Growth of apple-tree seedlings (*Malus domestica*) Borkh cv. Antonówka obtained from non-stratified embryos

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Abstract

The growth of seedlings (*Malus domestica* Borkh cv. Antonówka) obtained from embryos isolated from non-stratified and stratified (90 days at 4–5°C) seeds was compared. The lack of differences in the germination power of embryos isolated from non stratified and stratified seeds, and the observed normal growth of plants obtained from non-stratified seeds, point to the fact, that the seeds of the autumn apple 'Antonówka' do not pass the stage of embryonal dormancy.

INTRODUCTION

Seeds of many plant species are not able to germinate without previous stratification (Crocker and Barton, 1953). The reason of this fact is the physiological underdevelopment of embryo which is in the state of so-called embryonal dormancy.

The seeds which pass the state of embryonal dormancy, according to many authors, are characterized by the following features:

1. Embryos isolated by removing the seed coat and endosperm are able to germinate in an insignificant degree only,
2. Seedlings obtained from isolated embryos form dwarf forms (Crocker, Barton, 1953); Chailikhian, Nekrasova, Klopenkova, Loznikova, 1963).

Deformation of cotyledon, observed by Come (1967) during the germination of apple embryos is also accepted by some authors as the symptom of embryonal dormancy (Sińska, Lewak, 1970; Maciejewska, Maleszewski, 1976).

The present investigations have explained if seeds of autumn apple 'Antonówka' can be included into the group of seeds characterized by the strong embryonal dormancy.

MATERIALS AND METHODS

Apple seeds (*Malus domestica* Borkh cv. Antonówka) were obtained from Z.N.O.S. in Sinołęka and Kurów. The germination power of embryos isolated from non-stratified and stratified seeds, and the dynamics of seedlings growth were studied during 1971—1977. The seeds were stratified during 90 days at the temperature 4—5°C in sterile quartz sand moistened with water to 60% of capillary water capacity (CWC).

The embryos from non-stratified seeds were isolated by removing the seed coat and endosperm from seeds previously soaked in distilled water during 24 hr. The embryos were grown in lots of 30 on 10 cm diameter Petri dishes, on filter paper moistened with 5 ml of distilled water under controlled conditions: 16 hr photoperiod (fluorescent tubes Flora and Dzienne 1:1, 40 W, light intensity 10 Klux), day temperature 23—25°C, night temperature 18—20°C. The criterion of germination was 3 mm long embryo radicle. The germination ability was estimated after 10 days of experiment. The variants of experiments are presented in Table 1.

Table 1

Variants of the experiments

Place	Light conditions	Thermal conditions °C		Type of culture	Series	Period
		day	night			
Phytotron	Fluorescence tubes 40 W “Flora” and “Dziennę” 1:1 light intensity 10 Klux Photoperiod 16 h	23—25	18—20	hydroponic capacity 200 l	I	1971 July—February
				hydroponic capacity 1,5 l	II	1973 July—December
				garden soil	III	1976 December—June
					IV	1975 April—September
					I	1971 November—May
				II	1972 July—November	
III	1973 April—November					
Vegetation hall	Natural light	20—30	10—15	sand	I	1971 November—May
				garden soil	IV	1972 April—November
				sand	II	1972 April—November

Water cultures of plants were carried out on the mineral medium No 11, according to Bently (1959). The medium was changed every 6 weeks in 200-liter tanks, and every 3 day sin 1.5-liter flower-pots. The

cultures carried out in tanks were aerated automatically for 10 hours daily.

