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IN VITRO PROPAGATION RESULTS OF *SORBUS ARIA* 'GRAN SASSO'

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ABSTRACT
*Sorbus*es are deciduous trees or shrubs belonging to the family *Rosaceae*, mainly used as ornamental or medicinal, fruit plant. In this work, *in vitro* sterile culture of *Sorbus aria* 'Gran Sasso' was established on half-strength MURASHIGE AND SKOOG (MS, 1962) basal medium, and examined its shoot and root productions. The ideal cytokinin concentration and type for *in vitro* shoot development (with more than five shoot per plant specimen) were 0.4 and 0.8 mg L^{-1} BA or BAR, lower dosages or other cytokinins (especially kinetin - KIN) was not effective, however, KIN resulted the longest and widest leaves. After a short (48-hour) period of root stimulation on an induction medium containing 15 mg L^{-1} IBA auxin, the hormone-free medium containing 0.75 g L^{-1} activated charcoal proved to be the best, but even in this case, it was not possible to achieve a rooting rate better than 36.7%. Silver nitrate was not resulted rooting in every dose, although higher concentrations (2 and 5 mg/l, respectively) decreased average shoot number and effectively increased shoots' length and leaf sizes.

Keywords: *Sorbus*, shoot and root development, plant hormones, silver nitrate

INTRODUCTION

*Sorbus*es (commonly known as rowans or sorbs) occur in the northern temperate zone, widespread in areas with different ecological conditions and therefore, *Sorbus* taxa have diverse botanical characteristics, especially their leaves are varied (simple, undivided or pinnately compound with several leaflets) not only within the species/subspecies/hybrids but also in the individual specimens. Certain areas (for example in the Carpathian Basin), several natural hybrids (originated from wild population of *S. aucuparia, S. aria, S. torminalis* crossing) show large diversity and give great possibilities for gardening uses. Because of their attractive white flowers and usually colourful (mainly orange, red) fruits, sorbs are popular ornamental plants suitable in gardens, public parks or streets (TÓTH, 2012). In Asia, Europe and North America, folk medicine use the fruits, leaves and bark and their extracts to treat health problems. The use of *S. domestica, S. aucuparia, S. torminalis* and *S. aria* species as herbs is known in Europe (SOŁTYS ET AL., 2020). Fresh or preserved fruits (and food products like jam, vinegar, wine etc.) of Asian (*S. commixta, S. pohuashanensis*) and European (*S. aria, S. aucuparia, S. domestica,, S. torminalis*) species were also consumed (TARDÍO ET AL., 2006; 2012; LI ET AL., 2014; LEE ET AL., 2017).
Conventionally, wild and apomictic species (in Hungary: *S. bakonyensis, S. borbasii, S. borosiana, S. degenii, S. dacica, S. redliana, S. vertesensis* etc.) with high fruit yields were propagated by sowing (TÓTH, 2012). The main problems of this propagation way are the low germination rate and the absence of uniformity among the seedlings, which usually grow slow and start to produce fruits later (MIKO AND GAŽO, 2004). Most of the sorb cultivars (especially with strange canopy shape or with low seed production) are grafted onto different rootstocks (*Pyrus pyraster, Crataegus monogyna* or *Sorbus aucuparia, S. intermedia*). However, certain sorb taxa need special rootstock because of the different grafting results and sometimes, graft incompatibility causes poor growth and shorter lifespan of the scion (SCHMIDT AND TÓTH, 2004; TÓTH, 2012). Cutting propagation is

difficult because of the cuttings's poor rooting; young mother plants often give better, juvenile shoots with higher rooting ratios (HANSEN, 1990; SCHMIDT ET AL., 1995; SCHMIDT, 1997). Despite their importance and popularity, relatively few *Sorbus* taxa were micropropagated regularly, mainly *S. aucuparia* (CHALUPA, 1988, 2002; LALL ET AL., 2006) and *S. domestica* (ARRILLAGA ET AL., 1991, 1995; NIKOLAOU ET AL., 2008).
Now, the aim of this study was to find morphological differences between the *in vitro* multiplied and rooted *Sorbus aria* 'Gran Sasso' plants according to the media accessories (cytokinins, auxins, silver nitrate).

MATERIAL AND METHOD

Plant material, media, culture conditions

The sorb variety studied was *Sorbus aria* 'Gran Sasso', a drought tolerant, durable small tree with egg-shaped crown, round (upside: dark green, underside: white, pubescent) leaves, white flowers and red fruits, originated from the Gran Sasso d'Italia, selected and bred by Elemér Barabits in 1993 (TÓTH, 2012).
As initiation, hardwood twigs (harvested in early April) and newly developed, actively growing softwood shoots (gathered in mid-May) were collected from mature trees (more than 20-25 years old) located in the Buda Arboretum. Twigs and defoliated shoots were cleaned, chopped into single nodes or shoot tips and after washing with running tap water for one hour, soaked in 50 v/v % sodium hypochlorite for 5 minutes, followed by 70 v/v % of ethanol for 10 minutes and finally, rinsed in sterile distilled water three times. The starting medium was MS (MURASHIGE AND SKOOG, 1962) with ½ macronutrient concentrations, 20 g L^{-1} sucrose, 5.5 or 7 g L^{-1} agar, 0.05 mg L^{-1} IBA and 0.25 mg L^{-1} BA. Main phases of initiation were shown on *Figure 1*.
In the next step (*in vitro* multiplication), the same ½ MS basic medium was supplemented with four doses (0.1, 0.2, 0.4 and 0.8 mg L^{-1}) of four types of cytokinins: MT (meta-topolin), KIN (kinetin), BA (benzyladenine), BAR (benzyladenine-riboside), formed totally 16 groups with 55-60 individuals.
During *in vitro* rooting, the same number of shoots/group were established, with the use of other stock that included shoots previously grown on hormone-free ½ MS medium until 1.5 month. After that (as short induction phase lasted 48 or 72 hour), all shoots kept on ½ MS media containing 8 g L^{-1} agar, 20 g L^{-1} sucrose and 15 mg L^{-1} IBA, followed by a long, 2-3 or 4-month rooting period with almost the same, but hormone-free media supplemented with 0.5, 0.75 or 1 mg L^{-1} AC (activated charcoal, *Figure 2*). In another trial, shoots (from the same stock grown on ½ MS media without plant growth regulators) were transplanted onto ½ MS media with 8 g L^{-1} agar, 20 g L^{-1} sucrose plus 0.1 mg L^{-1} IBA, 0.5 mg L^{-1} MT and four levels (0.5, 1, 2 and 5 mg L^{-1}) of silver nitrate, in order to stimulate rooting.
In case of all media, the pH was adjusted to 5.6 with KOH (potassium hydroxide) and autoclaved for 35 minutes on overpressure (10^5 Pa). *In vitro* cultures were maintained at 22 ± 2°C under 16/8 photoperiod with a photosynthetic photon flux density of 40 µmol m^{-2} s^{-1}.

