Pazderu K *et al.* (2024)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 52, Issue 2, Article number 13561
DOI:10.15835/nbha52213516
Research Article

How the application of supporting substances affects the formation of fertile embryos of crossbred cherries and its germination

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Abstract

The seeds of early ripening cherry cultivars are generally of poor quality, thereby adversely affect the success of the entire breeding process. The current study aimed to examine the application of supporting substances, such as brown algae extract (ALGA), humic and fulvic acids + IAA (LEXIN), and gibberellic acid (GA_3), in the blooming and ripening of fruits. Successively, the same substances were applied to seeds within laboratory conditions, to enhance the quality of cherry embryos and promote their germination. For comparison, two cultivars, an early one 'Burlat' and late 'HL 13577' were used in a multi-year trial. The application of GA_3 at the time of blooming in the 'Burlat' cultivar had a positive effect on increasing the share of harvested fruits (31.6%), reduction of the share of bad seeds (17.7%) and improving seed germination (21.9%), compared to the untreated control. Cultivar 'HL 13577' had 16.6% of developed fruits, 15.0% share of bad seeds and seed germination 9.8%. No remarkable increase in germination was observed in any tested variants after the secondary application of preparations in the laboratory. The impact of the year was most explicit in the proportionality of developed fruits, while its effect on seed germination post-treatment remained uncertain. On the contrary, the treatment exhibited a greater effect on germination testing. Based on these findings, we conclude that direct application of GA_3 to the trees (from blooming to fruit harvesting) in the orchard was beneficial. However, the other substances tested did not produce positive effects on the parameters.

Keywords: breeding; earliness; germination; gibberellic acid; seed development; seed quality

Introduction

Cherries belong to the most popular and cultivated fruit species. This great popularity is based on consumers appreciation not only in terms of quality, but also in terms of nutrition (Proietti *et al.*, 2019). Fruits are a source of many nutrients, vitamins (Ferretti *et al.*, 2010) and antioxidants, which play a positive role in the prevention of cardiovascular and oncological diseases in humans (Cruz-Lopes *et al.*, 2022; Knapová and Bílková, 2022).

Received: 18 Dec 2023. Received in revised form: 19 Mar 2024. Accepted: 08 May 2024. Published online: 25 Jun 2024.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Based on market demand, breeders are currently trying to create very early and very late ripening cultivars. Breeding for earliness is primarily about the highest possible earliness of cultivars, but the quality of the seeds obtained by hybridization of early ripening cherry cultivars is poor (Žďárská and Suran, 2021). Research carried out at the Iasi Fruit Research Station in Romania over 23 years involved the pollination of 706,365 flowers. This effort yielded 133,150 hybrid fruits, equating to a fruit set rate of 18.8%. However, only 13,453 of these fruits provided viable seeds, resulting in 6,493 seedlings, which corresponds to an overall success rate of 0.91%. Yearly differences were notable, with fruit set rates ranging from 5.8% to 35.7%, and the success rate from flowers to seedlings varying between 0% and 2.9% (Gradinariu *et al*., 2008).

The breeding work in the Jerte Valley of Spain, analyzed for the years 2006 to 2008, began with 36,906 pollinated flowers. They harvested 6,334 fruits with an average fruit set rate of 17.2%, and a global success rate of 4.9%. Year-over-year, the fruit set fluctuated between 13.5% and 20.6%, and germination rates experienced a low of 1% in 2006 but rose to 44.7% and 46.9% in the following two years. Notably, three cultivars (Ambrunés, Hudson, and Pico Colorado) produced 98.7% of the resultant seedlings (López-Corrales *et al*., 2014).

At INRA-Bordeaux, the last 11-year period of breeding has yielded diverse outcomes (unpublished data). The fruit set percentage varied widely from 0 to 8.2%, averaging at 4.7%, while the germination rate was between 6.3% and 71.9%, averaging at 21%. The overall success rate was roughly 1%, with individual results ranging from 0.3% to 2.6%. Despite lower average fruit sets than the Romanian and Spanish programs, INRA showed comparable overall success rates due to its higher germination rates. The variability in fruit set and germination rates across 22 maternal genitors were significant, with fruit sets between 0-19.1% and germination rates from 0.3-84.9%. The cultivars 'Fertard', 'Regina', and 'Fermina' contributed to the lower average fruit set at INRA due to their high chilling requirements, though 'Fertard' compensated with a high germination rate of nearly 66% (Quero Garcia, 2019).

