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THE EFFECT OF *IN VITRO* CULTURE INITIATION TIME ON EXPLANT DEVELOPMENT ACTIVATION IN REPRESENTATIVES OF THE GENUS *CRATAEGUS* L.

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Досліджено дати виходу зі спокою та відновлення вегетації у маточних рослин видів роду Crataegus L., а також вплив їх фізіологічного стану на активацію розвитку експлантів у культурі in vitro. Встановлено, що оптимальний час для введення бруньок глоду в культуру in vitro збігається з початком відновлення вегетації маточних рослин у природних умовах.

Ключові слова*: глід, вид, експлант, культура in vitro*

Introduction. Microclonal propagation of plants using explants containing meristematic tissues or groups of meristematic cells allows for the rapid production of a large quantity of plant material that is genetically identical to the donor plant. This method plays a significant role in the accelerated cloning of fruit, berry, ornamental, and some woody plant species [1].

Among the undeniable advantages of this method for woody species are high multiplication rates, the ability to culture plants year-round, the sanitation of planting material, the possibility of selecting plant material with desirable traits in *in vitro* culture, and the shortening of the juvenile phase in the life cycle of perennial woody plants. Additionally, it enables the propagation of species that are difficult or impossible to root or graft under conventional conditions [1]. Microclonal propagation is also applied for the creation of collections of varieties and species needed for breeding and genetic research, the preservation of endangered and rare plant species, and the introduction of woody and ornamental plants into new growing environments [1, 2].

Review of Recent Studies and Publications. Most *Crataegus* L. species are valuable fruit, ornamental, and medicinal plants [2, 3]. In gardens and parks, hawthorn is cultivated as small trees or large shrubs – either individually, in alleys, or in groups – and is often used to form dense, impenetrable hedges. The fruits of many *Crataegus* L. species are edible when fresh and can also be used to prepare jams, jellies, and marmalades. Preparations made from the fruits and flowers of certain species are used to treat heart diseases [3].

Traditionally, *Crataegus* L. species are propagated by seeds. However, hawthorn seeds germinate very slowly – some may sprout in the first spring after sowing, others in the second, or even later. Up to 80% of seeds can be empty. Vegetative propagation is more successful through root suckers, while propagation by layering or grafting is less effective [2, 3].

The existence of established *in vitro* micropropagation techniques for many herbaceous plants, along with certain successful applications of this method for woody species [4, 5], provides a basis for considering its use in accelerating the propagation of *Crataegus* L. species. The main factors influencing explant development in *in vitro* culture include the donor plant's genotype, its physiological and ontogenetic state, the explant's size, the origin of the explant tissue, and the season when the explant is collected [4].

The physiological condition of perennial woody plants changes significantly throughout the year, especially in regions with seasonal cold periods, where it is associated with the plant's ability to survive unfavorable growing conditions. In the second half of summer, temperate climate plants gradually enter deep dormancy, characterized by slowed growth, lignification of shoots, and accumulation of carbohydrates in tissues. Buds of woody plants in deep dormancy do not sprout even under favorable growth conditions. As spring approaches, deep dormancy gradually transitions into enforced dormancy, during which the buds regain their ability to sprout. Identifying the dynamics of changes in the physiological state of woody plants will enable the determination of optimal timing for explant selection and their initiation in *in vitro* culture.

Research Methodology. The study was conducted during the winter-spring period when plants transition from deep dormancy to enforced dormancy. To determine the date of dormancy release from deep dormancy in donor trees, shoots were cut weekly and placed in containers with water at approximately 20 °C until bud break began. The date of dormancy release was recorded when the time from shoot cutting to bud break was no more than 7 days.

In vitro culture initiation was performed at three different dates: February 15, March 1, and April 1. One-year-old hawthorn shoots were cut and kept at room temperature in water-filled glasses until bud break began. Buds used as explants for *in vitro* culture initiation were excised, sterilized, and planted on nutrient media under sterile conditions. Cultivation was carried out on modified Murashige and Skoog medium. Explant development activation was assessed 20 days after planting by calculating the percentage of explants showing macroscopic growth structures relative to the total number of sterile explants. Simultaneously, phenological phases of donor trees were recorded according to phenological observation methods used in botanical gardens.