Data and statistical analysis

Two-four months after transplanting, morphological data (shoot and root number, leaf, shoot and root length, rooting ratio) were recorded. Data were evaluated by SPSS 23.0 (IBM Corp., USA). An analysis of variance (ANOVA) was conducted to calculate the

statistical significance of all data presented. When significant differences between treatments were found, the means were separated by Tukey's test at $p \leq 0.05$.

Figure 1. Initiation steps of *Sorbus aria* 'Gran Sasso'. Prepared from defoliated shoots (A), buds were washed with running tap water (B), disinfected with chemical agents such as 70 v/v % ethanol (C), followed by rinsing in sterile distilled water (D). After drying out of the buds (E), the explants were transplanted onto ½ MS medium (F)

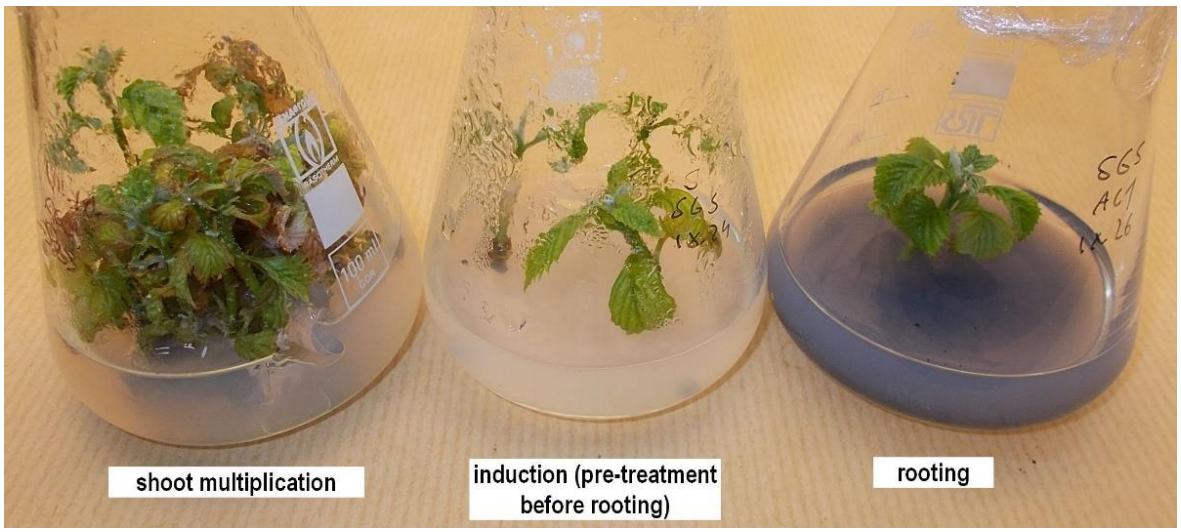

Figure 2. Rooting steps of *Sorbus aria* 'Gran Sasso'

RESULTS

Initiation

Softwood shoots were better for initiation, because of the easier cutting (hardwood twigs have massive, thick bark on their surface that make difficulties during single node-preparation) and higher sterilization ratio (90.9%). In case of hardwood twigs, only 41.8% of the buds were sterilized successfully. Healthy buds sprouted 2-3 weeks later after the

disinfection, and produced several lateral shoots (*Figure 3*) during the next month. These new parts were used for the next step (multiplication).

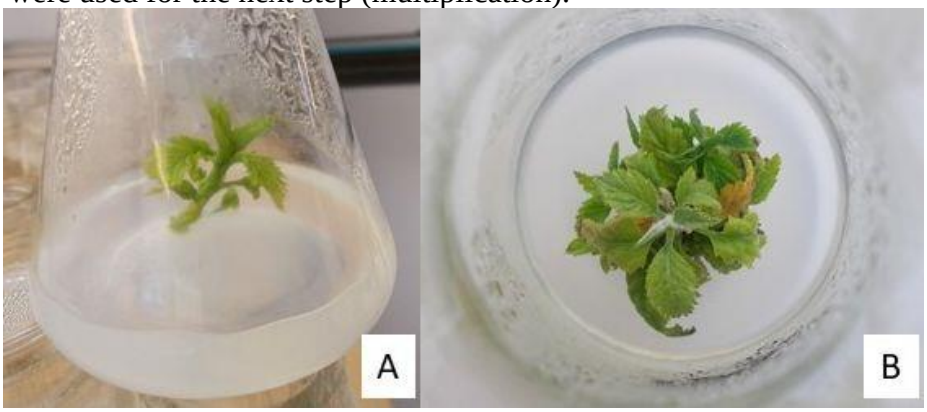

Figure 3. Successfully sterilized buds sprouted (A) and developed lateral shoots (B)