The seeds of early cultivars are not fully developed, because the fruits ripen earlier than the embryos, and the seeds often die during fruit ripening or have low germination (Blažková *et al*., 2003). Little or poor germination is characteristic especially for the early maturing cultivar 'Burlat', whose seeds show insufficient growth of embryos. Therefore, it is important to harvest the embryos, when the fruits are still immature (Lang, 2019). Low seed germination makes it difficult to obtain abundant populations and crossbreeding is inefficient. Early cultivars can form viable embryos, but seeds do not germinate due to poor cotyledon development during fruit ripening (Stanys, 1997). Ultimately, by gathering seeds from early ripening cultivars like 'Burlat', 'Earlise', 'Early Star', and similar cultivars, the application of in vitro embryo rescue techniques can enhance the germination rates of immature embryos (Balla and Brozik, 1996). However, it's important to note that the effectiveness of embryo culture can fluctuate significantly across different years and depending on the cultivar, as evidenced by the findings of Balla and Brozik in 1996. Not only genetic characteristics, but also environmental factors are important for the cultivation of fruit trees. A rich cherry crop is the result of a combination of internal and external conditions and depends on the successful completion of a sequence of reproductive events (Tosun and Koyuncu, 2007).

Dormancy, which is the result of adaptation to adverse conditions of the climate cycle, has a significant influence on seed germination. This is a period of rest when physiological processes are stopped or reduced (Şan *et al*., 2014). It is therefore a strong inhibitor of germination in stone fruit seeds (Stein *et al*., 2020). Seed dormancy affects the timing of germination and is greatly influenced by the environment and natural conditions in which the seeds mature. Baskin and Baskin (2004) introduced the dormancy model as a model of the influence of the external environment on seed formation and maturation. Germination inhibitors occur in different concentrations in different parts of the seed, and it is possible to reduce their proportion by removing one or several parts of the seeds, thereby increasing the percentage of germination (Şan *et al*., 2014). Germination, on the other hand, stimulates kinetin, which promotes cell division. Exogenous application of this hormone significantly increases germination percentage if applied in sufficient quantity (Selim *et al*., 1981). Cherries are naturally dormant stone fruits, but there are differences between years and between cultivars.

Cherry seed dormancy can be removed mainly by stratification, pre-cooling, or application of KNO_3 and gibberellic acid (GA_3) (ISTA, 2019; Çetinbaş and Koyuncu, 2006). However, a well-developed embryo is a prerequisite, which can be a problem with early cherry cultivars because the seeds do not have enough time to develop.

The aim of the study was to try to improve the quality of cherry seeds by applying supporting substances directly to trees from blooming to fruit harvesting period in the orchard and to increase the germination of these harvested seeds in laboratory conditions.

Materials and Methods

Field (orchard) trials

The experiment was conducted at Research and Breeding Institute of Pomology, Holovousy, Ltd., Czech Republic, between 2019 to 2023, with subsequent laboratory evaluation at Czech University of Life Sciences, Prague, Czech Republic. The trial included trees from the Holovousy locality, Eastern Bohemia (360 m above sea level; 50.383629, 15.576902). The long-term average annual temperature for this locality is 8.4 °C with an average annual precipitation of 663.5 mm. The growing conditions here correspond to the beet production type. According to agroclimatic zoning, the locality falls into the macro-region of moderately warm and the sub-region of moderately humid. The orchard is located on a gentle slope with southern exposure. The soil belongs to the group of brown soils, it is deep and non-stony.

The planting was established without supplemental irrigation in 2008 on 'Gisela 5' rootstock. The training system was open vase, with trees planted in a spacing of 5.0 × 1.5 m. The trees were managed using conventional production practices. Permanent grass cover was maintained between the rows, which was regularly mowed or mulched. Herbicides were used to maintain the alleyways. Basic fertilization is applied once a year with LAV 27 fertilizer (ammonium nitrate with limestone) at a rate of 1.1 q/ha. Additional fertilization is done by foliar spraying with DAM 390 fertilizer twice at a rate of 3.0 L/ha, three times at a rate of 4.7 L/ha, and during fruit ripening, STOPIT is applied twice at a rate of 10.0 L/ha. Pruning of trees is carried out in spring before tree blooming. Biomass from pruning is mulched in the inter-row spaces.

Two maternal cultivars of early and late ripening time were pollinated in a controlled conditions with paternal materials, both in an isolate. Cross combination was as follow: early ripening cultivar 'Burlat' × 'Tamara' and late ripening cultivar 'HL 13577' × 'Irena'. Blooming branches of pollen donor were placed into isolate in a bucket with water. Pollination was provided by bumblebees added in the hives to the isolates. A separate pollination isolate, covered with an insect-proof fiber net, was prepared for each variant of application onto trees.

