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Slow Growth Storage of *Berula erecta in vitro* – Effect of Sucrose, Sorbitol and Temperature

Jana Ambrožič-Dolinšek[1-3]*, Sanja Oblonšek[4], Terezija Ciringer[1]
[1]University of Maribor, Faculty of Natural Sciences and Mathematics, Koroška cesta 160, 2000 Maribor, Slovenia
[2]University of Maribor, Faculty of Education, Koroška cesta 160, 2000 Maribor, Slovenia
[3]University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, 2311 Hoče, Slovenia
[4]Brezje pri Oplotnici 22, 2317 Oplotnica, Slovenia

ABSTRACT

It has been established that *in vitro* storage of *Berula erecta* (Huds.) Coville by slow growth would rationalize the culture maintenance by reducing the number of transfers to fresh medium. Attempts of slow growth were made while maintaining viability by adding sugars and sugar alcohols to the MS medium and lowering the culture temperature in the growth chambers. The effect of low temperatures (13°C and 4°C), different combinations and concentrations of sorbitol (20, 40 g L^{-1}) and sucrose (30, 40 g L^{-1}), and light was tested to determine the viability and ability of *B. erecta* to recover growth. Increasing sucrose concentration did not inhibit or slow growth. At 23 ± 2°C, the addition of 20 g L^{-1} sorbitol successfully inhibited growth, while the addition of 40 g L^{-1} sorbitol resulted in plant senescence after two months of culturing. Storage of *B. erecta* at 13°C did not effectively inhibit growth and recovery ability in any treatment. The most effective way to maintain *B. erecta* for long-term slow growth storage in tissue culture while retaining a high level of viability with minimal growth was to culture the plants at 4°C in the dark. This kept the plants viable and capable of regeneration for several months.

Key words: slow growth storage, *Berula erecta*, viability, sucrose, sorbitol, temperature

INTRODUCTION

Rapid plant propagation in tissue culture, micropropagation, is an excellent method for rapid multiplication of selected plant species such as *Berula erecta* (Huds.) Coville (Apiaceae). *B. erecta* is micropropagated on solid MS medium (Murashige and Skoog, 1962) without growth regulators. Propagation without the use of growth regulators makes it suitable for a variety of research purposes (Mechora et al., 2016, 2017, 2020). The disadvantage of such propagation is the rapid depletion of the medium. Consequently, plant material in tissue culture needs to be subcultured onto fresh medium every 4 weeks. Frequent subcultivation requires a lot of laboratory work, it is costly, it can lead to somaclonal changes, and it usually carries a high risk of contamination and loss of culture (Ambrožič Dolinšek, 2017).

For long-term storage of *B. erecta* as a source of genetic diversity, we want to slow down micropropagation and make it less intense by subculturing on fresh medium at longer intervals. This type of micropropagation is called slow growth culture. Slow growth allows the plant material to be stored without labor-intensive subcultivation on fresh media for a period ranging from two months to several years, depending on the plant species (Kamińska et al., 2018). This technique also reduces the cost of culture (Dube et al., 2011). The "slow growth" technique is based on inhibiting metabolic

**Correspondence to:*
E-mail: jana.ambrozic@um.si

activity and slowing the growth rate while maintaining the viability of the plant material through cultivation on modified growth media and/or under modified growth conditions (Dube et al., 2011; Chauhan et al., 2019). The main advantage is the extended time between subcultivation onto a new medium (Chauhan et al., 2019). The technique is already well established and used in a wide range of genetic material conservation. It has a high regrowth rate and good genetic stability compared to long-term storage. Slow or minimal growth is one of the most common methods to keep living gene banks disease-free, with low space and labour requirements for culture transfer and survival testing (Cha-um and Kirdmanee, 2007).

Various chemical and physical factors are considered in the reduction of *in vitro* plants (Cha-um and Kirdmanee, 2007). External physical factors that slow growth are suboptimal temperature, reduced light intensity, and shortened photoperiod (Gopal and Chauhan, 2010). Low temperature is the most commonly cited factor for successful slow growth and medium-term storage of many plant species (Cha-um and Kirdmanee, 2007). Temperatures between 2°C (e.g., *Castanea* hybrid, *Prunus avium*, *Quercus* spp.) and 12°C (*Vitis* spp.) are commonly used for slow growth, while tropical species that are more temperature sensitive require temperatures between 15 and 20°C (Capuana and Di Lonardo, 2013). Another important factor is light, both intensity and photoperiod. Often, lowering the temperature is combined with lowering light intensity or by exposing plants to complete darkness (Chauhan et al., 2019). Both can be used as alternative factors to control plant growth and development. Reducing temperature and light intensity or leaving plants in the dark can also be combined with reducing nutrient concentration (Hassan and Bekheet, 2016; Trejgell et al., 2015). Mild water deprivation through the application of the osmotic substances is another alternative for slow growth *in vitro*. It is achieved by increasing the concentration of sugars and sugar alcohols in the culture medium (Cha-um and Kirdmanee, 2007). Sugars are sources of organic carbon that act as the osmotic substance in the medium. One of them is the sugar alcohol sorbitol (Marino et al., 2010). Osmotic substances such as sorbitol reduce mineral uptake due to the change in osmotic pressure, which inhibits plant growth (Hassan and Bekheet, 2016).