Multiplication

Most of the cytokinins resulted significantly more and longer shoots comparing with the hormone-free control, excepting KIN (in all doses) and the lowest concentration (0.1 mg L^{-1}) of BA or BAR (*Figures 4-6*). Higher cytokinin-levels usually increased shoot number, and the best results were achieved on medium supplemented with 0.4 mg L^{-1} BA (6.2 shoots) and 0.8 mg/l BAR (9.6 shoots); furthermore, the latter cytokinin resulted in the longest (35.8 mm) shoots. On hormone-free medium, the number of shoots was only 1.7 with 22.3 mm length. On the other hand, 0.1 and 0.2 mg L^{-1} MT considerably increased the leaves' length (up to 22.9 and 22.2 mm) and the shortest values (8.7 and 10.7 mm) were observed in the presence of 0.8 mg L^{-1} BAR and BA. Similar effect was recorded in the case of *S. redliana* 'Burokvölgy' (ÖRDÖGH ET AL., 2006). Thus, the length of the leaves was inversely proportional to the number of shoots. The optimal cytokinin type depends on the sorb species. The use of BA usually gave the best shoot multiplication when *S. commixta* (MOON, 1993), *S. aucuparia* (CHALUPA, 1988, 2002; LALL ET AL. 2006) and *S. torminalis* (JANA ET AL., 2009, MALÁ ET AL., 2009) were propagated *in vitro*, but for *S. aria* 'Gran Sasso', BAR was more effective. In case of KIN (similar to the reaction of *S. aria* 'Gran Sasso'), this cytokinin was also not effective for other sorbs' shoot production, such as *S. domestica* (ARRILLAGA ET AL., 1991) and *S. borbasii* 'Herkulesfürdő' (ÖRDÖGH ET AL., 2009).

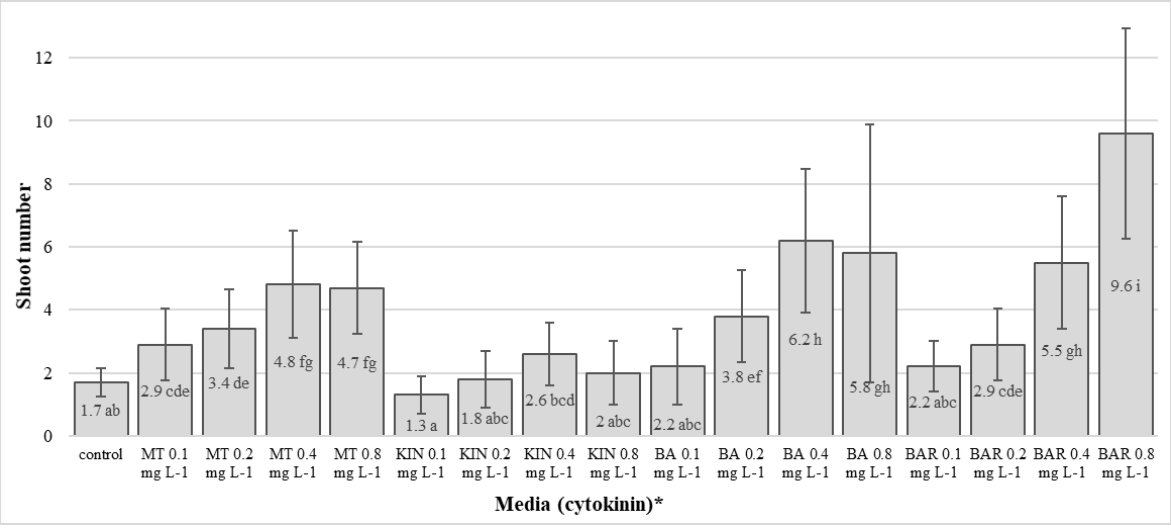

Figure 4. Shoot number of *Sorbus aria* 'Gran Sasso' on media with different cytokinins. Abbreviations: MT (meta-topolin), BA (benzyladenine), BAR (benzyladenine-riboside), KIN (kinetin). Means with different letter are significantly different by Tukey's test at $p \leq 0.05$

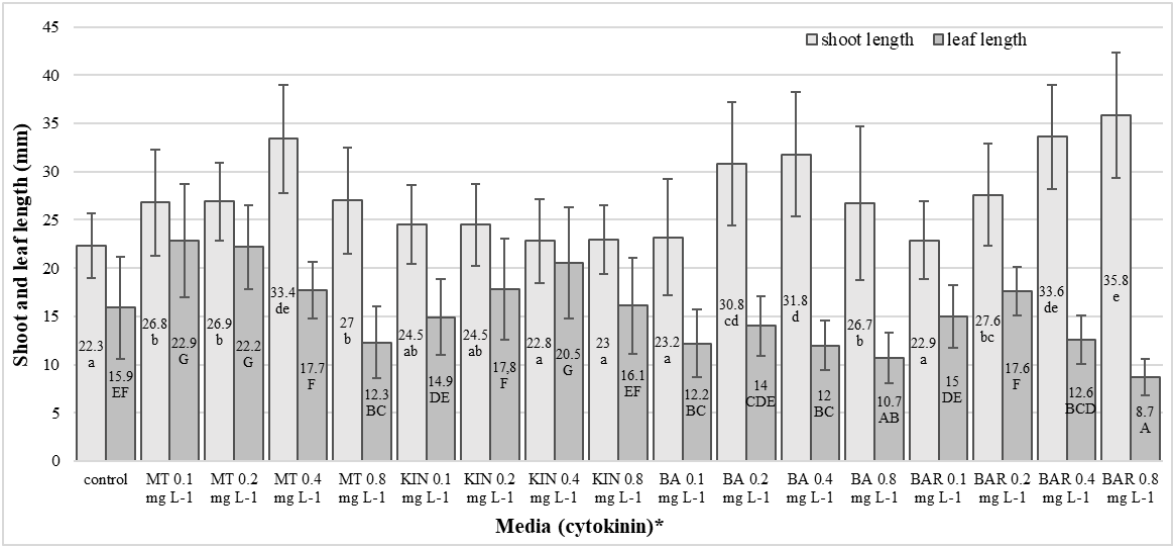

Figure 5. Shoot and leaf length of *Sorbus aria* 'Gran Sasso' on media with different cytokinins. Abbreviations: MT (meta-topolin), BA (benzyladenine), BAR (benzyladenine-riboside), KIN (kinetin). Means with different letter are significantly different by Tukey's test at $p \leq 0.05$

