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Comparison of growth development of micropropagated and generatively reproduced wild cherry (*Prunus avium* (L.) L.) on the Polná demonstration plot (Czech Republic) up to the age of 15 years

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ABSTRACT: Four clonal progenies of wild cherry obtained using an *in vitro* technique and control progeny of generative origin were tested on the Polná research plot in southeastern Bohemia. Growth characteristics of cherry trees were determined each year during the period 2000–2010. At the age of 15 years, the progenies reach mean heights of 4–9 m and diameter at breast height (DBH) of 5–10.5 cm. The determined differences in height and DBH between progenies are statistically significant. Cherry trees of generative origin and progeny of clone No. 14 grow markedly slower in comparison with clones Nos 24, 26 and 28. The values of growth characteristics for verified clones are comparable with similar findings from abroad. The *in vitro* plantlets of wild cherry achieve similar growth characteristics as generative seedlings.

Keywords: forest trees; vegetative propagation; *in vitro*; clone testing; field trials

With gradual intensification of human activities influencing the environment, efforts have also been strengthened to maintain the existence of valuable populations of forest trees constituting an *in situ* base for maintaining the genetic diversity of forest stands. Today, populations of certain species (e.g. *Taxus baccata* Linnaeus and *Abies alba* Miller) constitute mere fragments of their previous occurrences (e.g. Zatloukal et al. 2013). For other species (e.g. *Malus sylvestris* Miller, *Pyrus pyraster* (Linnaeus) Burgsdorff, *Prunus avium* (Linnaeus) Linnaeus, *Sorbus* spp.), their scattered or rare occurrence has a natural character (e.g. Máchová et al. 2013). Such species are endangered by standard forest management methods, so it is therefore necessary to pay increased attention to their reproduction and application in forest regeneration.

Some of the more rarely occurring trees (*Sorbus torminalis* (Linnaeus) Crantz, *P. avium*, *P. pyraster*, etc.) also demonstrate the ability to produce very valuable assortments of wood if they receive proper care as part of their tending (e.g. Kupka 2007). Wood is an important industrial material, and thus individual genotypes are selected in forestry practice according to their productivity and resistance. Abroad (e.g. in New Zealand, Australia, the USA, Japan, France), even so-called clonal forestry is a widespread practice, which consists in ligniculture of several clones with short rotation periods (e.g. Libby et al. 1993).

More serious problems with natural and artificial forest stand regeneration may occur especially in populations under various unfavourable influences (industrial pollution, pathogen infestation, and the like). It is also frequently necessary to deal with natural physiological barriers to tree reproduction, for example age of commencing fructification, intervals between seed years, and others (e.g. Schmidt 2006; Molina et al. 2016).

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One possible solution to overcome these issues or simultaneously to increase forest management efficiency in Central Europe, including the Czech Republic is to reproduce trees using *in vitro* biotechnological methods (e.g. Chalupa 1979; Malá et al. 2012, 2013, 2014; Máchová et al. 2013). Micropropagation can produce high-quality material for standard planting as well as for establishing clonal archives and seed orchards, repatriation, storage in a tissue culture bank, monitoring of genetic variability, and so forth. It is necessary for forestry purposes that the regenerated progeny is genetically identical to the donor tree – so-called "true-to-type" (Ahuja 1987). Selection of high-quality individuals (ortets) is subjected to an approval process in the Czech Republic in accordance with Act No. 149/2003 Coll. From donor trees it is possible to nondestructively remove sufficient plant material (usually several buds or immature cones) for reproducing enough copies required.

Verification of growth properties of forest trees reproduced *in vitro* has been carried out for a number of years in forest and non-forest environments. In the Czech Republic, the first comparative experimental plots were established in 1994 in southern Bohemia (*Picea abies* (Linnaeus) H. Karsten, *Pseudotsuga menziesii* (Mirbel) Franco). Later, in various parts of the Czech Republic, *Quercus robur* Linnaeus, *P. avium*, *Sorbus domestica* Linnaeus, *S. torminalis*, *Sorbus avium* Linnaeus, *Tilia cordata* Linnaeus, *Populus tremula* Linnaeus, and others were also planted in this way (e.g. Jurásek, Malá 2000; Cvrčková et al. 2007; Dostál et al. 2010, 2011). Research to date has recorded no significant differences in morphological characteristics (stem form, type of branching), health status or height growth between sets of variously reproduced cherry trees. The objective of this paper is to compare the growth development of wild cherry trees of *in vitro* origin and of generative origin on the Polná experimental plot (southeastern Bohemia).

