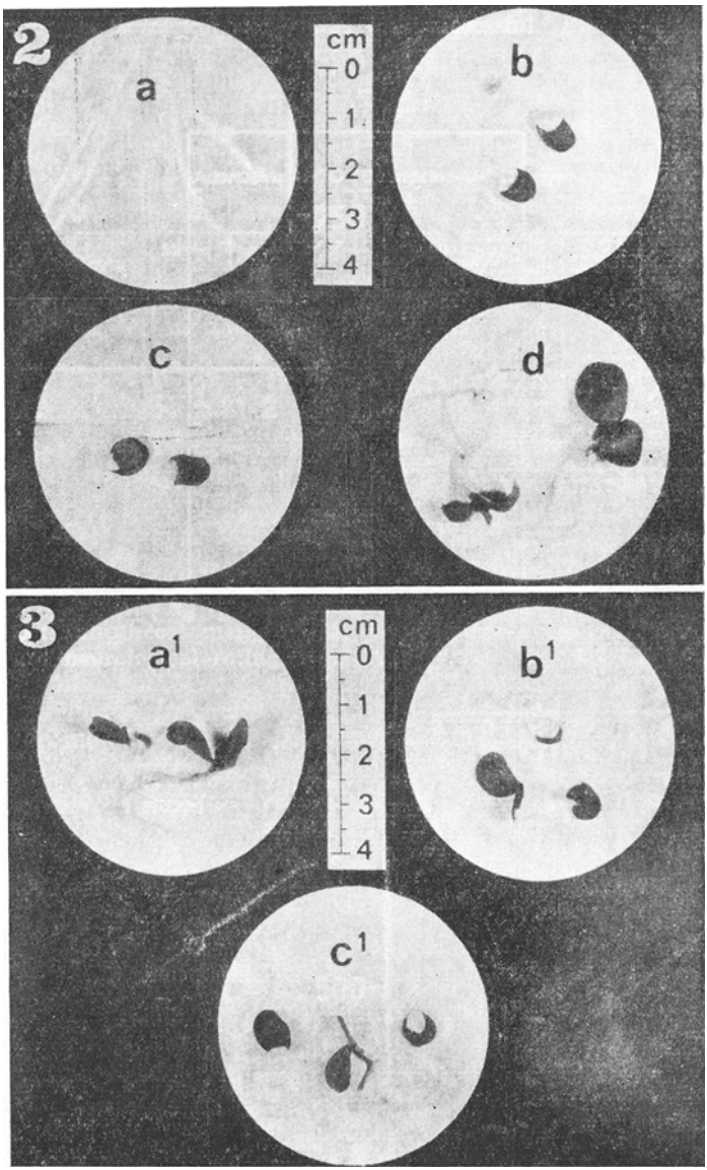

Fig. 2. Types of apple seedlings considering the area of greening of cotyledons: *a*. both cotyledons remained white; *b*. greening only of the lower cotyledon, the upper one remained white; *c*. greening on whole surface of the lower cotyledon and on the apical part of the upper one — the basal part of the surface of the upper cotyledon remained white; *d*. greening on the whole surface of both cotyledons.

Fig. 3. Types of apple seedlings considering the size ratio between both cotyledons: a^1, both cotyledons were of the same size; b^1, the lower cotyledon was less than twice larger than the upper one; c^1, the lower cotyledon was at least twice as large as the upper one.

3. The Changes in the Morphology of Embryonal Axis

Investigation of the embryonal axis growth was limited to measurement of its hypocotyl and main root elongation.

3.1. The growth of main roots and hypocotyls.

The data presented in Figs. 7 and 8 concern the changes in average length of main roots (Fig. 7) and hypocotyls (Fig. 8), in seedlings cultured for 3, 6