Figure 6. Multiplied *Sorbus aria* 'Gran Sasso' plants grown on media without hormones (A), with 0.4 mg L^{-1} BA (B) and 0.8 mg L^{-1} BAR (C)

Rooting

In vitro rooting of sorbs is not easy. Certain taxa, e.g. *S. vertesensis* 'Gánt' (KLAUSCH, 1991) did not develop roots *in vitro*, only if the shoots were threatened with 2 mg L^{-1} NAA 24 hour before planting them in greenhouse (*ex vitro*). In case of *S. borbasii* 'Herkulesfürdő', after a 72-hour-period induction with 5 mg L^{-1} IBA or NAA, only 1.8% of the shoots rooted on hormone-free media supplemented with 1 g L^{-1} AC (JÁMBORNÉ ÉS BENCZÚR, 2005). Higher concentration of auxin (15 mg L^{-1} IBA) and shorter induction time (48 hour) resulted better rooting ratio (almost 80%) of this sorb cultivar (ÖRDÖGH, 2011).

The best rooting values (30 and 36.7% ratio, 3 and 3.6 root/plant) of *S. aria* 'Gran Sasso' were observed on hormone-free media containing 0.5 and 0.75 g L^{-1} AC; however, there was no positive correlation between the roots' number and the length of growing period (*Figure 7*). Longer induction time (72 hour instead of 48) increased the percentage of

rooted specimens and their roots' length (up to 121.8 mm), even if the plants' growth time was shorter (2 or 3 months instead of 4). Besides, higher AC concentration also resulted longer roots (*Figure 8-9*). For *S. redliana* 'Burokvölgy' (ÖRDÖGH, 2011), induction was also essential with 15 mg L^{-1} IBA till 48 hour, and application of 0.75 g L^{-1} AC (with no hormones) generated the highest root production (46.4%, 3.8 roots). ARRILLAGA ET AL. (1991) described that a rooting rate of 80-87% can be achieved (in case of *S. domestica*), when juvenile (embryonal) shoot explants were used, otherwise this value was only 20-33% if buds had been collected from older, mature (25-30 year old) trees. CHALUPA (2002) also experienced better results (82% rooting) with the presence of young aged *S. aucuparia*. Presumably, relatively low (16.7-36.7%) rooting ratios of *S. aria* 'Gran Sasso' caused by the mother plants' higher age (>20-25 year). Therefore, not only the species/cultivars or the type or concentration of auxin/activated charcoal and the length of stimulation/growth period, but also the age of mother plants affected the success of sorbs' rooting.

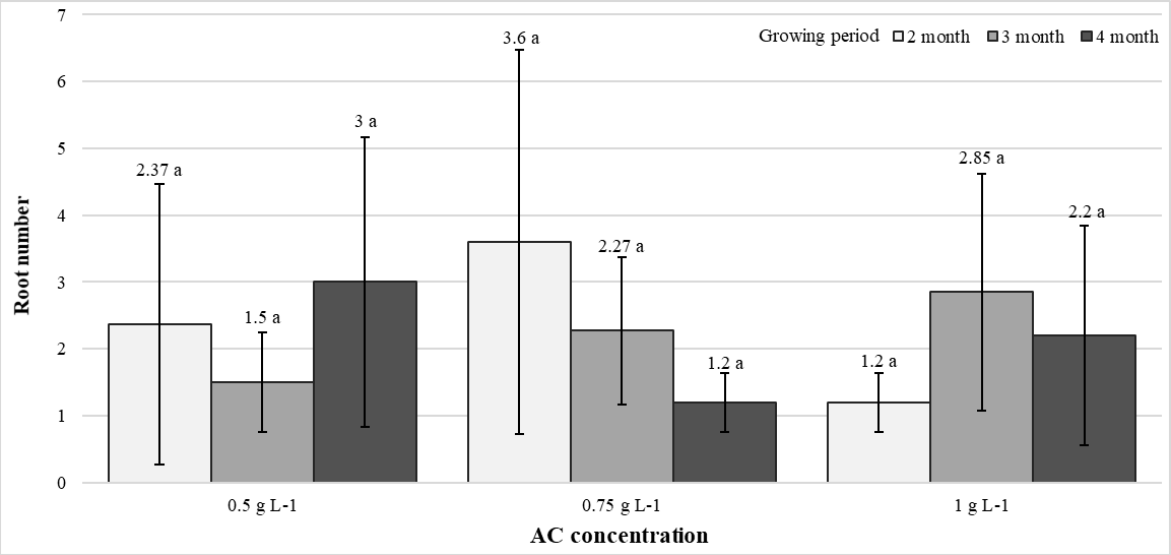

Figure 7. Root length of *Sorbus aria* 'Gran Sasso' on media with activated charcoal. Means with different letter are significantly different by Tukey's test at $p \leq 0.05$