MATERIAL AND METHODS

To acquire plantlets of *in vitro* origin, the micropropagated clones of the wild cherry were selected from the tissue culture archive of the Forestry and Game Management Research Institute (FGMRI), Czech Republic (Table 1). A previously developed system was applied to ensure successful growth of plantlets. This consists in a step-by-step process of (*i*) induction of rhizogenesis of shoots in cuttings in an agar medium, (*ii*) planting the rooted cuttings into cultivation pots with perlite, and (*iii*) planting the plantlets into garden soil in tried and tested conical pots with the perforated bottom and equipped on the internal side with longitudinal risers promoting proper development of the root system and acclimatization of plantlets.

A portion of the clones from the FGMRI archive in Strnady was micropropagated in semi-commercial conditions of the Olešná laboratory (South Bohemia). All *in vitro* plantlets were then grown and adapted to the external environment in the Olešná tree nursery. The quality of the plantlets was evaluated based on the same criteria as required for the seedling material of generative origin (Czech National Standard ČSN 48 2115:1998, and/or its revision ČSN 48 2115 Z1).

Verification of the plantlet growth was performed on the Polná experimental plot near Jihlava (forest stand 19e1b) in southeastern Bohemia and owned by the Forest Syndicate in Polná (49°27'55.919"N, 15°42'31.371"E). The stand is situated at the elevation of 560–570 m a.s.l. It has a northern exposure and a 2% slope. Its bedrock is composed of biotitic paragneisses overlaid by mesotrophic to oligotrophic brown soils. The location is classified as a moderately warm climate region and a moderately humid highland subregion. Average annual temperature is 7°C, and average annual precipitation is 650–700 mm. Average vegetation period is 153 days. Typologically, the plot corresponds to the conditions of Natural Forest Area (provenance zone) 16 – Bohemian-Moravian Highlands (forest stand group 53 – acidic sites of higher elevations, forest site type 5S – Nutrient-medium Fir-Beech).

Two-year tissue culture plantlets were planted in spring 1998. For comparison purposes, the three-year commercial material of generative origin was also planted on the plot in autumn 1998. As for the

Table 1. Characteristics of evaluated clones

Clone No.	Locality	Forest stand No.	Plus tree No.	Natural forest area*	Forest altitudinal zone**
14	Zadní Hrobce hill, Špička	430f6	–	8	3
24			740137		
26	Žofina Huť	71b5	740139	15	4
28			740143		

*according to Czech legislation (Decree No. 139/2004 Coll.) 41 natural forest areas (provenance zones) are defined, **according to the typological system of the Czech Republic (Decree No. 83/1996 Coll.) nine forest altitudinal zones are defined

experimental design, the row planting was used with 2 × 2 m spacing. The rows of cherries are replicated in a different number, clones 26 and 28 (two replications), clones 14 and 24 (four replications), and generative progeny were planted in 13 rows. Each of the four wild cherry clones is represented on the plot by 40 *in vitro* plantlets and the generative material by 100 seedlings (i.e. a total of 260 trees). The plot was fenced off for the entire monitoring period.

Plantlet development was assessed each year during 2000–2010 with the exception of the year 2006 when the plot was not evaluated. The subject of the evaluation was initially survival rate and root collar thickness and later height and DBH. Root collar thickness (2000–2001) was measured using a sliding calliper (to the nearest 1 mm) and DBH (2007 to 2010) with a millimetre calliper. Height was determined at first using a measuring rod (to the nearest 1 cm) and starting from 2007 with a Vertex III ultrasonic altimeter (0.1 m accuracy; Haglöf Sweden AB, Långsele, Sweden).

To evaluate differences between the compared experimental units, Kruskal-Wallis one-way ANOVA was calculated followed by the Kruskal-Wallis multiple comparison Z-value test using the statistical programme NCSS 10, Version 10.0.10 (NCSS, LLC., Kaysville, USA). The nonparametric test was used because data assumptions were violated.

RESULTS

Fig. 1 shows changes in the number of growing individuals of relevant clones from the beginning of measurements. Generative progeny and clone No. 24 are the best in the given indicator.