The following mixture of mineral salts was used for the sand cultures of plants:

CaCl ₂ · 6H ₂ O	— 0.3 g
KCl	— 0.2 g
MgSO ₄ · 7H ₂ O	— 0.3 g
Ca(H ₂ PO ₄) ₂ , H ₂ O	— 0.3 g
Iron citrate	— 0.01 g
NH ₄ NO ₃	— 0.4 g
Mixture of microelements	1 ml
(amounts for 5 kg of sand)	

Mixture of microelements:

CuSO ₄ · 5H ₂ O	— 50 mg
ZnSO ₄ · 7H ₂ O	— 100 mg
H ₃ BO ₃	— 550 mg
Al(SO ₄) ₃	— 50 mg
MnCl ₂ · 4H ₂ O	— 350 mg
NiSO ₄ · 4H ₂ O	— 50 mg
Co(NO ₃) ₂ · 6H ₂ O	— 50 mg
KJ	— 25 mg
KBr	— 25 mg
Na ₂ MoO ₄ · 2H ₂ O	— 50 mg
LiCl	2 drops of saturated solution
distilled water	900 ml

During the vegetation period the following measurements were taken:

1. Shoot length of plants — in weekly intervals
2. Fresh weight of plants from water cultures — in weekly intervals
3. Fresh and dry weight of shoots and roots of plants — at the end of experiments.

The growth dynamics of stratified and non-stratified plants was compared according to the Students test (significance of differences between two averages t).

RESULTS

1. Growth of apple-tree seedlings on garden soil

Two weeks old apple-tree obtained from non-stratified and stratified embryos were transplanted to Wagner vases containing 5 kg of garden soil. Moisture of soil was kept at the level 60% capillary water capacity (CWC), and corrected every day. The experiments were performed in

three parallel series in phytotron room (exp. I, II and III), and in one serie in vegetation hall (exp. IV).

The elongation growth of shoot during the vegetation period and the dynamics of weekly shoots increments are presented in Fig. 1 and 2. At the first two weeks the average shoot length of stratified plants exceeded (4–22 mm) the average shoot length of non-stratified plants and for experiments I, II and III the differences were statistically significant. At the next weeks the higher elongation rate of non-

Fig. 1. Shoot elongation rate of plants grown upon garden soil

-stratified plants caused the leveling of shoot length of plants in both groups, and disappearance of statistically significant differences.

The cyclic character of the elongation growth was observed. The minimal and maximal shoot length increments occurred in both groups of plants (stratified and non-stratified) at almost the same period (Fig. 2).

The results obtained at the end of experiment are presented in Table 2. The differences of shoot length, wet and dry weight of stratified and non-stratified plants were insignificant statistically.

Fig. 2. Weekly shoot length increments of plants grown upon garden soil
Explanations as in Fig. 1

Table 2
Growth of apple-tree seedlings upon garden soil

Series	Age of plants (weeks)	Height (mm)			Fresh weight (g)				Dry weight (g)				
		S	NS	Signif. differ.	shoot		S	NS	shoot	Signif. differ.	root		s/r
					S	NS					S	NS	
I	24	386	370	—					5.76	5.48	3.90	3.72	1.47
II	20	305	363	—	8.65	12.73	3.98	5.35	4.23	5.45	3.98	5.35	2.61
III	20	562	640	—	15.98	14.26	6.44	6.19	7.22	6.23	2.03	1.75	3.55
IV	28	577	441	—	27.25	19.99	37.09	28.45	13.28	9.88	14.37	12.84	0.92

(Average values from 5 stratified and 10 non-stratified plants).

S — stratified plants.

NS — non-stratified plants.

The ratio dry shoot weight to dry root weight was different in particular experiments, but the same for stratified and non-stratified plants. The water balance of plants grown in the vegetation hall was more negative compared to the water balance of plants grown in phytotron room; so the growth of roots of plants grown in the vegetation hall was much more rapid than the growth of shoots and the ratio shoot weight to root weight was the lowest (less than 1).

Phot. 1. Habit of 12-week old plants grown upon garden soil

The growth of individual plants in both investigated groups was differentiated in all experiments. For example, at the last week of experiment III the height of stratified plants was ranging from 265 — 900 mm, and non-stratified ones from 260 — 850 mm. Habit of plants grown on garden soil is presented on Phot. 1 and 2.

2. Growth of apple-tree seedlings on sand

The apple-tree seedlings obtained from stratified and non-stratified embryos were grown in Wagner vases containing 5 kg of quartz sand. Moisture of sand was kept at the level 60% capillary water capacity (CWC) and corrected every day.

The first experiment, carried out in phytotron, lasted from November 1971 to May 1972. The second experiment, carried out in the vegetation hall, lasted from April to November 1972.