The experimental design included species of the genus *Crataegus* L. from the collection of the National Dendrological Park “Sofiyivka” of the National Academy of Sciences of Ukraine: *C. almaatensis* A. Pojark. – Almaty hawthorn, *C. arnoldiana* Sarg. – Arnold hawthorn, *C. chlorosarka* Maxim. – green-fleshed hawthorn, and – pear hawthorn.

Species *C. almaatensis* A. Pojark., *C. chlorosarka* Maxim., and *C. phaenopyrum* (L. f.) Medic. are promising for use in landscaping and park construction due to their decorative qualities [6]. *C. arnoldiana* Sarg. is notable not only for its ornamental value but also for producing large and tasty fruits [6], which may justify its involvement in fruit breeding programs.

Research Results. The study of dormancy release dates in the investigated *Crataegus* L. species (Table 1) showed that *C. chlorosarka* Maxim. was the first to exit deep dormancy.

Table 1. Dates of deep dormancy release and onset of vegetation in *Crataegus* L. species (2023–2024)

Species	Year of observation	Date of deep dormancy release	Bud swelling start	Bud break
C. chlorosarka Maxim.	2023	3.01	30.03	10.04
	2024	30.12	18.03	24.03
C. arnoldiana Sarg.	2023	31.01	29.03	9.04
	2024	12.01	16.03	21.03
C. almaatensis A. Pojark.	2023	7.02	30.03	12.04
	2024	31.01	17.03	22.03
C. phaenopyrum (L. f.) Medic.	2023	21.02	1.04	15.04
	2024	12.02	19.03	26.03

When cut shoots were transferred to room conditions, bud break occurred within one week on shoots cut on January 3. Nearly a month later, deep dormancy ended in *C. arnoldiana* Sarg. Bud break within seven days was observed on shoots of this species cut on January 31. In *C. almaatensis* A. Pojark. and *C. phaenopyrum* (L. f.) Medic, the exit from deep dormancy was recorded on February 7 and February 21, respectively.

Table 1 shows that despite the considerable variation in dates of deep dormancy release, bud *C. arnoldiana* Sarg. and *C. almaatensis* A. Pojark. started vegetation in 2023 on March 29–30. Only *C. phaenopyrum* (L. f.) Medic. showed signs of vegetation renewal slightly later, on April 1. This can be explained by the cool spring of 2023, which delayed the onset of vegetation and thus narrowed the differences in bud swelling dates among the species.

Further observations revealed that the genetic differences among species became more distinct in the timing of subsequent phenological phases. The bud break phase began first in *C. arnoldiana* Sarg. and *C. chlorosarka* Maxim. on April 9 and April 10, respectively, followed by *C. almaatensis* A. Pojark. on April 12. The last species to enter the bud break phase was *C. phaenopyrum* (L. f.) Medic., which also had the latest transition from deep to enforced dormancy.

In 2024, the dates of deep dormancy release, bud swelling, and bud break for all studied *Crataegus* L. species occurred earlier compared to 2023. As in 2023,

C. arnoldiana Sarg. was the first to reach these phenological phases, and *C. phaenopyrum* (L. f.) Medic. the last.

Analysis of explant development activation in the studied *Crataegus* L. species showed significant differences when explants were introduced into *in vitro* culture at various times during the winter-spring period, both between the different dates of introduction and among species (Table 2).

Table 2. Activation of explant development in *Crataegus* L. species depending on the timing of *in vitro* culture initiation (2023–2024)

Species	Year of observation	Date of *in vitro* culture initiation	Explant development activation, %
C. almaatensis A. Pojark.	2023	15.02	0
		1.03	10
		1.04	80
	2024	5.02	1
		5.03	14
		26.03	84
C. arnoldiana Sarg.	2023	15.02	3
		1.03	45
		1.04	85
	2024	5.02	2
		5.03	42
		26.03	86
C. chlorosarka Maxim.	2023	15.02	6
		1.03	40
		1.04	85
	2024	5.02	5
		5.03	44
		26.03	88
C. phaenopyrum (L. f.) Medic.	2023	15.02	0
		1.03	5
		1.04	80
	2024	5.02	0
		5.03	6
		26.03	85