The application of supporting substances (Table 1) took place at the same time, depending on the progress of the phenophases, in a total of 4 repetitions. The first dose was sprayed at full bloom, the second after flowering during the fall of the flower petals, and two more applications at 10-days intervals during fruit development. For each variant, the number of flowers, their reduction and the proportion of fruits obtained after pollination when applying supporting preparations were counted. The number of flowers after the 1st application of the preparations and the number of developed fruits after the 4^{th} application were calculated. The evaluation was carried out on 6 branches for each variant. This data was used to compare the effect of treatment variants on fruit set by determining the percentage of developed fruits from the flower set. A whole tree of each variant was harvested for the germination trials.

At the same time, all variants were also treated against *Monilinia* blossom blight and brown fruit rot during maturation. In some years, *Monilinia* infection was decisive to the amount of obtained fruit and the quality of embryos.

Table 1. Scheme of application of supporting substances

Treatment	Compounds
Control	Tap water only,
ALGA	Alga 600, 1 g/1 liter of suspension, brown algae extract, Agrobiosfer, Ltd.
GA_3	0.01% solution of gibberellic acid GA_3, Carl Roth, Gmbh
LEXIN	Lexin (humic and fulvic acids 6%, IAA 0.25%) 0.08% solution, Lexicon plus, Ltd.

Seed material

Sweet cherry fruits from the F1 generation of each variant were harvested at full maturity. The soft exocarp and mesocarp were removed by mechanical peeling and stone fruits were surface disinfected with 1% sodium hypochlorite. The hard endocarp was removed mechanically (by cracking) and seeds with a seed coat were disinfected with fenhexamid (500 g/l). Seeds were not stratified or otherwise stimulated to break dormancy in order to clarify, in particular, the effect of the application of supporting substances on the development of the seeds on the trees.

Seeds were divided into 4 categories according to stage of embryo development (Figure 1):

Category 1 – a well-developed embryo, that fills whole or most of the pit, 7-9 mm long

Category 2 – partially developed embryo, fills half of the pit, 4-6 mm long

Category 3 – poorly developed embryo, fills a quarter or less of the pit, 1-3 mm long

Category 4 – missing embryo or only wrinkled seed coat

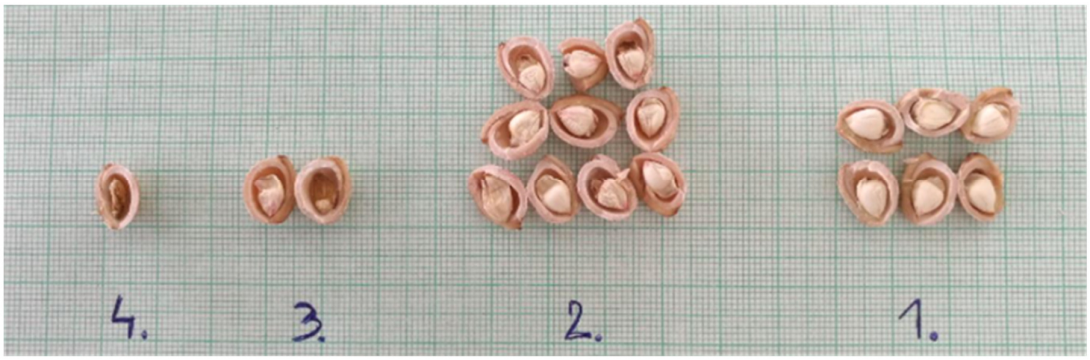


Figure 1. Categories of developed seeds

Treatment in laboratory

Seeds of categories (1+2+3) of each variant after mechanical removal of hard endocarp (by cracking) with seed coat were soaked 1 day in the same all supporting substances each: tap water only (control), ALGA, LEXIN and GA_3, all solutions with the same concentration, except GA_3 (0.1% for soaking of seeds).

Seed germination testing was carried out in pleated filter paper (Hahnemuhle, 110 grams per sqm) in 4 replications at 20 degrees with continuous light (BP method – ISTA, 2019). All three seed categories (1+2+3) were germinated together. Every 2 weeks, seeds were transferred to a new filter paper until final germination or seed destruction. The total duration of the germination test was 3 months (12 weeks). Seeds with green cotyledons and with developed minimum 3 mm long root were counted as germinated.