Phot. 2. Habit of 24-week old plants grown upon garden soil

The elongation growth, and the dynamics of weekly shoot increments are presented in Fig. 3 and 4. During all the experiments the elongation shoot increments of non-stratified plants were distinctly smaller than increments of stratified ones. No cyclic changes were noted in weekly shoot length increments in both groups of plants grown in the greenhouse and in the phytotron (Fig. 4). The results obtained at the end of the experiment are presented in Table 4. Clearly poorer growth of non-

Fig. 3. Shoot elongation rate of plants grown upon sand substrate
Explanations as in Fig. 2

Fig. 4. Weekly shoot length increments of plants grown upon sand substrate
Explanations as in Fig. 2

Table 3
Growth of apple-tree seedlings upon sand substrate

Series	Age of plants (weeks)	Height (mm)		Fresh weight (g)				Dry weight (g)							
		S	NS	Signif. differ.	shoot		root	shoot			root		s/r		
					S	NS		S	NS	Signif. differ.	S	NS	Signif. differ.	S	NS
I	24	362	188	+				3.12	2.00	+	2.08	1.54	—	1.50	1.29
II	28	411	229	+	16.87	10.07	45.02	8.26	4.38	+	16.27	10.41	+	0.50	0.42

(Average values from 5 stratified and 10 non-stratified plants)

S — stratified plants

NS — non-stratified plants

Table 4
Growth of apple-tred seedlings in water cultures

Series	Age of plants (weeks)	Height (mm)		Fresh weight (g)				Dry weight (g)								
		S	NS	Signif. differ.	shoot		root		shoot			root				
					S	NS	S	NS	S	NS	S	NS	Signif. differ.	S	NS	Signif. differ.
I	32	570	650	+					11.17	12.63	+	6.50	9.48	+	1.71	1.33
II	17	196	168	—	6.45	6.60	5.48	4.92	3.14	3.18	—	1.40	1.25	—	2.24	2.54
III	24	684	791	—	47.87	52.53	42.64	41.75	17.78	19.13	—	8.53	8.72	—	2.08	2.19
IV	24	817	1225	+	54.68	63.55	18.89	22.21	21.66	27.60	+	3.89	5.28	+	5.56	5.22

S — stratified plants

NS — non-stratified plants

SN
S

Average values from: I's—16 stratified and 15 non-stratified plants
II's—20 stratified and 22 non-stratified plants
III's—18 stratified and 14 non-stratified plants
IV's—5 stratified and 10 non-stratified plants

Phot. 3. Non-stratified 24-week old dwarf plant obtained on sand substrate

-stratified plants could have been caused by their higher sensitivity to the negative water balance, compared with the sensitivity of stratified plants, which higher resistance to the water stress resulted of low temperature during the stratification. In the group of non-stratified plants grown in phytotron — one dwarf plant was noted. It is presented on Phot. 3. General habit of stratified and non-stratified plants is presented on Phot. 4.

3. Growth of apple-tree seedlings in water cultures

The experiments were carried out during 1971—1977.

The apple-tree seedlings, obtained from stratified and non-stratified embryos were grown in hydroponic vessels containing 200 l of mineral medium (exp. I, II and III) and in pots containing 1.5 l of mineral medium (exp. IV).

The shoot elongation growth is presented in Fig. 5. Examples of weekly shoot length increments obtained in experiments III and IV are presented in Fig. 6.

The changes in the shoot length increments of stratified and non-stratified plants, grown in hydroponic vessel are parallel. The maximal increments of stratified plants in 9th and 14th week of growth were

observed. The maximal increments of non-stratified plants were delayed 7 days.

In the experiments with plants grown in hydroponic pots the character of shoot elongation was different, and no parallel changes observed.

Phot. 4. Habit of 24-week old plants grown upon sand substrate

In experiments II and III there were no statistically significant differences between the shoot length and fresh and dry weight of stratified and non-stratified plants (Table 4).

In experiments I and IV the shoot length and fresh or dry weight of non-stratified plants exceeded shoot length and weight of stratified ones. The differences were statistically significant.