The goal of this study was to rationalize the maintenance of *B. erecta* culture by reducing the number of transfers to fresh medium by slow growth storage of *B. erecta* while retaining viability and the ability to recover growth of *B. erecta*. We strive to achieve this by lowering the culture temperature in the growth chambers and adding sugars and sugar alcohols to the MS medium. In our study, we want to determine the effect of low temperatures (13°C and 4°C), different combinations, concentrations of sorbitol (20, 40 g L^{-1}) and sucrose (30, 40 g L^{-1}) in combination with a modified light regime.

Material and methods

Plant material and growth conditions

Berula erecta (Huds.) Coville (Apiaceae) is a creeping, fast-growing terrestrial or aquatic perennial that grows to 40 cm high and thrives in fresh water. It has a subcosmopolitan distribution, growing in most parts of the Northern Hemisphere and is native to Slovenia (Mechora et al., 2016). Shoots propagated *in vitro* were cultured on the Murashige and Skoog (1962) solid medium (MS) without growth regulators. The MS medium supplemented with 0.8% Difco - Bacto agar and 3% sucrose was adjusted to a pH of 5.7-5.8 before autoclaving. Two shoots, each with a fresh weight of approximately 90 mg, were placed on the surface of the medium in 175 mL jars with transparent lids. The plants were grown under controlled conditions at 23 ± 2°C, through a photoperiod of 16 hours at 38-50 μmol m^{-2} s^{-1} (Osram L 58W/77 - Fluora), and at 50% relative humidity. Growth and development were measured monthly over a period of six months and compared with each other and with the control with 30 g L^{-1} sucrose. The experiment with 5 replicates per treatment was repeated twice. Different treatments (Table 1) and environmental conditions (Table 2) were compared. The effects of different temperatures (23, 13, and 4°C), different combinations and concentrations of sorbitol (20, 40 g L^{-1}), sucrose (30, 40 g L^{-1}), and different exposures to light regimes were determined (Table 1, 2).

Table 1: The amount of sucrose and sorbitol in MS medium varied between treatments

Treatments	Sucrose [g L^{-1}]	Sorbitol [g L^{-1}]	Denotes
1 (control)	30	-	MS 30
2	40	-	MS 40
3	30	20	MS 30 + 20
4	30	40	MS 30 + 40
5	40	20	MS 40 + 20
6	40	40	MS 40 + 40

Table 2: Growth conditions

Treatments	Temperature [°C]	Photoperiod [h]
Fridge	4 ± 1	24 dark
Growth chamber	13 ± 2	16 light, 8 dark
Growth chamber	23 ± 2	16 light, 8 dark

Viability with growth and development was measured monthly during a six-month experimental period. Several parameters were monitored: fresh and dry weight, shoot and root length, and number of shoots and stolons. The parameters were measured at the beginning of the experiment on 20 plant samples from each treatment to obtain baseline values, and then weekly for each treatment on 12-14 plant samples from the treatment.

Statistical analysis

Viability, growth, and development results are presented by means and standard deviations (±SD) and were statistically analyzed by independent samples Kruskal-Wallis 1-way ANOVA (k samples) test and Dunn-Bonferroni pairwise post hoc test using SPSS 27 software (SPSS Inc., Chicago, IL, USA). Different letters or * indicate significant differences ($P < 0.05$) between means.

Results

Growth and development

Growth and development were assessed after two, four and six months in three different growth chamber with three different light and temperature regimes. After two months, the differently treated *B. erecta* shoots from the 23°C growth chamber were compared (Figure 1). In the control medium MS 30 with 30 g L^{-1} sucrose, it grew throughout the medium. In the medium MS 40 with 40 g L^{-1} sucrose, the plants also grew beyond the medium, but the leaves were visibly darker, slightly purple in color, and there was some chlorosis present. Shoots were shorter than in the control group. In the MS 30 + 20 treatment, 20 g L^{-1} sorbitol reduced growth compared to the control, however in the MS 30 + 40 treatment, the plant stopped growing and the leaves became dark purple or chlorotic. The medium MS 40 + 20 resulted in dark green leaves and short shoots. In MS 40 + 40 the shoots became chlorotic and hardly grew. It was obvious that the addition of 40 g L^{-1} sorbitol stressed the plant.