Statistical analysis

All data obtained were evaluated by the ANOVA method (analysis of variance) as a 3-factor full model (treatment × cultivar × year) with interactions, separately for data both from the stand (trees) and data from laboratory testing. Differences between means were assessed by Tukey's Honestly Significant Difference (HSD) method. The SAS system, version 9.4, was used for evaluation (SAS Institute, USA). From the entire five-year experiment, only the years 2019 and 2022 with enough number of seeds from individual variants were

used for statistical evaluation, when it was possible to evaluate the importance of tested factors on seed quality and their ability to germinate.

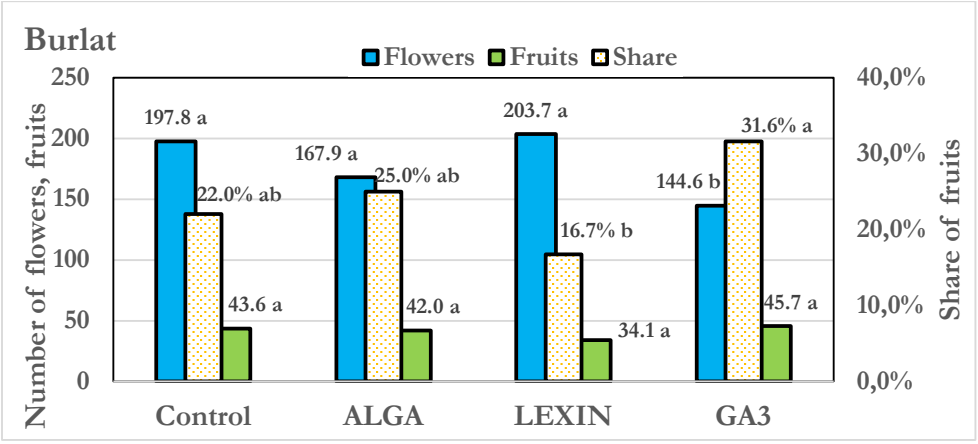
Results

Fruit development

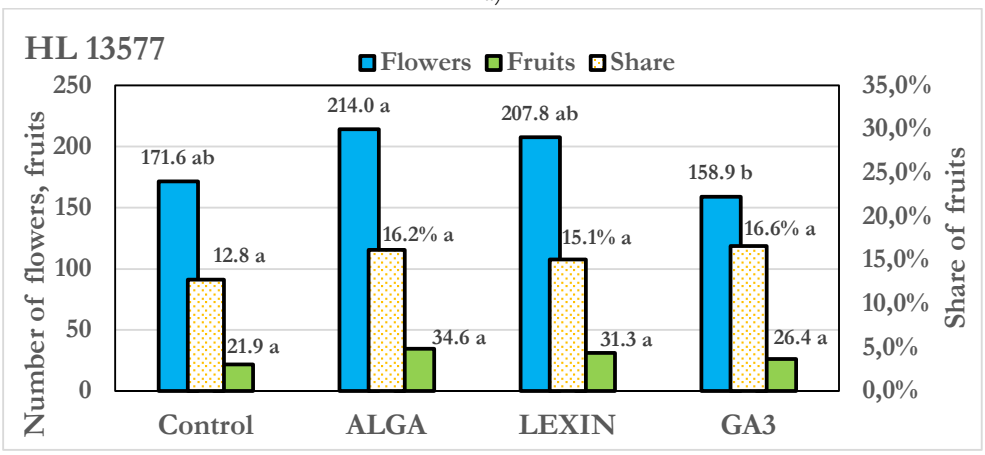
The 'Burlat' cultivar showed the highest proportion of developed fruits following the GA_3 spraying (31.6%), which was demonstrably superior to all other treatments. The lowest number of flowers 144.6 (per branch) was also found for the variant GA_3. Flower to fruit reduction in the GA_3 variant was 68.4%, while in the untreated control 78.0%.

In the case of the 'HL 13577' cultivar, the differences between the treatments were inconclusive, except for the number of flowers after treatment in the GA_3 variant, where the number of flowers per branch (158.9) was significantly reduced compared to the other variants.

A detailed look at individual donor progenies shows Figure 2a and b.



a)



b)

Figure 2. a) Fruit development after treatment with supporting substances onto trees. Differences among means are marked with small letters (separately for each category), HSD Flowers = 55.4., HSD Fruits = 23.0, HSD Share = 8.3; b) Fruit development after treatment with supporting substances onto trees. Differences among means are markted with small letters (separately for each category), HSD Flowers = 13.2, HSD Fruits = 20.4 HSD Share = 11.5.