Fig. 5. Shoot elongation rate of plants grown in water cultures
 Explanations as in Fig. 1

The ratio shoot weight to root weight of non-stratified and stratified plants were very similar, as in the experiments previously described. The ratio shoot weight to root weight was ranging from 1—3 times in different experiments.

Among the populations of both investigated groups of plants significant differences in growth of particular individuals were observed. Phot. 5 illustrates maximal differences of growth 14 weeks old stratified and non-stratified plants. Phot. 6, 7 and 8 present growth of plants in water cultures in 200 l vessels and in 1.5 l pots.

Fig. 6. Weekly shoot length increments of plants grown in water cultures
 Explanations as in Fig. 1

DISCUSSION

According to the general opinion, the lack of germination ability of seeds of many plant species, observed even under optimal conditions is connected with the state of embryonal dormancy. The germination of

embryos is possible, when the seed coat and endosperm are removed, but obtained plants remain in dwarf form, until the growing point reaches the process of secondary development, induced by staying at the low temperature during few weeks. The physiological maturity is obtained by the embryos during the stratification process, which terminates the intensive seed formation and proper plant development.

Phot. 5. Scattering of shoot elongation rate of 14-week old plants grown in a 200 l hydroponic vessel

Table 5

Number of germinating embryos after 10 days of culture

Year	% of germinated embryos	
	S	NS
1971	97.5	85.0
1975	63.3	59.6
1976	83.3	71.1
1977		60.2

(Average values from 300 stratified and non-stratified embryos)

S — stratified

NS — non-stratified

Our studies on the germination power of non-stratified embryos, and comparative studies on the growth of plants obtained from stratified and non-stratified seeds indicate that the embryos of autumn apple 'Antonówka' do not pass the state of embryonal dormancy.

Phot. 6. Habit of 24-week old non-stratified plants grown in a 200 l hydroponic vessel

The data shown in Table 5, confirmed by Duczmal and Duczmalowa (1963) indicate the lack of differences between the germination of embryos isolated from non-stratified and stratified seeds. Differences in the amount of embryos germinated after 10 days of experiment, occurred in some particular years could have been caused by varying environmental conditions during the embryogenesis, as have been shown by the other authors (Birecka, Tuchołka, 1956; Szukalski, 1961; Szukalski, 1962) as well as by different seeds storage conditions.

The percentage of germination of apple-tree 'Antonówka' embryos, after 10 days of experiment, observed by Smoleńska and Lewak (1973) was 50, and in experiment of Lewak and coworkers (1975) even smaller — 30. These results are inconsistent with our results presented in Table 5. The differences could have been caused by different ger-

mination conditions, different photoperiod (4 hr longer day in case of our experiments) and different spectrum of used light.

There were no differences of shoot length and fresh and dry weight between the plants obtained from non-stratified and stratified embryos,

Phot. 7. Habit of 24-week old stratified plants grown in a 200 l hydroponic vessel grown on garden soil, or in water cultures; and in some cases the growth of non-stratified plants exceeded even the growth of stratified ones (Tab. 2 and 4).

Weekly increments of shoot length and fresh and dry weight of non-stratified plants exceeded weekly increments of stratified ones, what was the reason that differences of growth occurring in the early phase of growth disappeared in the later phase.

In our opinion the more dynamic growth of stratified seedlings observed in the early phase of growth was caused by the fact that stratified seeds were physiologically older, and their germination started earlier. After 12-weeks stratification a large amount of germinated seeds developed several cm long roots, while the embryos isolated from non-stratified seeds were just prepared for germination.

Phot. 8. Habit of 24-week old plants grown in 1.5 l hydroponic pots

The results obtained by Maciejewska and Maleszewski (1976) indicating the more intensive incorporation of ^{14}C in photosynthese, during first 10 days of growth of stratified plants, compared with non-stratified ones, can be also explained by the differences observed in degree of development of stratified and non-stratified seeds.

The worst growth of both groups of investigated plants was observed, when sand medium was used. In experiments in phytotron (1971) and vegetation hall (1972) on this medium, the more intensive increments of shoot and fresh and dry weight of stratified than non-stratified plants noted. The character of weekly increments of shoot length of both groups of plants was practically the same (Fig. 4). Among 20 non-stratified plants only one plant formed dwarf form.

