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ORIGINAL PAPER

Early growth of saplings of selected chestnut (*Castanea* spp.) taxa raised in vegetation cells, hydrogel enriched substrate, or soil

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ABSTRACT

Climate change mitigation strategies, which include distributing ecological risk over a greater number of tree species in forest ecosystems, increase the attractiveness of cultivating chestnut in selected areas of Slovakia. Artificial restoration practices are important tools for preservation and improvement of the current status of chestnut. However, ongoing technology advances have tended to provoke their revision and actualization. The aim of this study was to examine the effect of sowing on the early growth of saplings of selected chestnut taxa. Chestnut seeds collected from *Castanea mollissima* (*C. moll.*), *C. crenata* (L7) and hybrids *C. crenata* × *C. sativa* (C6, B12) were germinated and sowed into the following treatments: vegetation cells (VC), growing substrate enriched by hydrogel (H), or loam soil (C), then raised under standard cultivation conditions over one growing season. During the growing season, the aboveground height was measured every 2 weeks. Despite slightly higher values of height of the aboveground parts of saplings *C.moll.*, B12, and C6 grown in VC, and L7 grown in H, than saplings grown in C, analysis of variance did not reveal these differences as statistically significant. On the other hand, significant differences were observed in the aboveground height of the chestnut taxa. Saplings of *C. moll.* (28.9 cm) and L7 (25.3 cm) grew significantly taller than hybrids C6 and B12 (17.3 cm and 13.2 cm). Our findings suggest that cultivation of chestnut saplings in VC or H under standard growing conditions does not stimulate growth in height during early growth stages.

KEY WORDS

chestnut hybrids, direct sowing, forest restoration, juvenile, large seeds

Introduction

In Slovakia, the presence of chestnut trees can be dated to the 17th century. Introduction of this non-native tree species into the new territories contributed to the extension of local sources of raw material, mainly food supply but also wood and bark (Tokár, 2007; Bolvanský *et al.*, 2008). Nowadays, another important function is the contribution chestnut trees can make in distributing

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ecological risk over a greater number of tree species (Grossiord, 2019). Thus, in areas most affected by climate change, predominantly in the southern parts of Slovakia (Fendeková *et al.*, 2017), cultivation of chestnut trees has become attractive again.

The current status of chestnut populations in Slovakia could be improved by wider use of artificial regeneration techniques (Fitzimons and Oram, 2006; Tokár, 2007; Corredoira *et al.*, 2017). While commercial production of planting material is focused predominantly on horticulture, characteristics of chestnuts including a high germination rate (exceeding 85%), the large size of seeds, and a long radical root, make direct seeding in forestry applications easier and less labour-intensive (Bolvanský *et al.*, 2007; Benedetti *et al.*, 2012; Corredoira *et al.*, 2017). However, the fate of the sapling after depletion of supplies stored in the endosperm of the seed is determined by interactions with the environment (*e.g.*, substrate, water, temperature, and other living organisms) (Grossnickle, 2012). Recently, increases in temperature and drought frequency during the growing season has drawn attention to development of strategies to improve adaptability of seedlings and saplings through an extended period after planting (Crous, 2017; Tučeková *et al.*, 2018; Repáč *et al.*, 2021).

Recent studies suggest that application of special substances called hydrogels, which retain and provide plentiful water for plants, would be an efficient means for mitigation of seedling vulnerability to water supply imbalance during early growth stages (Crous, 2017). According to Landis and Haase (2012), improved management of water balance after hydrogel application also can be connected with the formation of a layer with properties similar to natural polymeric mucilage produced by healthy roots. However, Bowman *et al.* (1990) and Mikkelsen (1994) suggested that adsorption capacity as well as the lifespan of the hydrogel molecule (specified by the manufacturer usually for laboratory conditions) can be markedly modified by selected attributes of environment in the field. Hence, the demand for a more reliable concept for improving early growth of tree plantings led to more advanced technology, *i.e.*, direct sowing into the vegetation cells, which technology was successfully verified in tough conditions of reforestation of large salvage-felled areas (Tučeková, 2011; Tučeková *et al.*, 2018). A vegetation cell consists of substrate, vegetative cover, or other optional compounds (*e.g.*, hydrogel, fertilizer, growth regulators, stimulators) that provide favourable physical and chemical conditions, as well as a suitable hydrothermal regime for development of inserted seed (Tučeková, 2011). Furthermore, walls of the vegetation cell could protect against negative environmental factors or biological pests (*e.g.*, weeds) (Tučeková, 2011; Tučeková *et al.*, 2018).