The year and 3-factor interaction year x treatment x cultivar had the greatest effect on the number of developed fruits, and also the year, followed by cultivar, and the 2-factor interaction of year and cultivar had significant influence on the share of developed fruits (relative to the number of flowers). The effect of the treatment on the average of both cultivars, early 'Burlat' and late 'HL 13577', was inconclusive (Table 2).

Table 2. F-values and P-values of factors influencing the number of developed fruits and the share of developed fruits (average of 2019 and 2022 years)

Factor	Number of developed fruits		Share of developed fruits	
	F-value	P	F-value	P
Year (A)	10.30	<0.01	142.08	<0.01
Treatment (B)	0.29	NS	0.82	NS
Cultivar (C)	7.68	<0.01	35.86	<0.01
A × B	0.77	NS	1.14	NS
A × C	0.06	NS	27.94	<0.01
B × C	2.53	NS	5.82	<0.01
A × B × C	12.96	<0.01	14.60	<0.01

In the case of an additive ANOVA model, the F-values show the relative proportions of the factors to the total variability of the model. NS – not significant.

Effects of factors on share of bad seeds and seed germination

The application of supporting substances in the flowering period had a significantly positive effect both on the reduction of the share of bad seeds (category 4) and on the germination of embryos (Table 3). The effect of cultivar was higher for the proportion of wrong seeds than in seed germination after treatment in flowering. The year of production had a significant effect only on the proportion of bad seeds; to the germination, the effect of year was inconclusive, and the effect of cultivar was less significant than the effect of treatment.

Table 3. F-values and P-values of factors influencing the share of bad seeds and germination after treatment in flowering (both years 2019 and 2022 and cultivars)

Factor	Share of bad seeds (category 4)		Germination after treatment in flowering	
	F-value	P	F-value	P
Year (A)	43.67	<0.01	1.85	NS
Treatment (B)	31.57	<0.01	11.66	<0.01
Cultivar (C)	109.87	<0.01	4.86	<0.05
A × B	8.70	<0.01	21.17	<0.01
A × C	51.50	<0.01	1.64	NS
B × C	22.16	<0.01	5.90	<0.01
A × B × C	9.89	<0.01	12.46	<0.01

In the case of an additive ANOVA model, the F-values show the relative proportions of the factors to the total variability of the model. NS – not significant.

The proportion of bad seeds (category 4) in the 'Burlat' cultivar (Table 4) after mechanical removal of the endocarp was significantly the lowest after the application of GA_3 (17.7%). On the contrary, the variant with the application of lexin had the highest proportion of bad seeds (58.6%).

On average, across all variants, including the control, the share of bad seeds for the early cultivar 'Burlat' was 36.9%.

The germination of harvested seeds after spraying on trees without subsequent treatment in the laboratory was the highest for the GA_3 variant (21.9%), on the contrary, after the application of lexin, it was the lowest (2.2%). Only GA_3 was significantly different compared to the untreated control.

The subsequent application of supporting substances to the seeds did not have a significantly positive effect, either in the case of the untreated variant in the stand (control in flowering) or in other variants of the application in flowering, but some variants were comparable in comparison to the control.

The highest germination of laboratory treated seeds was 16.6% for the combination GA_3/ GA_3 (tree/lab), closely followed by the alga/alga combination with a similarly high germination (15.9%).

Table 4. 'Burlat'- Early cultivar, share of wrong seeds and seed germination after treatment in flowering and after treatment in laboratory

Treatment in flowering	Share of bad seeds (%)		Germination (%)		Treatment in laboratory	Germination (%)	
Control	39.4.	B	6.6.	BC	Control	6.6	ab
					Alga	6.9	a
					Lexin	2.1	b
					GA_3	6.1	ab
Alga	31.8.	B	12.3	B	Control	12.3	ab
					Alga	15.9	a
					Lexin	1.9	c
					GA_3	5.0	bc
Lexin	58.6	A	2.2	C	Control	2.2.	a
					Alga	1.6	a
					Lexin	0.8	a
					GA_3	3.9	a
Gibberellic acid	17.7	C	21.9	A	Control	21.9	a
					Alga	13.3	a
					Lexin	1.0	b
					GA_3	16.6	a
HSD 0.05	11.6		8.6				
2019	45.1	A	15.4	A			
2022	26.1	B	8.6	B			
HSD 0.05	6.1		4.5				

Differences among treatments in flowering are marked with capitals, differences among treatments in laboratory are marked with small letters (separately for each treatment on the trees). HSD for laboratory treatment, HSD Control = 4.6, HSD Alga = 8.9, HSD Lexin = 4.3, HSD Gibberellic acid = 8.6

The late cultivar 'HL 13577' (Table 5) generally had a lower number of bad seeds (category 4) after spraying the trees. Among the treated variants, the best outcome was observed with gibberellic acid (15%), while the untreated control had the highest part of underdeveloped seeds (22.5%). On an average, across all the treatments, the late cultivar performed better than 'Burlat', with only a 19% of defective seeds.