In spite of decreased energy of growth, 12-weeks old non-stratified plants grown on sand (experiment in vegetation hall) were 2,5-times as high as the stratified plants grown on soil obtained by Duczmal and Duczmalowa (1963).

More pronounced differences, over 6-fold, were noted between the length of shoot of non-stratified plants obtained in our experiments and by Duczmal (1963).

The decrease of the growth intensity of non-stratified plants grown on sand, compared with the growth on soil and in water cultures could have been caused by their increased sensitivity to water shortage. The further studies on this subject are necessary.

The special attention should be paid to the differentiation of growth dynamics of apple-tree 'Antonówka' plants, observed not only during the embryo germination, but also in the further steps of ontogenic development of both groups investigated plants.

The studies carried out during 1971—1977 on 89 plants obtained from stratified and 121 plants obtained from non-stratified embryos indicate, that the non-stratified embryo of apple-tree 'Antonówka' with removed coat seed and endosperm is able to germinate as well as stratified one, and the development of seedlings obtained from embryos of both groups is the same.

The presented results show, that the seeds of apple-tree 'Antonówka' can not be used as a material for studies of embryonal dormancy of seeds.

The deep physiological changes of enzyme activity and hormone concentration observed by Sińska and Lewak (1970) and Smoleńska and Lewak (1973) are probably caused by the low temperature (4—5°C) treatment, but can not be connected with the embryonal dormancy.

The differences of the activity of photosynthetic apparatus of apple-tree 'Antonówka' seeds, observed by Maciejewska and Maleszewski (1976) are also probably caused by the long-lasting low temperature treatment during the stratification.

CONCLUSIONS

1. There was no difference between the germination of embryos isolated from non-stratified and stratified seeds.
2. The germination of embryos in same particular years was different what could have been caused by the different conditions of embryogenesis and seeds storage.
3. There was no difference between the growth (expressed as the shoot elongation, and fresh and dry weight) of plants obtained from non-

stratified and stratified embryos, and grown on garden soil, or in hydroponic system. The statistically significant differences between the growth of seedlings from stratified and non-stratified embryos were found when sand as a growth medium was used. However, no dwarf forms among non-stratified plants observed.

4. The obtained results prove that the autumn apple 'Antonówka' seeds do not show the embryonal dormancy, on the experimental conditions.

SUMMARY

During 1971—1977 the growth of apple-tree seedlings (*Malus domestica* Borkh cv. Antonówka) was studied. The growth of seedlings obtained from embryos isolated from non-stratified and stratified (90 days at 4—5°C) seeds was compared. The plants were grown on sand, garden soil, or mineral medium in the vegetation hall, or in the air-conditioned room lighted with the fluorescent tubes ("Flora" and "Dzienne" 1:1) of 40 W, the light intensity about 10 Klux.

There were no differences observed in the shoot length and fresh or dry weight of the plants obtained from embryos isolated from non-stratified and stratified seeds, grown garden soil.

When sand as a growth medium was used, the growth (expressed as the elongation of shoot, and fresh or dry weight) of plants from stratified embryos was statistically more intensive than the growth of plants obtained from non-stratified embryos.

No dwarf forms noted in the group of non-stratified plants grown on sand (except one plant), but the growth was less intensive than the growth in the stratified group.

In the case of water cultures of the plants, the growth of non-stratified plants was practically the same as the growth of stratified plants, except two experiments, when the growth of the plants from stratified embryos was more intensive (statistically significant).

The number of germinating embryos isolated from non-stratified seeds and from seeds stratified for 90 days was the same.

The lack of differences in the germination power of embryos isolated from non-stratified and stratified seeds, and the observed normal growth of plants obtained from non-stratified seeds, point to the fact, that the seeds of the autumn apple 'Antonówka' do not pass the state of embryonal dormancy.