Although chestnut trees could contribute to more diverse forests, and hence reduce overall ecological risk under climate change, recently developed restoration practices for chestnut have not been thoroughly investigated in Slovakia as yet. The aim of this study is to examine the effects of sowing treatments on the early growth of saplings of selected chestnut taxa.

Material and methods

The experiment was established in field conditions of the research plot of the Slovak Academy of Sciences in Nitra, Slovakia (48°18'28" N, 18°05'40" E). Chestnut nuts of similar size collected from four maternal trees (10 nuts from each maternal tree) (Table 1) in October 2018 were pregerminated and sown on 12th March 2018 with the germ 3-8 cm long using the following sowing treatments:

i) Sowing into vegetation cells (VC) (40 seeds)

ii) Sowing into the prepared sowing substrate with admixed hydrogel (H) (40 seeds)

iii) Sowing into loam soil (sand 50%, silt 40%, clay 10%) (C) (40 seeds)

Regardless of sowing treatment, germinated saplings were irrigated only in March and April with irrigation dose of 6 l m^{-2} applied twice a week. In May, June, and July no additional water was used; atmospheric precipitation during these months was sufficient (Table 2). The growing substrate used in the experiment was prepared by a commercial manufacturer and consisted of a mixture of peat and silicic sand. VC used in the experiment consisted of plastic tubes (length 15 cm, diameter 8 cm) filled with growing substrate and capped by a plastic cover. The installation of vegetation cells was carried out according to a standard protocol described by Tučeková (2011): a manually dug hole (depth 10 cm, diameter 8 cm) was filled with a 5 cm layer of growing substrate. The seed was placed on the layer of growing substrate, then covered by another layer of growing substrate and encased by a plastic tube capped with a plastic cover.

The application of hydrogel was carried out by mixing 1000 g of dry granules of STOCKOSORB Micro hydrogel with 40 l of growing substrate (25 g dm^{-3}). Hydrogel enriched substrate was put into the 40 seed holes with dimensions 10×10×10 cm prepared within the seedbed.

Seeds in the experimental sowing treatments were arranged in rows spaced 70 cm apart. In each row, 10 nuts from individual maternal trees in the row of *C.moll*, B12, L7, and C6 were planted in this order. The distance among the seeded nuts in a row was 20 cm. Seedling assessment was focused on detailed description of height growth of saplings, during June and July, a period characterized by completion of the most intensive growth processes. The first record of the aboveground height of saplings was first recorded on 5 June, then subsequently on 19 June, 4 July, 17 July, and 31 July.

Two-way analysis of variance with repeated measures followed by Tukey's studentized range test (HSD) (p<0.05) was used to detect significance of differences in sapling height, with

Table 1.

List of seed sources of chestnut nuts used in the experiment. The nuts of all maternal trees come from open pollination

Maternal tree	Origin	Locality
C. crenata (L7)	Pontevedra, Spain	Príbelce, Slovakia chestnut orchard
C. mollisima (C.moll.)	Dolenje, Slovenia	Nitra, Slovakia private garden
C. sativa × *C. crenata* (C6)	*C. sativa* Pontevedra, Spain *C. crenata* Pontevedra, Spain	Príbelce, Slovakia chestnut orchard
C. sativa × *C. crenata* (B12)	*C. sativa* Tlstý vrch, Slovakia *C. crenata* Pontevedra, Spain	Príbelce, Slovakia chestnut orchard

Table 2.

Monthly sum of precipitation totals and average monthly temperature over the experimental period (March-July of 2018). Data were derived from the local meteorological station of the University of Agriculture in Nitra, less than 1 km from the research plot

Month	Total sum of precipitation [mm]		Average temperature [°C]	
	2018	normal (1981-2010)	2018	normal (1981-2010)
March	40	44	3.5	5.1
April	37	43	15.4	11.0
May	92	68	19.1	16.0
June	89	69	21.3	18.8
July	81	73	22.5	21.0

sowing treatment, chestnut taxa, and their interaction as fixed effects. Statistical analysis was performed using STATISTICA 12 (StatSoft, Inc., Tulsa, USA).