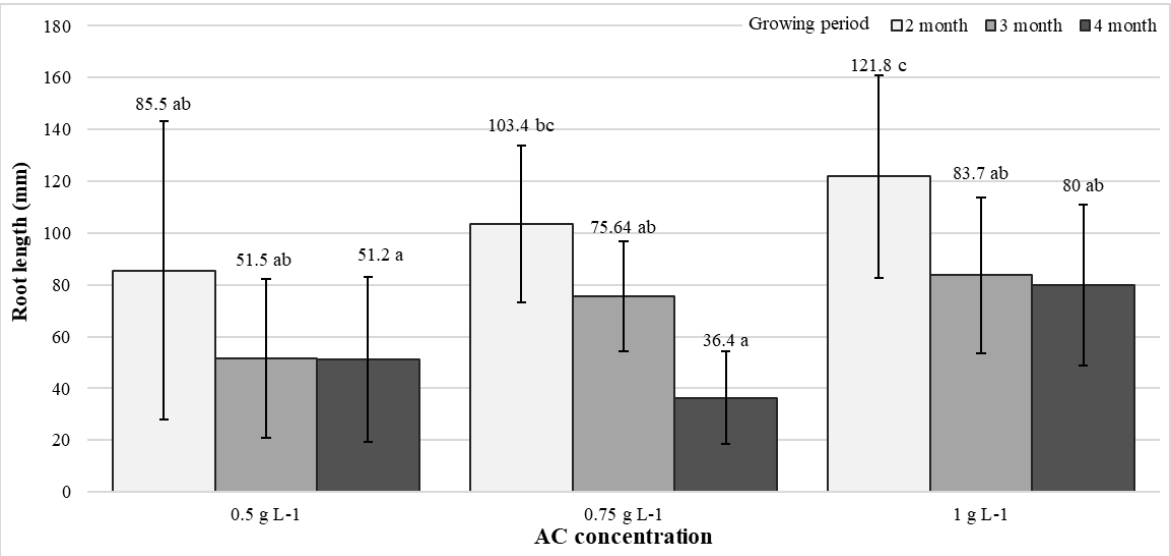

Figure 8. Root length of *Sorbus aria* 'Gran Sasso' on media with activated charcoal. Means with different letter are significantly different by Tukey's test at $p \leq 0.05$

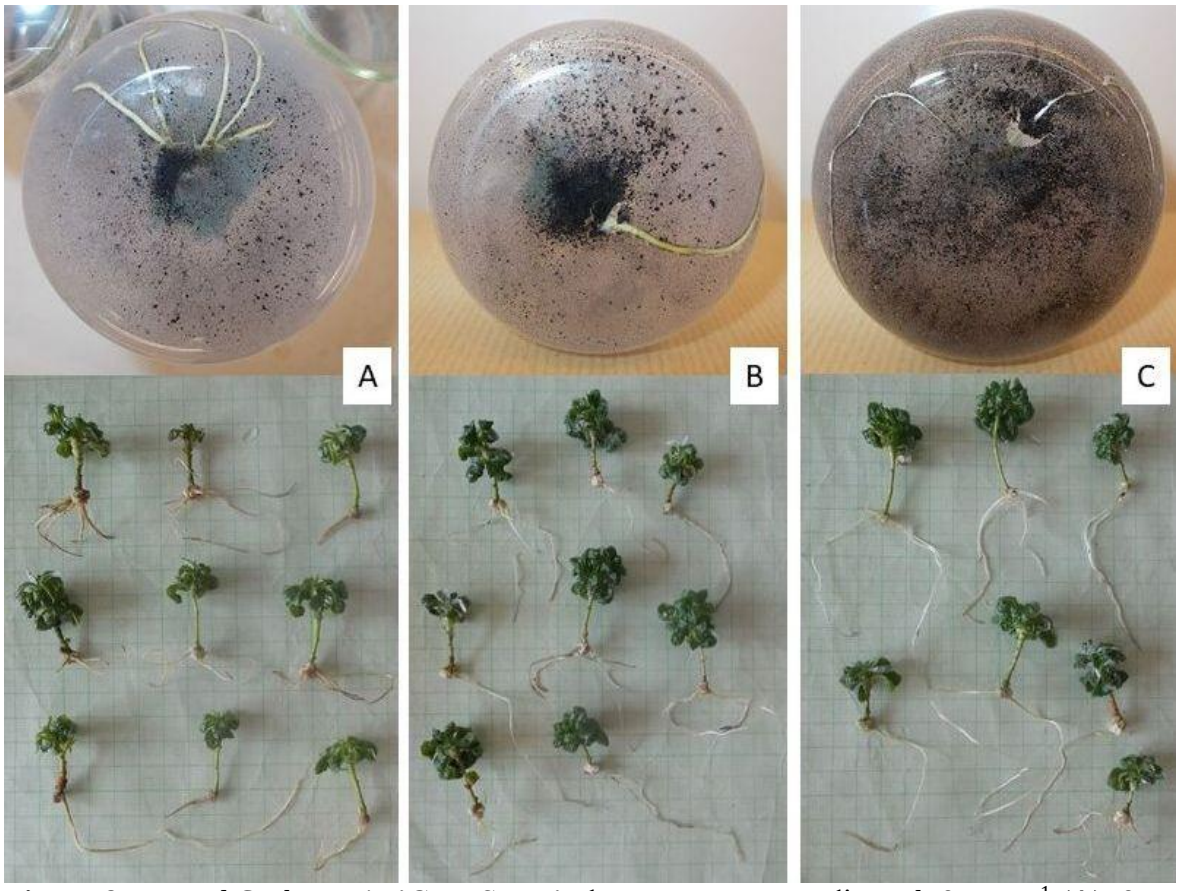

Figure 9. Rooted *Sorbus aria* 'Gran Sasso' plants grown on media with 0.5 g L^{-1} (A), 0.75 g L^{-1} (B) and 1 g L^{-1} (C) activated charcoal

The effect of silver nitrate

The use of silver nitrate showed variable effects in different *in vitro* trials. Among others, this agent effectively stimulated the emergence and elongation of lateral shoots from the explants of Virginia-type peanut, and reduced callus development (OZUDOGRU ET AL., 2005). During *Pistacia vera* 'Kirmizi' *in vitro* culturing, also positive results were shown with lower callus formation, better shoot multiplication, especially when not only $AgNO_3$ but growth regulators (BA, GA_3) were used (OZDEN-TOKATLI ET AL., 2005). In a surface sterilization process of the sour cherry cultivar 'Obla**činska' buds, 1** g L^{-1} **$AgNO_3$ (for 20 minutes) was the best for controlling the infection (**MIHALJEVIĆ ET AL., 2013). In case of a Hungarian sorb variety (*S. degenii* 'Csákvár'), this medium accessory (in 2 mg L^{-1} dosage) reduced vitrification (hyperhydricity), shoot/leaf deformities (MÁNDY ET AL., 1997) and similar results were achieved during potato (ALVA TICONA AND OROPEZA, 2013) and carnation (GUTIÉRREZ-MICELI ET AL., 2010) micropropagation.