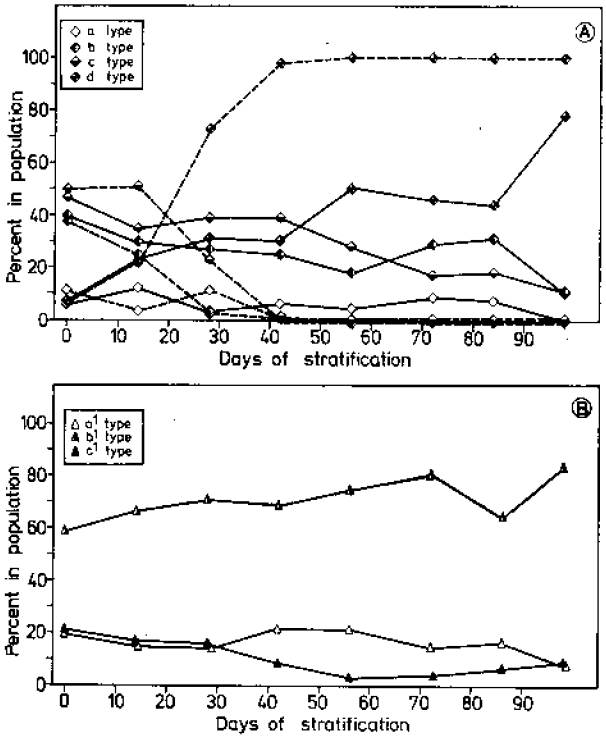

Fig. 4. The distribution of the types of apple seedlings in 9-d old population in relation to the period of seed stratification; harvest (———) 1974, (— — —) 1975. *A* — considering the area of greening of cotyledons; *a*. both cotyledons remained white; *b*. greening only of the lower cotyledon, the upper one remained white; *c*. greening on whole surface of the lower cotyledon and on the apical part of the upper one — the basal part of the surface of the upper cotyledon remained white; *d*. greening on the whole surface of both cotyledons; *B* — considering the size ratio between both cotyledons; a^1. both cotyledons were of the same size; b^1. the lower cotyledon was less than twice larger than the upper one; c^1. the lower cotyledon was at least twice as large as the upper one.

and 9 d from embryos isolated after various periods of stratification. Those data indicate that since the half of the stratification period more intensive growth of roots and hypocotyls is observed. The beginning of intensive growth of roots forestalls that of the hypocotyls.

The longer the period of stratification the greater the standard deviation in investigated samples, which implies the increasing differentiation of seedlings in relation to the growth of embryonal axis.

3.2. The distribution of main roots and hypocotyl occurrence in various length classes.

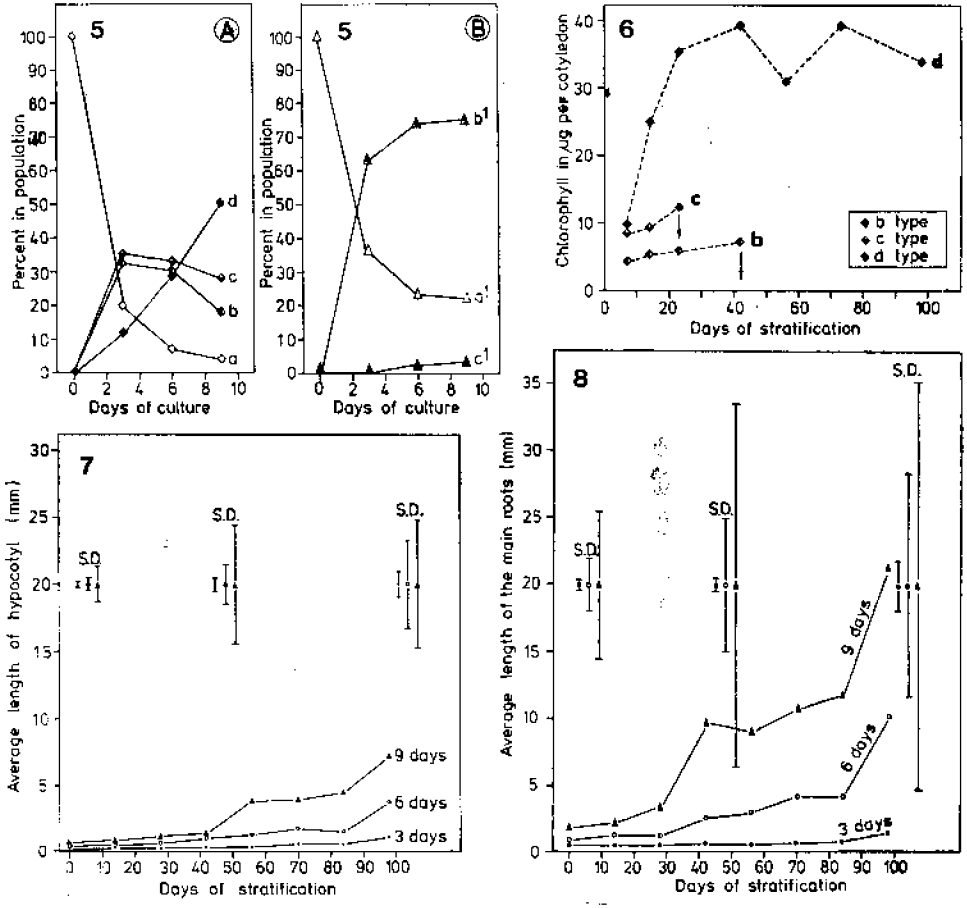

Fig. 5. The distribution of the types of apple seedling during the culture of embryos isolated from seeds collected in 1974 and stratified for 56 d: *A* — considering the area of greening of the cotyledons; *a*. both cotyledons remained white; *b*. greening only of the lower cotyledon, the upper one remained white; *c*. greening on whole surface of the lower cotyledon and of the apical part of the upper one — the basal part of the surface of the upper cotyledon remained white; *d*. greening on the whole surface of both cotyledons; *B* — considering the size ratio between both cotyledons; a^1. both cotyledons were of the same size; b^1. the lower cotyledon was less than twice larger than the upper one; c^1. the lower cotyledon was at least twice as large as the upper one.
Fig. 6. The amount of chlorophyll in cotyledons of 9-d old seedling cultured from embryos isolated from seeds collected in 1975 after various periods of stratification: *b*, *c*, *d*: (◆) in cotyledons of type *b*. *c*, *d* seedlings, respectively. ↓ The last term of occurrence of seedlings at a given type.
Fig. 7. The changes in average length of main roots in seedlings cultured for 3, 6 and 9-d from embryos isolated from seeds collected in 1974 after various periods of stratification.
Fig. 8. The changes in average length of hypocotyls in seedlings cultured for 3, 6 and 9-d from embryos isolated from seeds collected in 1974 after various periods of stratification.