Results and discussion

Although analysis of variance did not reveal a significant effect (p=0.089) of sowing treatment or chestnut taxa × sowing treatment interaction (p=0.172) on the height growth of chestnut saplings, the results of this study suggest that VC as well as H might provide more favourable conditions for early development of selected chestnut taxa (Fig. 1). *C. mollissima* (*C.moll.*) and hybrids *C. sativa* × *C. crenata* (B12 and C6) reacted similarly and grew best in VC. On the other hand, *C. crenata* seedlings (L7) grew best in the H, whereas plants in VC and the C variant reached lower values of height of the aboveground parts (Fig. 2). The highest values of aboveground height recorded for VC treatment of chestnut saplings (*C.moll.*, and C6) (Fig. 2) are in accordance with findings of Tučeková *et al.* (2018) and Tučeková and Takáčová (2018), that also documented better growth of seeds sowed into VC for European beech on windthrow areas in Kysucké Beskydy Mts. and improved survival for European oak, black walnut, and common walnut on sites with salinized soils. Moreover, Tučeková *et al.* (2018) also reported that European beech saplings raised in the VC were capable of producing higher growth increments than one year old containerized seedlings four consecutive years after plantation establishment.

In this study, despite regular irrigation of cultivated saplings, *C. crenata* saplings grown in H tended to reach slightly higher values of the aboveground part than individuals cultivated in C (Fig. 2). Under non-limiting water conditions, Salaš (1996) and Puhlová and Šmelková (1998) suggest that the positive effect of hydrogel on plant growth could be associated with a more balanced moisture level of the growing medium as well as lower risk of seed drying. Further explanation may be connected with observations that particles of hydrogel that are in close contact with the plant roots form a layer with properties similar to natural polymeric mucilage produced by healthy roots (Landis and Haase, 2012). Carminati and Moradi (2010) observed that this mucilage weakens the drop in water potential at the root-soil interface, increasing the conductivity of the flow path across soil and roots and reducing the energy needed by the plant to take up water.

The negligible effect of VC or H on the early aboveground growth in height of cultivated chestnut taxa in this study is partially a consequence of favorable growing conditions. According to our study findings, even loam soil was able to provide an appropriate setting for satisfactory height growth of chestnut saplings. Taking into account our results as well as findings published in other studies (Beniwal *et al.*, 2011; Orikiriza *et al.*, 2013; Tučeková *et al*,. 2018) the beneficial effects of hydrogel and VC probably would have more distinctive effects on development of

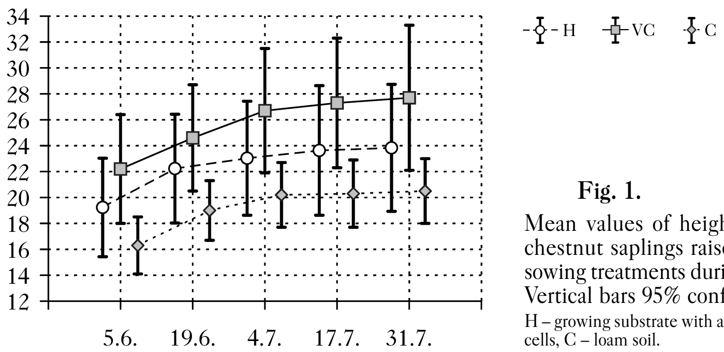

Fig. 1.

Mean values of height of aboveground part of chestnut saplings raised from seeds in different sowing treatments during the first growing season. Vertical bars 95% confidence intervals.

H – growing substrate with admixed hydrogel, VC – vegetation cells, C – loam soil.

reproductive material of tree species in harsher environmental conditions (Beniwal *et al*., 2011; Orikiriza *et al*., 2013; Crous, 2017; Tučeková *et al*., 2018).

The remarkable fact in this study was the difference in the growth of aboveground part of cultivated chestnut taxa. In this study saplings of chestnut taxa reached significantly different height of aboveground part ($p<0.001$) during the first growing season. The highest values were recorded for *C. mollissima* (*C. moll.*) 28.9 cm, and *C. crenata* (L7) 25.3 cm, while hybrids of *C. sativa* × *C. crenata* (B12 and C6) were significantly smaller and their average aboveground height reached only 17.3 cm and 13.2 cm (Fig. 2). Similar growth dynamics of juvenile *C. sativa* × *C. crenata* individuals, characterized by values for aboveground height from 9.5-13.8 cm, was observed also by Bolvanský *et al*. (2007).

In spite of encouraging results concerning early growth of chestnut saplings raised from seed in this study, direct seeding of chestnut on a larger scale should be practiced very cautiously. High attractiveness of chestnut seeds to rodents observed also in our experiment as well as by Skousen *et al*. (2013) may lead to significant damage. In extreme situation described by Skousen *et al*. (2013) with only six germinated seeds out of 250 sowed, almost complete destruction of seeded material can be experienced.