Germination in the late cultivar was the highest in the GA_3 variant and the lowest in the alga variant, but the differences were not conclusive. The lower germination rate in comparison with the 'Burlat' cultivar was caused by a higher infestation of the seeds by *Monilinia* infection, which could not be eliminated despite the use of disinfectants.

Additionally, the second application of the preparations in the laboratory did not result in a demonstrable increase in germination in any of the variants, although the seeds in some application variants had slightly higher germination than the control variant (without treatment in the laboratory).

Table 5. 'HL 13577' - late cultivar, share of bad seeds and seed germination after treatment in flowering and after treatment in laboratory

Treatment in flowering	Share of bad seeds (%)		Germination (%)		Treatment in laboratory	Germination (%)	
Control	22.5	A	7.1	A	Control	7.1	a
					Alga	7.3	a
					Lexin	8.6	a
					GA_3	3.4	a
Alga	20.1	A	5.4	A	Control	5.4	a
					Alga	9.6	a
					Lexin	6.9	a
					GA_3	4.5	a
Lexin	18.2	A	5.8	A	Control	5.8	a
					Alga	6.6	a
					Lexin	7.9	a
					GA_3	7.0	a
Gibberellic acid	15.0	A	9.8	A	Control	9.7	a
					Alga	14.2	a
					Lexin	11.9	a
					GA_3	8.9	a
HSD 0.05	9.0		8.8				
2019	18.5	A	8.6	A			
2022	19.6	A	6.4	A			
HSD 0.05	4.8		4.7				

Differences among treatments in flowering are marked with capitals, differences among treatments in laboratory are marked with small letters (separately for each treatment on the trees). HSD for laboratory treatment, HSD Control = 5.9, HSD Alga = 6.2, HSD Lexin = 6.2, HSD Gibberellic acid = 8.5

In both cultivars, fully developed large seeds germinated first (category 1), on the contrary, small seeds germinated rarely during the duration of the germination test.

In the overall 4-factor model (year, cultivar, treatment on tree, treatment in lab), germination was 10.1% in 2019, respectively 6.4% in 2022 (significantly different means of years).

Discussion

The filter paper (BP method) germination test was chosen as a standard method of germination evaluation (ISTA, 2019) to compare the effect of the application of supporting substances on the quality of cherry seeds without being influenced by other methods affecting germination, especially stratification. Germination values obtained by this traditional method are comparable to, for example, Dulič *et al*. (2016), who reports from 35 to 8.3% of seeds with seed coat developed into plants at 'Burlat'. Similarly, Stein *et al*. (2021) found 14.9% of germinated seeds without treatment. However, Du Hyun (2019) found that whole seeds of *Prunus yeodensis* Matsumura with seed coat did not germinate, neither in BP nor in sand. Additionally,

various sources have noted a wide range of germination rates, which can vary from 0 to over 80% depending on the cultivars, as documented by Quero Garcia (2019).

The impact of application of supporting substances affecting the development, ripening and germination of seeds is recognized in field production (Procházka *et al*., 2016). However, it has not been previously tested in the orchard as a spray in blooming and ripening to affect the quality of cherry seeds. In the control variant, the percentage of fruit set (from flower to fruit) ranged from 12.8 to 22% depending on the cultivar. Published data from breeding programs show an average fruit set percentage ranging from 1% to 18.8% (Quero Garcia, 2019). Our study revealed a significant difference in the percentage of fruit set among treated trees of different cultivars. While the share for the early 'Burlat' cultivar was between 16.7 and 31.6%, the late HL 13577 cultivar ranged from 12.8 to 16.6%.

Cherry seed development is influenced by the presence of abscisic acid, which is produced during seed formation and ripening. A high level of ABA in the pith increases the sugar content in the fruit and affects the fruit growth rate, maturation, and colouring of sweet cherry (Kondo and Gemma, 1993). On the other hand, poor development and strong dormancy of the formed seeds are important problems that affects the effectiveness of breeding, especially in the case of early cultivars (Blažková *et al*., 2003; Žďárská and Suran, 2021). Typically, cherry seeds are in various stages of development and dormancy when the fruits are harvested. This was also verified by Finch-Savage *et al*. (2002). To remove it, it is best to use pretreatment with GA3 in naked embryos followed by 1 month of cold stratification (Pipinis *et al*., 2012; Al-Absi, 2010). In our case, however, the subsequent application of GA3 to the seeds in the laboratory did not have an additional positive effect.