In 2023, the species *C. arnoldiana* Sarg. and *C. chlorosarka* Maxim., which exited deep dormancy during January, demonstrated low morphogenic activity (3–6%) already at the first *in vitro* culture initiation date – February 15. When these species were introduced into *in vitro* culture on March 1, explant development activation increased to 40–45%. Explants of *C. almaatensis* A. Pojark. and *C. phaenopyrum* (L. f.) Medic. showed no development when cultured on February 15. The donor plants of *C. almaatensis* A. Pojark. exited deep dormancy only 8 days

before this first culture initiation date, while *C. phaenopyrum* (L. f.) Medic. plants were still in deep dormancy (see Table 1). When introduced into culture on March 1, 10% of *C. almaatensis* A. Pojark. explants and 5% of *C. phaenopyrum* (L. f.) Medic. explants showed morphogenic capacity, which was 30–40% lower than the activation rates of *C. arnoldiana* Sarg. and *C. chlorosarka* Maxim. at the same date.

Initiating *in vitro* culture of hawthorn explants on April 1, 2023, led to increased development activation rates in all studied species, reaching 80–85%. At this time, donor plants were ending the period of enforced dormancy and undergoing vegetation renewal. Comparing the results of *in vitro* culture initiation in 2024 with those of 2023 showed only minor differences in the quantitative activation of explant development, while the overall trend depending on the timing of culture initiation remained consistent.

Conclusions. The conducted research demonstrated that the optimal timing for initiating *in vitro* culture of hawthorn buds is close to the onset of vegetation renewal in donor plants under natural conditions. Initiation of *in vitro* culture during deep dormancy and the early phase of enforced dormancy was ineffective.

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Annotation

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The effect of in vitro culture initiation time on explant development activation in representatives of the genus Crataegus L.

This study investigated the physiological dormancy dynamics and optimal timing for in vitro culture initiation of four Crataegus L. species (C. almaatensis, C. arnoldiana, C. chlorosarka, C. phaenopyrum) from the National Dendrological Park "Sofiyivka" collection. Research was conducted during winter-spring period, when plants transition from deep dormancy to enforced dormancy. To determine dormancy release dates, shoots were sampled weekly and incubated in water at ~20 °C until bud break within seven days occurred. In vitro culture initiation was performed on three dates: February 15, March 1, April 1. One-year-old hawthorn shoots were collected, buds sterilized, and placed on modified Murashige and Skoog nutrient medium. Explant morphogenesis was assessed 20 days after culture initiation by calculating percentage of explants showing visible growth structures.

Results demonstrated significant variation in dormancy release timing among species: C. chlorosarka exited deep dormancy first (January 3, 2023), followed by C. arnoldiana (January 31), C. almaatensis (February 7), and C. phaenopyrum (February 21). Despite differences in dormancy release, bud swelling in all species began almost simultaneously by late March 2023. Bud break phases showed greater genetic differentiation, with C. arnoldiana and C. chlorosarka initiating earlier and C. phaenopyrum last. In 2024, all phenological phases occurred earlier than in 2023, maintaining the same interspecies pattern.

Explant development activation in vitro varied substantially with culture initiation timing and species. Early initiation during deep dormancy (February 15) resulted in minimal morphogenic activity (0–6%), especially for species still dormant. Cultures started March 1 showed moderate activation (5–45%), with C. almaatensis and C. phaenopyrum lagging behind C. arnoldiana and C. chlorosarka. Cultures initiated April 1, near vegetation renewal, exhibited high activation rates (80–85%) across species. Similar trends appeared in 2024 with minor quantitative differences. The study concludes that initiating in vitro culture of hawthorn buds is most effective when timed close to natural vegetation renewal. Initiation during deep dormancy or early enforced dormancy phases is ineffective for explant morphogenesis, highlighting the importance of physiological state monitoring for successful micropropagation of Crataegus L. species.

Key words: *hawthorn, species, explant, in vitro culture*

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