Height was first measured at the age of 5 years in 2000 (Table 2, Fig. 2). The lowest growth was observed in generative progeny whereas the highest average height was reached by micropropagated clones originating from Žofina Huť (clones Nos 24, 26, 28). An analogous situation was repeated at 6 years of age in the next year. There was a change in 2002, when clone No. 14 from Zadní Hrobce first showed the fastest height growth. This trend has continued to the present day. The respective order of the best-growing clones from Žofina Huť has remained relatively unchanged since the beginning. In total, the height differences between groups of different origin (i.e. the three clones from Žofina Huť, clone No. 14, and the material of generative origin) increased with age and in 2010, at 15 years of age, reached 2–5 m. Kruskal-Wallis one-way ANOVA for each year of data collection rejects the null hypothesis of equal mean values of height at the $\alpha = 0.05$ level of significance. Results from the Kruskal-Wallis multiple comparison Z-value test for individual years are documented in Table 3.

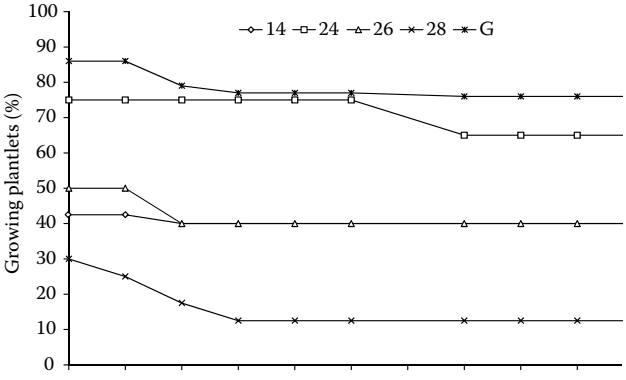

Fig. 1. Percentages of growing plantlets by progeny type and year on Polná research plot (14, 24, 26, 28 – clone No., G – commercial material of generative origin)

Diameter at breast height was evaluated on the plot at 12 years of age in 2007 (Table 2, Fig. 2). In this indicator, clones originating from the Žofina Huť basin stood out for the entire evaluation period, followed with a significant lag by the material of generative origin. For the entire period, the growth in thickness was demonstrated by clone No. 14 from Zadní Hrobce. Results from Kruskal-Wallis one-way ANOVA for DBH reject the null hypothesis in all evaluation years at the $\alpha = 0.05$ significance level. According to the Kruskal-Wallis multiple comparison Z-value test (Table 4), clones 24, 26 and 28 differed from clone 14 and the generative seedlings in all evaluation years. In 2007 and 2008, clone 28 demonstrated a difference from clone 14, but this difference was not recorded in subsequent years.

DISCUSSION

Findings from abroad (e.g. Meier-Dinkel 1997) have shown the growth character of wild cherry reproduced generatively and by micropropagation to be comparable. In experiments at the clonal level, for example Cornu and Chaix (1981) in France and Hammatt (1999) in the UK, however, larger heights were recorded in cherry trees of *in vitro* origin. The differences are explained by the genetic properties of the selected initial clones.

The quality of *in vitro* plantlets was validated by field trials, for example in poplar, cherry and walnut in France; birch or oak in Sweden and Norway (Pilate et al. 2002).