Conclusion

The results of this study show that sowing of chestnut seeds into vegetation cells as well as hydrogel enriched substrate do not necessarily improve the height growth of saplings raised under

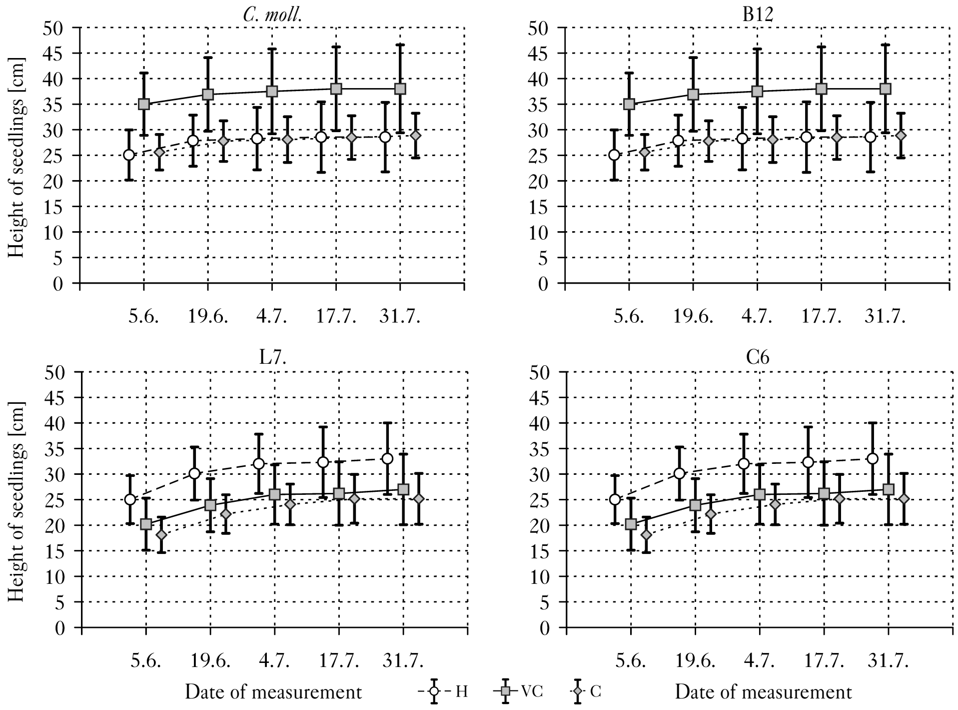

Fig. 2.

Mean values of height of aboveground part of *C. mollissima* (*C. moll.*), *C. sativa* × *C. crenata* (B12), *C. crenata* (L7) *C. sativa* × *C. crenata* (C6) saplings raised from seed in different sowing treatments during the first growing season. Vertical bars denote 95% confidence intervals.

H – growing substrate with admixed hydrogel, VC – vegetation cells, C – loam soil non-limiting cultivation conditions. However, slightly better growth of *C. mollisima* (*C. moll.*) and hybrids *C. sativa* × *C. crenata* (B12, C6) in vegetation cells as well as *C. crenata* (C7) in hydrogel-enriched substrate compared to individuals raised in loam soil may suggest potentially beneficial effects of testing sowing treatments under more harmful environmental conditions. The remarkable finding of this study concerning distinctive differences in early growth rate of selected chestnut taxa, as well as the variability of interaction with formulated sowing treatments implies differential adaptability of chestnut saplings that would deserve further investigation.

Authors' contribution

M.P. and J.P. – conceived of the presented idea; J.P. – developed the theory and performed the computations; M.B. and A.T. – verified the analytical methods; A.T. – encouraged; M.P. – to investigate and supervised the findings of this work; All authors – discussed the results and contributed to the final manuscript.

Conflicts of interest

The authors declare the absence of potential conflicts of interest.

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STRESZCZENIE

Wczesny przyrost wysokości sadzonek różnych taksonów kasztana (*Castanea* spp.) hodowanych w komórkach wegetacyjnych, na substracie wzbogaconym hydrożelem i w glebie

Wprowadzenie strategii łagodzenia zmian klimatu, w tym promowanie rozproszenia ryzyka ekologicznego na większą liczbę gatunków drzew w ekosystemach leśnych, zwiększa również atrakcyjność uprawy kasztana na wybranych obszarach Słowacji. Sztuczne zabiegi odnawiania stanowią ważne narzędzie zachowania i poprawy obecnego stanu kasztanów, jednak stały postęp technologiczny powoduje, że istnieje potrzeba ich rewizji i aktualizacji.