In this study, higher doses (2 and 5 mg L^{-1}) resulted significantly fewer shoots (1.4 and 1.34), whereas their average length (19.05 and 18.7 mm) was also definitely longer on these media than in the other cases. Although the plants have no roots (*Figure 10*), the leaf lengths show that the highest values were obtained at a concentration of 2 mg L^{-1}, similar to the shoots' length (*Figure 11*). Thus, silver nitrate did not have positive effect on the plants' root development, and it stimulated the growth of existing shoots and leaves rather than new shoot development, particularly when applied in higher concentrations.

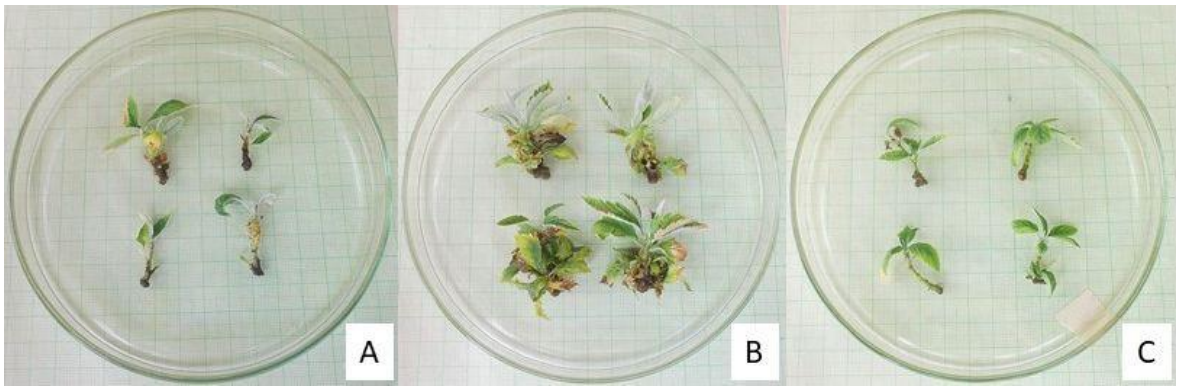

Figure 10. *Sorbus aria* 'Gran Sasso' plants' shoot and leaf characteristics effected by 0.5 (A), 1 (B) and 2 mg L^{-1} (C) silver nitrate

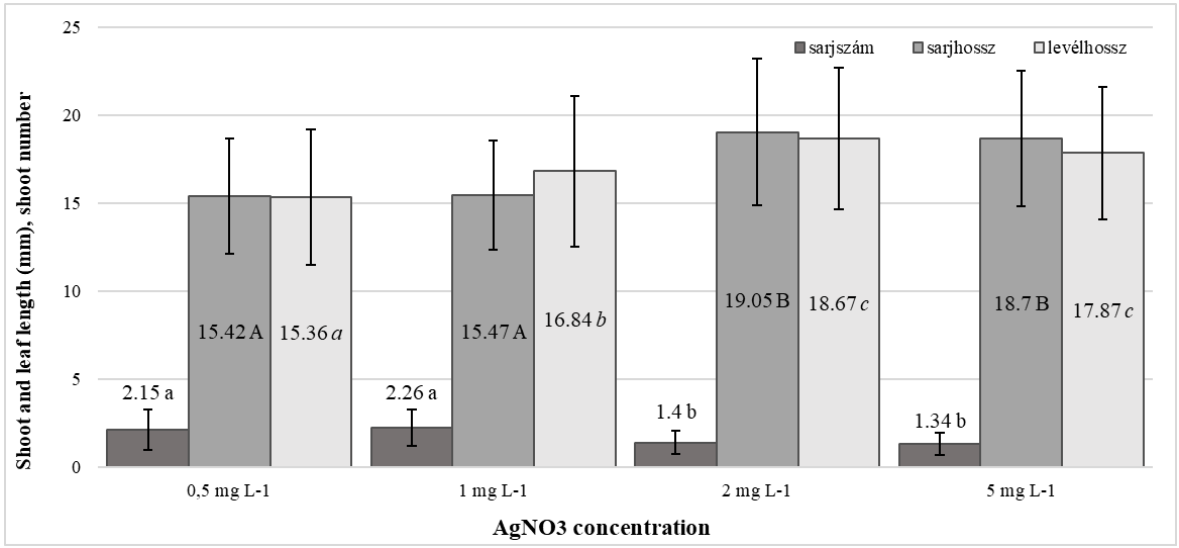

Figure 11. Shoot and leaf length, shoot number of *Sorbus aria* 'Gran Sasso' on media with silver nitrate. Means with different letter are significantly different by Tukey's test at $p \leq 0.05$

DISCUSSION

For the first phase (initiation), hardwood twigs or softwood shoots collected from *Sorbus aria* 'Gran Sasso' mother plants were equally good sources for successful surface sterilization of single nodes with one bud, although the latter plant parts was better for starting *in vitro* culture with higher (more than 90%) disinfection rate. In the next step (multiplication), different cytokinins were added to the ½ MS media in order to stimulate new shoots' development, and higher doses (0.4 and 0.8 mg L^{-1}) of BA, BAR resulted the best shoot production (moreover, 0.8 mg L^{-1} BAR elongated effectively the shoots' length). BA and BAR stimulated shoot proliferation better than KIN and MT (which were more effective for leaf development). The best rooting values were achieved on hormone-free ½ MS media supplemented with 0.5 and 0.75 g L^{-1} AC, but root induction (as pre-treatment with the use of 15 mg L^{-1} IBA supplementation until 48 or 72 hour) was essential. Longer induction time (72 hours instead of 48) resulted higher rooting ratio and longer roots. Silver nitrate was tested at four concentrations, but none of the treatments stimulates

rooting, however, in lower doses (not more than 2 g L^{-1}) it was optimal for shoot and leaf development.