The seedlings were classified according to the length of main roots and hypocotyls.

The classification was based on the assumption that the classes differed by 5 mm; the class of non-growing roots (ca. 1 mm) and the one of non-growing hypocotyls (ca. 0.5 mm) were separated. Classes that followed consisted of roots between 1 and 5 mm length and of hypocotyls between 0.5 mm and 5 mm length.

Owing to the remarkable differentiation of main roots and hypocotyls occurring after 9 d embryo culture, the analysis of distribution of main roots and hypocotyl occurrence in each class was carried out on the basis of individual measurements.

The curves illustrating distribution in particular classes of root length in 9 d old seedlings as related to the period of seed stratification (Fig. 9A) indicate the apple seed population to be inhomogeneous, consisting of 3 different physiological groups. This is evidenced by the 3 maxima in the

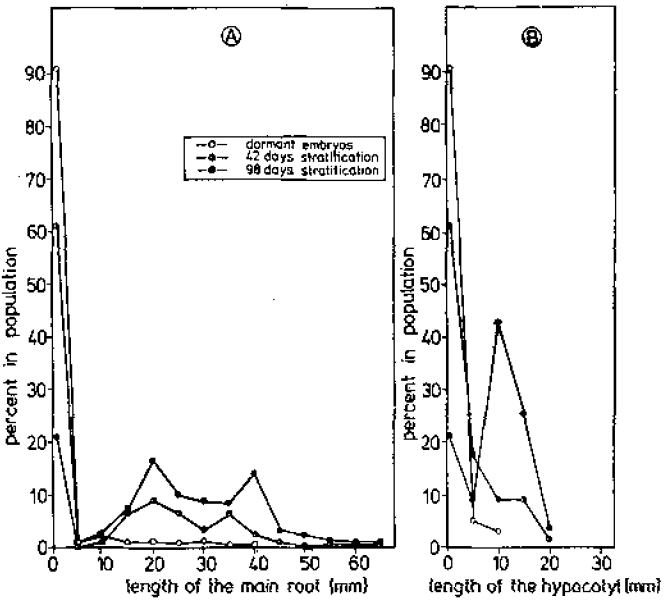

Fig. 9A. Distribution of frequency of main roots occurrence in various length classes: (○) dormant embryos; (◑) 42 d stratification; (●) 98 d stratification.
Fig. 9B. Distribution of frequency of hypocotyl occurrence in various length classes: (○) dormant embryos; (◑) 42 d stratification; ●) 98 d stratification.

curves presented for seedlings from partly and completely stratified seeds. Among seedlings from dormant seeds where the "i" type distribution of root length is apparent the group of deepest dormancy predominates. Along with increasing after-ripening the amount of those seeds decreases and seeds in less deep dormancy appear in the population, beginning to prevail.

The data presented in Fig. 9B indicate that the distribution of hypocotyl occurrence changes from that of the "i" type towards the normal one after complete stratification. According to the distribution the population can be divided into 2 physiological groups: fully dormant seeds and the various extents of non-dormant seeds.

Discussion

The comparison of data concerning various morphological elements of young seedlings cultured from apple embryos isolated from seeds after various periods of stratification implies that along with the after-ripening the population becomes more and more homogenoous. That could be traced from the distribution of several features similar to the normal one and the decreased number of seedlings with remarkable deformities.

The changes of some morphological elements converge with the changes of germination ability. Similarly to the latter full greening and the chlorophyll level change, too.

Data concerning the main roots and hypocotyl length are not to that extent unequivocal. Even in this case the relation between the depth of dormancy and the speed of roots and hypocotyls growth is evidenced.

Therefore some characteristic features of seedling development should be taken into account such as the embryonic dormancy criterion along with the ability to germinate. Cotyledon morphology is a convenient one; the growth of embryonal axis is a valid one but technically difficult to realize.

The results together with data presented by CÔME (1970), FLEMION (1934, 1956), NIKOLAEVA (1967), PAILLARD (1974) and other authors justify determination of the features characteristic of abnormal development of apple seedlings due to incomplete removal of dormancy. Those features are traceable in the early period of culture and are therefore already relevant. Abnormal development may be attributed to all the seedlings the cotyledons of which present remarkable differences both regarding the surface and the area of greening. Deformed seedlings can be obtained only by culture of isolated embryos.

Three stages of seed after-ripening were distinguished according to the ability to germinate and to the changes in hormonal equilibrium and in some metabolic activities (LEWAK *et al.* 1975). The obtained results allow some of the investigated morphological features to be divided into these 3 stages. Both the observation of cotyledons greening and data concerning the total chlorophyll content indicate that the previously described inhibition in the photosynthetic apparatus formation occurs in the first stage of after-ripening.

Acknowledgements

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