Table 2. Growth characteristics of wild cherry plantlets on Polná research plot

Progeny type	Year	Age	Live (pcs)	Mortality (%)	Height (m)			DBH (cm)		
					mean	SD	median	mean	SD	median
14	1998	3	40	–	–	–	–	–	–	–
	2000	5	17	58	0.79	0.07	0.85	0.88*	0.06*	1.00*
	2001	6	17	0	0.71	0.07	0.68	0.79*	0.06*	1.00*
	2002	7	16	6	1.79	0.20	1.84	–	–	–
	2003	8	16	0	1.96	0.25	2.07	–	–	–
	2004	9	16	0	2.11	0.27	2.35	–	–	–
	2005	10	16	0	2.46	0.32	2.80	–	–	–
	2007	12	16	0	3.23	0.38	3.70	3.81	0.64	4.00
	2008	13	16	0	3.53	0.38	4.20	4.02	0.68	4.25
	2009	14	16	0	3.74	0.43	4.40	5.00	0.79	5.50
	2010	15	16	0	4.21	0.53	4.95	5.19	0.83	5.65
24	1998	3	40	–	–	–	–	–	–	–
	2000	5	30	25	1.27	0.05	1.27	1.17*	0.04*	1.10*
	2001	6	30	0	1.30	0.05	1.31	1.18*	0.05*	1.10*
	2002	7	30	0	2.86	0.14	3.23	–	–	–
	2003	8	30	0	3.48	0.18	3.95	–	–	–
	2004	9	30	0	3.82	0.20	4.20	–	–	–
	2005	10	30	0	4.22	0.23	5.00	–	–	–
	2007	12	26	13	6.18	0.30	6.65	8.07	0.50	8.90
	2008	13	26	0	6.47	0.30	6.90	8.76	0.53	9.50
	2009	14	26	0	7.01	0.34	7.45	9.95	0.62	11.25
	2010	15	26	0	8.23	0.42	8.70	10.23	0.65	11.50
26	1998	3	40	–	–	–	–	–	–	–
	2000	5	20	50	1.35	0.07	1.40	1.04*	0.06*	1.00*
	2001	6	20	0	1.22	0.07	1.40	0.93*	0.06*	0.95*
	2002	7	16	20	3.29	0.19	3.30	–	–	–
	2003	8	16	0	3.91	0.25	3.98	–	–	–
	2004	9	16	0	4.27	0.27	4.30	–	–	–
	2005	10	16	0	4.73	0.32	5.00	–	–	–
	2007	12	16	0	6.39	0.38	6.80	8.66	0.64	8.85
	2008	13	16	0	6.72	0.38	6.88	9.25	0.68	9.10
	2009	14	16	0	6.95	0.43	6.88	9.67	0.79	9.60
	2010	15	16	0	8.86	0.53	9.40	10.55	0.83	10.80
28	1998	3	40	–	–	–	–	–	–	–
	2000	5	12	70	1.21	0.18	1.21	0.70*	0.17*	0.70*
	2001	6	10	17	0.96	0.09	0.76	0.88*	0.08*	0.80*
	2002	7	7	30	3.16	0.53	3.16	–	–	–
	2003	8	5	29	4.11	0.45	4.00	–	–	–
	2004	9	5	0	4.52	0.49	4.30	–	–	–
	2005	10	5	0	4.86	0.56	4.70	–	–	–
	2007	12	5	0	6.72	0.68	6.60	8.00	1.15	8.80
	2008	13	5	0	6.87	0.69	6.90	8.60	1.22	9.50
	2009	14	5	0	7.28	0.78	7.20	9.70	1.41	10.50
	2010	15	5	0	8.36	0.96	8.40	9.88	1.49	11.00
G	1998	3	100	–	–	–	–	–	–	–
	2000	5	86	14	0.48	0.03	0.45	0.48*	0.03*	0.50*
	2001	6	86	0	0.46	0.03	0.42	0.47*	0.03*	0.50*
	2002	7	79	8	2.09	0.08	2.20	–	–	–
	2003	8	77	3	2.44	0.12	2.80	–	–	–
	2004	9	77	0	2.78	0.12	3.10	–	–	–
	2005	10	77	0	3.18	0.14	3.55	–	–	–
	2007	12	76	1	4.43	0.18	5.05	4.51	0.31	5.20
	2008	13	76	0	4.63	0.18	5.25	4.85	0.31	5.40
	2009	14	76	0	5.06	0.20	5.70	5.81	0.36	6.00
	2010	15	76	0	6.01	0.25	6.90	6.02	0.38	6.20

*diameter of root collar (2000–2001), 14, 24, 26, 28 – clone No., G – commercial material of generative origin, SD – standard deviation