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CAN THE DISTRIBUTION OF RED FOX BURROWS INDICATE THE CHAFER LARVAE DENSITY?

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ABSTRACT
Due to their general occurrence the European badger and the red fox have an important role in most of the ecosystems of the Carpathian basin. Both species use burrows for resting and cub rearing. Based on the previous studies, differences were found in the burrow site selection of these predators. The disparity was proven regarding the vegetation type, the soil texture and the density of primary food sources. This knowledge is essential for wildlife managers and nature conservationists, but could be useful for other sectors (e.g. agriculture, forest management) as well. In the present study, we took a plant protection approach. Our question was the following: does the chafer larvae density differ in the surrounding of badger and red fox burrows? The study area is located between Gödöllő and Valkó, in the Gödöllő Hills. Its size is 3728 ha and mainly (96%) covered by forests. Two methods were used during the study. At first strip transect method was implemented to localize the burrows. 81 burrows were found in total, from which 14 were used by badger and 14 by red fox, 53 of them were abandoned. The second method was the chafer larvae density and biomass measurement. Eight samples were taken per each active burrow, it means 224 samples in total. Our results showed higher chafer larvae density and biomass in case of red fox burrows, compared to badger burrows. We conclude that the soil texture could be in the background of this difference.

Keywords: chafer larvae, red fox, European badger, burrow, plant protection

INTRODUCTION

The European badger (*Meles meles*) and the red fox (*Vulpes vulpes*) are common species in the Carpathian basin (HELTAI, 2010). They have stable and dens populations (CSÁNYI ET AL., 2019), which highlights their important role in most of the ecosystems in Hungary (HELTAI, 2010). Although the two predator species belong to the same guild, they coexist in the same area. In the background, fine-scale niche segregation could be found, which manifests in the burrow site selection of these predators (MÁRTON ET AL., 2014). Previous studies have found differences in the pattern of the vegetation type, the soil texture and the density of primary food sources in the surrounding of the burrows (MÁRTON, 2018). For example the density of earth worms - as primary food sources of badger (GOSZCZYŃSKI ET AL., 2000, CLEARY ET AL., 2009) - was higher in the surrounding of badger burrows compared to red fox burrows (MÁRTON, 2018). In the Mediterranean region of Europe, studies have shown the importance of insect larvae in the badger's diet (CIAMPALINI AND LOVARI, 1985; LUCHERINI AND CREMA, 1995). This knowledge is important for wildlife managers and nature conservationists, but could be useful for other sectors as well, for instance in case of agriculture (crop production) and forest management. Larvae of certain insect species are also known as plant pests, for example the common cockchafer (*Melolontha melolontha*), which can also cause serious damage in agricultural and forested areas (BENKER AND LEUPRECHT, 2005).
In present study we took plant protection approach based on this knowledge. Perhaps the distribution of badger burrows can indicate the chafer larvae hotspots in the field. Our hypothesis was the following: the density and biomass of chafer larvae are higher in the surrounding of European badger burrows compared to red fox burrows.

MATERIALS AND METHODS

Study area

The study area is located between Gödöllő and Valkó, in the Gödöllő Hills. Its size is 3728 hectare and mainly (96%) covered by forests (*Figure 1*). The dominant tree species is the black locust (*Robinia pseudoacacia*, 24%), but the Turkey oak (*Quercus cerris*, 19%) and the English oak (*Quercus robur*, 19%) also have high proportions. Based on the soil texture the area could be separated into two classes: loamy soil (northern part), sandy soil (southern part).