More developed embryos were able to germinate shortly after removal from the seed. Arbeloa *et al*. (2009) confirm that they found higher germination in larger embryos. Small embryos rarely germinated in this experiment. The low germination of the less developed embryos of 3 cultivars was also shown by Dulič *et al*. (2016).

Despite breeders acknowledging the significant influence of the year, particularly on yield and fruit quality (Predieri, 2003), there hasn't been any efforts for experimental multi-year study to evaluate the exact effects of applying supporting substances during blooming and ripening on the quality of cherry embryos (Blažek *et al*., 2022).

The effect of multiple cultivars on embryo germination was reported by Dulič *et al*. (2016) and Stein *et al*. (2021).

Conclusions

The application of supporting substances in the blooming and ripening periods influenced the formation of fertile embryos in cherries, especially in the early crossbred. GA3 increased the proportion of developed fruits and reduced the share of bad seeds. Seed germination after spraying GA3 on trees was the highest from all tested variants.

In the multi-factor experiment, a more significant influence of the year on the number of flowers and on the number of ripe fruits was found, compared to the cultivar. The share of bad seeds was more affected by the cultivar than by the year; seed germination was most influenced by the used treatment and the cultivar, and the influence of the year was found to be inconclusive.

Authors' Contributions

Conceptualization: KP; Data curation: KP; Formal analysis: KP, PS, IZ and TB; Investigation: KP, PS, IZ, TB and LZ; Methodology: KP, PS; Project administration: LZ; Resources: PS, IZ and TB; Supervision: LZ; Validation: TB; Writing – original draft: KP, PS and LZ; Writing – review & editing: KP, PS and LZ. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by the research project NAZV, Czech Republic, grant number QK1910290.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Al-Absi KM (2010). The effects of different pre-sowing seed treatments on breaking the dormancy of mahaleb cherries, *Prunus mahaleb* L. seeds. Seed Science and Technology 38:332-340. *https://doi.org/10.15258/sst.2010.38.2.06*

Arbeloa A, Daorden E, Garcia E, Andreu P, Marin JA (2009). In vitro culture of myrobalan (*Prunus cerasifera* Ehrh.) embryos. HortScience 44:1672-1674. *https://doi.org/10.21273/HORTSCI.44.6.1672*

Balla I, Brozik S (1996). Embryo culture of sweet cherry hybrids. Acta Horticulturae 410:385-386. *https://doi.org/10.17660/ActaHortic.1996.410.60*

Baskin JM, Baskin CC (2004). A classification system for seed dormancy. Seed Science Research 14:1-16. *https://doi.org/10.1079/SSR2003150*

Blažek J, Zelený L, Suran P (2022). Sweet cherry research world overview 2015-2017. Horticultural Science (Prague) 49:121-146. *https://doi.org/10.17221/70/2021-HORTSCI*

Blažková J, Erbenová M, Susová V (2003). Preliminary results using in vitro techniques for obtaining hybrid seedlings of sweet cherries. Scientific Papers of Pomology 18:67-76.

Çetinbaş M, Koyuncu F (2006). Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea. Horticultural Science (Prague) 33:119-123. *https://doi.org/10.17221/3750-HORTSCI*

Cruz-Lopes L, Dulyanska Y, Domingos I, Ferreira J, Fragata A, Guiné R, Esteves B (2022). Influence of pre-hydrolysis on the chemical composition of *Prunus avium* cherry seeds. Agronomy 12(2):280. *https://doi.org/10.3390/agronomy12020280*

Du Hyun K (2018). Practical methods for rapid seed germination from seed coat-imposed dormancy of *Prunus yedoensis*. Scientia Horticulturae 243:451-456. *https://doi.org/10.1016/j.scienta.2018.08.039*

Dulič J, Ogujanov V, Ercisli S, Miodragovič M, Barač G, Ljuboevič M, Dorič D (2014). *In vitro* germination of early ripening sweet cherry cultivars (*Prunus avium* L.) at different fruit ripening stages. Erwerbs-Obstbau 58:113-118. *https://link.springer.com/article/10.1007/s10341-016-0265-y*

Ferretti G, Bacchetti T, Belleggia A, Neri D (2010). Cherry antioxidants: from farm to table. Molecules 15: 6993-7005. *https://doi.org/10.3390%2Fmolecules15106993*

Finch-Savage WE, Clay HA, Dent KC (2002). Seed maturity affects the uniformity of cherry (*Prunus avium* L.) seed response to dormancy-breaking treatments. Seed Science and Technology 30:483-497.