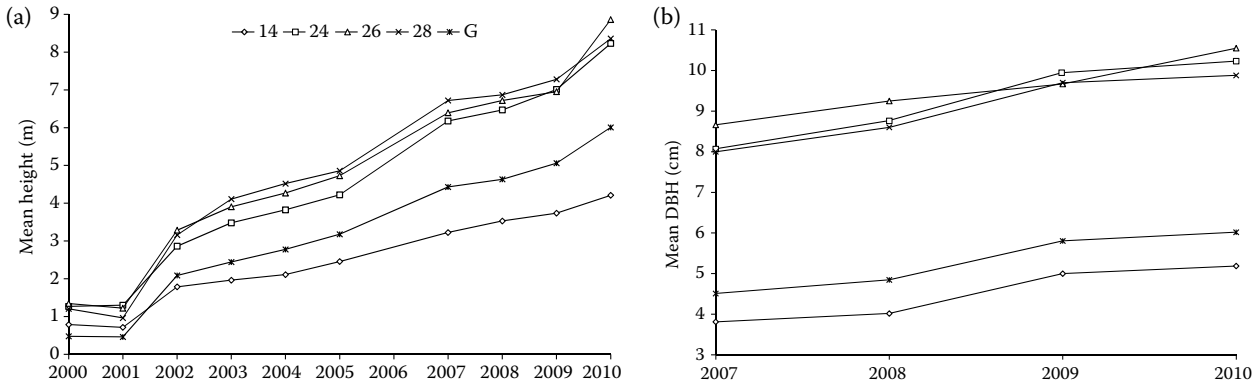

Fig. 2. Development of mean height (a), mean DBH (b) by progeny type and year on Polná research plot (14, 24, 26, 28 – clone No., G – commercial material of generative origin)

Equivalent or slower growth of *in vitro* plantlets has been observed with loblolly pine, peach and cherry (Frampton, Isik 1987; Navatel, Burrain 1989). Differences between *in vitro* wild cherry clones accounted for a large part of the observed variance in the traits measured (Ducci et al. 2006).

Jurásek and Malá (2000) considered the growth rates of generative and micropropagated plantlets to be equivalent. On the other hand, e.g. Bartoš et al. (2007) determined the better growth of *in vitro* cherry plantlets in comparison with generative ones on the agricultural land six years after plantation. In the previous measurement of the "Polná" plot also Cvrčková et al. (2007) determined highly significant differences in height growth in favour of *in vitro* plantlets. The new evaluation of the same plot presented in this paper performed at the level of individual clones (Tables 3 and 4) confirms that finding. Clone No. 14, whose origin differed from that of the other clones at Polná, grows markedly more slowly in comparison with the other clones, as do the generatively reproduced cherry trees. Negative values of height increments for clones 14, 26 and 28 in 2001 (Fig. 2) were caused by withering of terminal buds or death among taller individuals. This effect was not observed in the next years.

Comparison with domestic and foreign studies can be made with reference to specific values of growth variables. A detailed analysis of growth dynamics on research plots with wild cherry was performed by Stojecová and Kupka (2009). The periodic height increment over 7 years of monitoring was 1.9 m. The difference between suppressed (0.7 m) and dominant or co-dominant trees (2.4 m) was highly significant although those authors did not report a specific age of the juvenile experimental material. For the slowest growing clone No. 14, the height increment over 7 years (2002–2010) was 2.42 m, while for the best-growing clone No. 26 this increment was to 5.57 m. Of course, it is necessary to account for the fact that the growth conditions on the research plot are better than those in conventional stands.

According to mensuration tables (Anonymous 1989), values valid for European beech can be applied to wild cherry. For the best sites with quality class +1 the table height for 15 years of age exceeds 10 m. Progenies on the Polná plot reach mean heights of 4–9 m at that age (Fig. 2), thereby corresponding to quality classes 5 to +1.

Santi et al. (1998) evaluated the properties of 33 clonal wild cherry progenies at 7 years of age. These were progenies of selected trees from the northern part of France reproduced by an *in vitro* technique. At various study locations, average cherry tree heights ranged approximately between 2 m and 8 m. On the Polná plot, cherry tree heights at

Table 3. Kruskal-Wallis multiple comparison test (height)

Group	Difference from groups (α = 0.05)									
	2000	2001	2002	2003	2004	2005	2007	2008	2009	2010
G	14, 24, 26, 28	14, 24, 26, 28	24, 26	24, 26, 28	24, 26, 28	24, 26, 28	14, 24, 26, 28	14, 24, 26, 28	14, 24, 26, 28	14, 24, 26, 28
14	24, 26, G	24, 26, G	24, 26, 28	24, 26, 28	24, 26, 28	24, 26, 28	24, 26, 28, G	24, 26, 28, G	24, 26, 28, G	24, 26, 28, G
24	14, G	14, 28	14, G	14, G	14, G	14, G	14, G	14, G	14, G	14, G
26	14, G	14, G	14, G	14, G	14, G	14, G	14, G	14, G	14, G	14, G
28	G	24, G	14	14, G	14, G	14, G	14, G	14, G	14, G	14, G

G – commercial material of generative origin, 14, 24, 26, 28 – clone No.