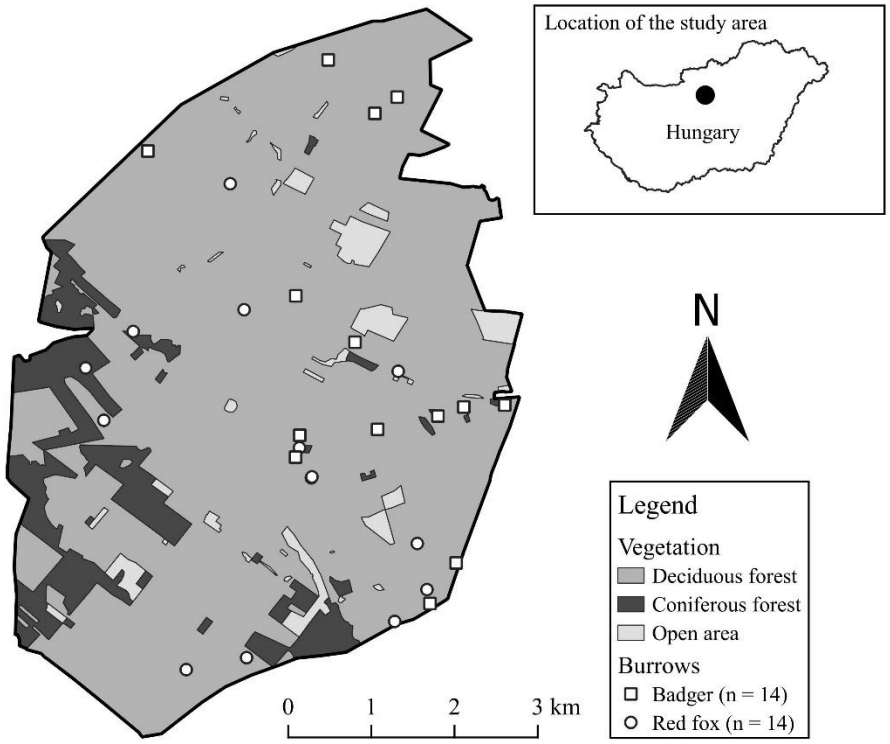

Figure 1. Study area and burrow locations

Data collection and processing

Two field methods were used during the study. In March of 2016 and 2017, strip transect method was implemented to localize the badger and red fox burrows (HELTAI AND SZEMETHY, 2010). The status (used or abandoned) of burrows found in 2016 were checked again in 2017. 81 burrows were found in total. In 2017, 14 of them were used by badger and 14 by red fox (*Figure 1*), 53 of them were abandoned. The second method was the chafer larvae density and biomass measurement. Eight sample plots were taken per each active burrow, it means 224 samples in total. The spatial distribution of the sample plots per active burrow were the following: two sample plots were taken per each cardinal directions. The closer was 100 meters and the further was 300 meters from the burrow. One sample plot was 50x50 cm wide and 15 cm deep (MÁRTON, 2018). We have counted the number of larvae and then the biomass was measured in every case. We did not use species-specific identification of larvaes. Typical control sites were not involved, because the whole study area could be used by badger and red fox. We have had no chance to use exclosures during the study. For data processing we have used Quantum GIS 3.4.4, Microsoft Excel 2016, R 3.3.1 and GraphPad InStat 3 software. Both badger and red fox

burrows and also the loam and sand soil texture were compared by the chafer larvae density and biomass. According to the results of Kolmogorov-Smirnov normality test Mann-Whitney U test was used for this analysis (REICZIGEL ET AL., 2010). Fisher's exact test was used to investigate the distribution of two carnivores' burrows (REICZIGEL ET AL., 2010) based on the environmental factors (vegetation, soil type, depth of the topsoil, soil texture). The spatial pattern data of these factors were originated from forest stand data of the local forestry (Pilisi Parkerdő Zrt.).

RESULTS

The total number of chafer larvae was 52 in case of badger burrows and 133 regarding red fox burrows. In the first step of the analysis our results showed higher chafer larvae density (Mann-Whitney U test: U = 5148.500, U' = 7395.500, p = 0.018, n = 224) and biomass (Mann-Whitney U test: U = 5105.000, U' = 7439.000, p = 0.014, n = 224) in case of red fox burrows, than in case of badger burrows (*Table 1*).

Table 1. The chafer larvae density and biomass in the surrounding of burrows. Legend: Min = minimum, Q1 = lower quartile, Q3 = upper quartile, Max = maximum, SD = standard deviation, n = number of sample plots, grey cellcolor = rows containing statistics data equal with zero only

Statistics	Chafer larvae density (thousand pcs./ha)		Chafer larvae biomass (kg/ha)	
	Badger (n = 112)	Red fox (n = 112)	Badger (n = 112)	Red fox (n = 112)
Min	0	0	0	0
Q1	0	0	0	0
Median	0	0	0	0
Q3	0	40	0	20
Max	280	520	200	260
Mean	19	48	9	27
SD	47	93	29	56

As a second step of the analysis, we have investigated the factors could explain the discrepancy in chafer larvae density. No significant difference was found regarding the distribution of two predator's burrows based on the vegetation (Fisher's exact test: p > 0.999, n = 28), the soil type (Fisher's exact test: p = 0.098, n = 28) and the depth of the topsoil (Fisher's exact test: p = 0.516, n = 28). However, significant difference (Fisher's exact test: p = 0.021, n = 28) was found in case of soil texture. The badger burrows were mainly located on loam soil (10/14 pcs., 71%) in contrast, the red fox most frequently used the sand soil texture (11/14 pcs., 79%). *Table 2* shows the results of comparisons the soil texture classes (based on the chafer larvae density and biomass). Significantly higher values are proved in case of sand soil texture than on loam (density: Mann-Whitney U test: U = 4160.500, U' = 8022.500, p < 0.001, n = 224, biomass: Mann-Whitney U test: U = 4118.500, U' = 8064.500, p < 0.001, n = 224).

Table 2. The chafer larvae density and biomass in the soil texture categories. Legend: Min = minimum, Q1 = lower quartile, Q3 = upper quartile, Max = maximum, SD = standard deviation, n = number of sample plots, grey cellcolor = rows containing statistics data equal with zero only

Statistics	Chafer larvae density (thousand pcs./ha)		Chafer larvae biomass (kg/ha)	
	Loam (n = 131)	Sand (n = 93)	Loam (n = 131)	Sand (n = 93)
Min	0	0	0	0
Q1	0	0	0	0
Median	0	0	0	0
Q3	0	40	0	20
Max	320	520	220	260
Mean	21	50	13	26
SD	62	88	41	50

DISCUSSION

The analysis showed contrary results to our hypothesis. The density and biomass of chafer larvae were higher in the surrounding of red fox burrows compared to badger burrows. Another primary result was the overlap between the spatial pattern of red fox burrows and the chafer larvae density. This overlap could not be explained by the predator-prey relation. Low importance of invertebrates was shown by most of the studies on red fox' diet (HELTAI ET AL., 2000; LANSZKI ET AL., 2006). Possible reasons are the following: (1) food sources for chafer larvae are denser on sandy soils than on loam soils. The black locust is the dominant tree species on sand. These forests could have higher density herbaceous layer thus more consistent root system (BARTHA ET AL., 2008), than the oak forests with closed canopy which are dominating on loam soils. (2) In case of red fox the burrow digging could be easier on sandy soils than on loam soils. It is important because the vixens can save more energy for cub rearing (MÁRTON AND HELTAI, 2017). (3) Badger regulates the chafer larvae density by consumption on loam soils. Majority of studies showed that the primary food sources for badgers are invertebrates (CIAMPALINI AND LOVARI, 1985; LUCHERINI AND CREMA, 1995; LANSZKI, 2002, VIRGÓS ET AL., 2004, ALVES ET AL., 2007).

Further studies are needed to clarify the reasons. However, one thing is certain: forest managers can use the red fox burrows as indicators of higher chafer larvae density in the present study area. Based on this knowledge they can make detailed measurements to predict the gradation of different chafer beetles (DEMIÁN ET AL., 2014) or to make decision about the treatment of larvae (ÉGETŐ, 2014).

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