REFERENCES

- Bentley M., 1959. Commercial hydroponics. Bendon Books-Johannesburg.
- Birecka H., Tuchołka Z., 1956. Doświadczenia nad wpływem następczym żywienia roślin fosforem. Roczn. Nauk Roln., 73-A-1:1.
- Chiliakhian M., Nekrasova T., Klopenkova L., Loznikova V., 1963. Role of gibberellins in processes of photoperiodism, vernalization and stratification of plants. Fiziol. Rast., 10: 465—476.
- Come M., 1967. L'inhibition de germination des graines de Pommier *Pirus malus* L. non dormantes. Role possible des phénols tegumentaires. Ann. Sc. nat. bot., 8: 371—378.
- Crocker W., Barton L., 1953. Physiology of Seeds, Waltham.

- Duczmal K., Duczmalowa, 1963. Obserwacje nad kiełkowaniem nasion jabłoni. Biul. IHiAR, 1 (52): 89—93.
- Lewak S., Rychter A., Żarska-Maciejewska B., 1975. Metabolic aspects of embryonal dormancy in Apple seeds. *Physiol. Vég.*, 13 (1): 13—22.
- Maciejewska U., Maleszewski S., 1976. Embryonal dormancy and photosynthetic carbon metabolism in Apple seedlings. *Z. Pflanzenphysiol.*, 79: 300—309.
- Sińska I., Lewak S., 1970. Apple seeds gibberellins. *Physiol. Vég.*, 8: 661—667.
- Smoleńska G., Lewak S., 1974. The role of lipases in the germination of dormant Apple embryos. *Planta (Berl)*, 116: 361—370.
- Szukalski H., 1961. Wpływ zasobności nasion w fosfor na rozwój i plon roślin. *Roczn. Nauk Roln.*, a) 84-A-3:464, b) 84-A-4:789.
- Szukalski H., 1962. Wpływ zasobności nasion w fosfor na rozwój i plon roślin. *Roczn. Nauk Roln.*, 85-A-1:100.

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*Wzrost siewek jabłoni Malus domestica Borkh cv. Antonówka
otrzymanych z nie stratyfikowanych zarodków*

Streszczenie

W latach 1971—1977 przeprowadzono porównawcze badania wzrostu siewek jabłoni *Malus domestica* Borkh cv. „Antonówka” otrzymanych z zarodków izolowanych z nasion niestratyfikowanych oraz z nasion poddanych przez okres 90 dni stratyfikacji w temperaturze 4—5°C. Hodowle roślin prowadzono na podłożu piaszkowym, glebie ogrodowej i na pożywce mineralnej w warunkach hali wegetacyjnej oraz w sklimatyzowanym pomieszczeniu przy sztucznym świetle świetlówek 40 W („Flora” i „Dzienne” 1:1) o natężeniu około 10 Klux.

Rośliny otrzymane z zarodków izolowanych z nasion nie stratyfikowanych prowadzone na glebie ogrodowej nie różniły się wysokością łodygi ani świeżą i suchą masą od roślin otrzymanych z zarodków izolowanych z nasion stratyfikowanych.

Wzrost elongacyjny łodygi oraz świeża i sucha masa roślin wyprowadzonych ze stratyfikowanych zarodków był na podłożu piaszkowym intensywniejszy od wzrostu roślin wyprowadzonych z zarodków nie stratyfikowanych a występujące różnice były statystycznie istotne.

Przedstawione wyniki wskazują, że nasiona jabłoni odmiany „Antonówka” nie są obiektem przydatnym do badań nad spoczynkiem zarodkowym nasion. Obserwowane przez Sińską i Lewak (1970), Smoleńską i Lewak (1974) głębokie zmiany fizjologiczne wyrażające się zmianą aktywności enzymów oraz zawartością hormonów są spowodowane działaniem przez dłuższy czas temperaturą 4-5°C, nie mogą być jednak z przyczyn wyżej wymienionych uważane za zmiany warunkujące ustępowanie spoczynku embrionalnego. Długotrwałe działanie obniżonej temperatury podczas stratyfikacji nasion jabłoni odmiany „Antonówka” jest również przyczyną występujących różnic w aktywności aparatu fotosyntetycznego siewek, obserwowanych przez Maciejewską i Maleszewskie-go (1976).