Table 4. Kruskal-Wallis multiple comparison test (DBH)

Group	Difference from groups (α = 0.05)			
	2007	2008	2009	2010
G	24, 26, 28	24, 26, 28	24, 26, 28	24, 26, 28
14	24, 26, 28	24, 26, 28	24, 26, 28	24, 26, 28
24	14, G	14, G	14, G	14, G
26	14, G	14, G	14, G	14, G
28	14, G	14, G	14, G	14, G

G – commercial material of generative origin, 14, 24, 26, 28 – clone No.

7 years of age reached 2–3 m, which is the lower level of growth recorded for French clones. This also confirms that systematic selection of the reproductive material from a larger area leads to more significant results. In the Czech Republic, horizontal transfer of reproductive material across natural forest areas 1 to 34 (i.e. within most of the state's territory) is acceptable for wild cherry by Czech legislation (Decree No. 139/2004 Coll.). Of course, it is also necessary to adhere to the rules for vertical transfer.

A total of 19 clones of wild cherry originating from Belgium were evaluated by Curnel et al. (2003). The specimens constituted 119 *in vitro* plantlets planted originally at a total of 14 locations. Height reached 100.5 cm at 2 years, 239.9 cm at 5 years, and 516.4 cm at 10 years. Large differences between locations were observed. On the Polná plot, heights at 10 years ranged between 2.3 m and 5 m. In comparison with the material from Wallonia, the growth of clones Nos 24, 26, and 28 was quite comparable while the progeny of clone No. 14 and the generatively reproduced cherry grew more slowly.

Kupka (2007) published results for the growth of wild cherry originating from Natural Forest Area 17 – Polabí on a research plot established in 1998 in the same natural forest area at 370 m a.s.l. (forest site type 2K, i.e. Acidic Beech-Oak, average annual temperature 10.1°C, average annual precipitation 650 mm). The average height of unpruned cherry trees was 2.6 m at 12 years, 2.9 m at 13 years, 3.2 m at 14 years, 3.3 m at 15 years, 3.5 m at 16 years, and 4.0 m at 17 years. Breast height diameter of the same trees was 1.9 cm (12 years), 2.6 cm (13 years), 3.0 cm (14 years), 3.2 cm (15 years), 3.4 cm (16 years), and 3.8 cm (17 years). On the Polná plot, all monitored progenies at 15 years reached greater average heights and DBH.

The study of the German authors Springmann et al. (2011), who evaluated cherry trees on a research plot on former farmland in the Rhineland (200 m a.s.l., average annual temperature 10°C, average annual precipitation 690 mm), enables yet another comparison. Average height of unpruned cherries under the given conditions reached 7.0 m at 15 years and 8.3 m at 16 years. Average DBH was 6.6 cm at 14 years, 8.3 cm at 15 years, and 10.0 cm at 17 years. The acquired data are comparable with the best-growing clonal progenies on the Polná plot. The outstanding growth of cherries in the Rhineland may be attributed, among other factors, to better climatic conditions.

Hajnala et al. (2007) performed clonal tests with clones of wild cherry plus trees. *In vitro* plantlets were planted in the period 2000–2001 in 11 locations at three forest districts in South Bohemia, but only six of these tests could be evaluated. A 6-year-old clonal trial with 13 clones was evaluated during the summer 2004. Observed traits were stem height, stem diameter (at the base), health status, and mortality. At this age, only one clone outperformed the remaining ones in volume production.

CONCLUSIONS

On a research plot with verified clonal progenies of wild cherry reproduced using an *in vitro* technique and with generatively reproduced material, statistically significant differences were determined in height and DBH. Growth characteristics recorded at 15 years of age document slower growth among generative seedlings and micropropagated clone No. 14 in comparison with clones Nos 24, 26, and 28. Comparison with works of other authors demonstrated that the growth characteristics of verified micropropagated clones are comparable, including those reported from abroad. It appears that suitable selection of clones may prove highly effective in efforts to increase volume production in wild cherry trees. Combined with a focus on morphological characteristics, such practice may result in significant economic benefits to the forest owners. Cherry trees of *in vitro* origin have been planted also at other experimental plantations in the Czech Republic. They will still be evaluated collectively with the accent on qualitative characteristics in the coming years.

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