Gradinariu G, Petre L, Corneanu G, Istrate M, Corneanu M, Gradinariu F, Budan S (2008). New sources of germplasm used in sweet cherry breeding at Iasi Fruit Research Station, Romania. Acta Horticulturae 795:141-145. *https://doi.org/10.17660/ActaHortic.2008.795.17*

ISTA (2019). International Rules for Seed Testing, Edition 2019, International Seed Testing Association, Bassersdorf, Switzerland.

Knapová P, Bílková A (2022). Antioxidant characteristics of sweet cherry cultivars bred in Holovousy. Scientific Papers of Pomology 28:10-18.

Kondo S, Gemma H (1993). Relationship between abscisic-acid (ABA) content and maturation of the sweet cherry. Journal of the Japanese Society for Horticultural Science 62:63-68. *https://doi.org/10.2503/jjshs.62.63*

Lang GA (2019). Achieving sustainable cultivation of temperate zone tree fruits and berries: Volume 2: Case studies. Burleigh Dodds Science Publishing Limited.

López-Corrales M, Mateos JR, Alarcón MV, Bañuls P, Pérez F, Serradilla MJ, Manzano MA (2014). Sweet cherry (*Prunus avium* L.) breeding program in Southern Spain. Acta Horticulturae, 1020:53-56. *https://doi.org/10.17660/ActaHortic.2014.1020.4*

Pipinis E, Milios E, Mavrokordopoulou O, Gkanatsiou C, Aslanidou M, Smiris P (2012). Effect of pretreatments on seed germination of Prunus mahaleb L. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 40:183-189. *https://doi.org/10.15835/nbha4027887*

Predieri S, Dris R, Sekse L, Rapparini F (2003). Influence of environmental factors and orchard management on yield and quality of sweet cherry. Journal of Food, Agriculture and Environment 1:263-266. *https://doi.org/10.1234/4.2003.377*

Procházka P, Štranc P, Pazderů K, Štranc J (2016). The influence of pre-sowing seed treatment by biologically active compounds on soybean seed quality and yield. Plant and Soil Environment 62:497-501. *https://doi.org/10.17221/655/2018-PSE*

Proietti S, Moscatello S, Villani F, Mecucci F, Walker RP, Famiani F, Battistelli A (2019). Quality and nutritional compounds of *Prunus cerasus* L. var. austera fruit grown in central Italy. HortScience 54:1005-1012. *https://doi.org/10.21273/HORTSCI13960-19*

Quero Garcia J (2019). Cherry breeding in the world: current analysis and future perspectives. Italus Hortus 26(1):9-20. *https://www.soihs.it/public/02/02/02%20Quero%20Garcia_01.pdf*

Şan B, Yildirim AN, Yildirim F (2014). An *in vitro* germination technique for some stone fruit species: The embryo isolated from cotyledons successfully germinated without cold pre-treatment of seeds. HortScience 49:294-296. *https://doi.org/10.21273/HORTSCI.49.3.294*

Selim HH, Ibrahim FA, Fayek MA, El-Deen SS, Gamal NM (2016). Effect of different treatments on germination of Romi red grape seeds. VITIS-Journal of Grapevine Research 20:115. *https://doi.org/10.5073/vitis.1981.20.115-121*

Stanys V (1997). *In vitro* techniques to increase the output of cherry seedlings from early-ripening parents. In: III International Cherry Symposium 468:203-208. *https://doi.org/10.17660/ActaHortic.1998.468.25*

Stein M, Serban C, McCord P (2020). Exogenous ethylene precursors and hydrogen peroxide aid in early seed dormancy release in sweet cherry. Journal of the American Society for Horticultural Science 146:50-55. *https://doi.org/10.21273/JASHS04952-20*

Tosun F, Koyuncu F (2007). Investigations of suitable pollinator for 0900 Ziraat sweet cherry cv.: pollen performance tests, germination tests, germination procedures, *in vitro* and *in vivo* pollinations. Horticultural Science (Prague) 34:47-53. *https://doi.org/10.17221/1851-HORTSCI*

Žďárská I, Suran P (2021). Využití *in vitro* kultur pro zvýšení klíčivosti semen raných třešní. [Use of *in vitro* cultures to increase seed germination of early ripening sweet cherry varieties]. In: Proceedings Seed and Seedlings, XV, CZU Prague, pp 24-30.

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