Najnowsze badania sugerują, że zastosowanie specjalnych substancji zwanych hydrożelami, które zatrzymują duże ilości wody i dostarczają ją roślinom, stanowiłoby skuteczny sposób łagodzenia wrażliwości siewek na brak równowagi w zaopatrzeniu w wodę w ich wczesnym stadium wzrostu. Jednak na zdolności adsorpcyjne i trwałość cząsteczek hydrożelu w terenie znaczący wpływ wywierają niektóre cechy środowiska. Dlatego też zapotrzebowanie na mniej zawodne koncepcje poprawiające wczesny wzrost materiału sadzeniowego gatunków drzew doprowadziło do opracowania bardziej zaawansowanej technologii bezpośredniego siewu do komórek wegetacyjnych – indywidualnych plastykowych osłonek o wymiarach 10×5 cm zagłębionych w glebie i wypełnionych podłożem oraz innymi opcjonalnymi składnikami (np. hydrożel, nawozy, regulatory wzrostu), zapewniających korzystne warunki fizyczne i chemiczne, a także reżim hydro-

termiczny dla rozwoju zasianych nasion. Technologia ta była opracowana i wstępnie testowana w Republice Czeskiej w początkach XXI wieku. Głównym celem pracy było zbadanie wpływu różnych sposobów siewu, w tym stosowania hydrożelu i komórek wegetacyjnych, na wczesny wzrost sadzonek różnych taksonów kasztana. Nasiona kasztana, zebrane z 18-letnich osobników *Castanea mollissima* (*C. moll.*), *C. crenata* (L7) i mieszańców *C. crenata* i *C. sativa* (C6, B12) (tab. 1), skiełkowano i wysiano na następujących poletkach badawczych: komórki wegetacyjne (VC), podłoże wzbogacone hydrożelem (H) oraz gleba gliniasta (C). Komórki wegetacyjne mają postać indywidualnych osłonek w kształcie rurki o długości 15 cm i średnicy 8 cm, w których na warstwie substratu grubości 5 cm wysiewane są 2-3 nasiona przykrywane kolejną warstwą substratu, piasku, perlitu lub gleby, a następnie zamykane przykrywką z plastyku lub siatki w celu ochrony przed gryzoniami i ślimakami. Następnie przygotowany w ten sposób materiał hodowano w standardowych warunkach uprawy przez jeden rok. W okresie wegetacji wysokość części nadziemnej mierzono co 2 tygodnie. Istotność różnic w wysokości części nadziemnej siewek w zależności od sposobu siewu, taksonu kasztana i ich interakcji jako efektów stałych oceniano za pomocą dwuczynnikowej analizy wariancji z powtórzonymi pomiarami, a następnie testu Tukeya opartego na studentyzowanym rozstępie (HSD) ($p<0,05$). Chociaż analiza wariancji nie wykazała istotnego wpływu ($p=0,089$) sposobu wysiewu, jak również interakcji jednostki taksonomicznej kasztana i sposobu wysiewu ($p=0,172$) na przyrost wysokości ocenianych sadzonek kasztana, to wyniki badań sugerują, że zarówno poletko VC, jak i H stwarzają korzystniejsze warunki dla wczesnego rozwoju wybranych taksonów kasztana (ryc. 1, 2). *C. mollissima* (*C. moll.*) oraz mieszańce *C. sativa* i *C. crenata* (B12 i C6) reagowały podobnie i najlepiej rosły w przypadku poletek VC. Z kolei siewki *C. crenata* (L7) najlepiej rosły na podłożu wzbogaconym hydrożelem H (ryc. 2). Istotnym stwierdzeniem w tych badaniach była różnica we wzroście części nadziemnej taksonów uprawianego kasztana. Sadzonki ocenianych taksonów kasztana w pierwszym sezonie wegetacyjnym osiągały istotnie różną wysokość części nadziemnej ($p<0,001$). Najwyższe wartości odnotowano dla *C. mollissima* (*C. moll.*) – 28,9 cm i *C. crenata* (L7) – 25,3 cm, natomiast mieszańce *C. sativa* i *C. crenata* (B12 i C6) były istotnie mniejsze, a ich średnia wysokość części nadziemnej osiągnęła zaledwie 17,3 cm i 13,2 cm (ryc. 2). Podsumowując: uprawa sadzonek kasztana na poletkach VC lub H w nieograniczonych warunkach uprawy nie ma istotnego wpływu stymulującego na przyrost wysokości we wczesnej fazie wzrostu. Jednak wyraźne różnice we wczesnym tempie wzrostu badanych taksonów kasztanów, a także pewna zmienność interakcji z określonymi metodami siewu mogą sugerować odmienne zdolności adaptacyjne sadzonek badanych taksonów kasztanów, co wymagałoby dalszych badań.