After four months, the differently treated shoots from the 23°C chamber were compared again (Figure 1 a, b). The media were severely dehydrated, and the experiment was terminated. On the control medium MS 30 containing 30 g L^{-1} sucrose, the plant grew throughout the medium and became completely chlorotic and/or necrotic. On the medium MS 40 containing 40 g L^{-1} sucrose, the plant was slightly smaller and the leaves were purple in color or chlorotic and/or necrotic. On the medium MS 30 + 20 with 20 g L^{-1} sorbitol, the plants did not grow out completely, but they became purple and chlorotic and/or necrotic. A similar observation was made on MS 40 + 20 with the combination of increasing sucrose and adding 20 g L^{-1} sorbitol, except that *B. erecta* grew less here. On the MS 30 + 40 and MS 40 + 40 media, *B. erecta* grew less. The addition of 40 g L^{-1} sorbitol was lethal to the plants, which eventually died.

After four months, the differently treated shoots from the 13°C chamber behaved quite similarly to the plants in the 23°C chamber (Figure 1 c, d). Plants remained viable only on the medium MS 40 with a higher sucrose content of 40 g L^{-1}, while the plants on the control medium MS 30 became chlorotic and/or necrotic. On the media enriched with 20 g L^{-1} sorbitol *B. erecta* was not viable and died, and the same was observed on media containing the combination of 40 g L^{-1} sucrose and sorbitol.

In the 4 °C chamber, there were no visible differences between treatments after all six months of the experiment (Figure 1 e, f). Growth slowed or even stopped and plants turned lighter green but remained visibly viable.

Dry weight

Compared to the control, the dry weight of plants from the 23°C chamber increased in time (Figure 2a). Compared to the baseline condition, the increase after 6 months was highest on the medium MS 30 and MS 40 and lowest on the medium MS 40 + 40. After two months, the highest dry weight of plants was observed on MS 40, followed by the control on the medium MS 30 difference being small. The lowest dry weights were obtained on MS 30 + 40 and MS 40 + 40 treatments.

After four months in the 23°C chamber, the average plant dry weight on MS 40 was the highest, followed by the average plant dry weight on MS 30 with a smaller difference (Figure 2a). The lowest plant dry weight after six months was on the MS 40 + 40 medium. Increased concentration of sorbitol significantly decreased plant dry weight. The more sorbitol added, the lower the plant dry weight. The combination of higher amounts of sucrose and sorbitol had a similar effect.

Comparing each treatment to the MS 30 control, the average dry weight of plants from the 13 °C chamber increased over time (Figure 2b). The exception were the MS 30 and MS 30 + 20 treatments, where the average dry weight of plants was higher after 2 months than after 4 months. The MS 30 + 40 treatment was also an exception, where the average dry weight of the plants was lower after 6 months than after 4 months.

After two months in the 13°C chamber, the highest dry weight of plants was obtained with the MS 30 + 20 treatment, followed by the MS 30 control, the MS 40, the MS 40 + 20, and the MS 30 + 20 treatments (Figure 2b). The lowest average dry weight was obtained with the increased sucrose concentration treatment (MS 40 + 40, 40 g L^{-1} sucrose) and in the case of 40 g L^{-1} sorbitol added.

The MS 30 control and the MS 30 + 20 treatment both had lower dry weight after four months than after two months. In the other treatments, the dry weight of the plants after four months was higher than after two months. It was highest in the MS 40 treatment and lowest in the MS 30 + 20 treatment. After 6 months, the highest dry weight of the plants was in the control and the lowest in MS 30 + 20.

We observed a trend of increasing dry weight of plants from the 4°C chamber as a function of the duration of the experiment (Figure 2c). Exceptions were the MS 30 + 20 and MS 40 + 40 media, where the dry weight of the plants was lower after 6 months than after 4 months. The greatest difference between the baseline condition and the condition after six months was found in MS 30 + 40, where plant dry weight was consistently the highest compared to the other treatments. There were no significant differences between the other treatments.

Sorbitol inhibited plant growth. As the sorbitol concentration increased, the dry weight of the plants decreased (Figure 3a). Increased sucrose concentration did not affect dry weight (Figure 3b). Lowering the cultivation temperature inhibited plant growth. As the temperature decreased, the average dry weight of the plants decreased sharply (Figure 3c).

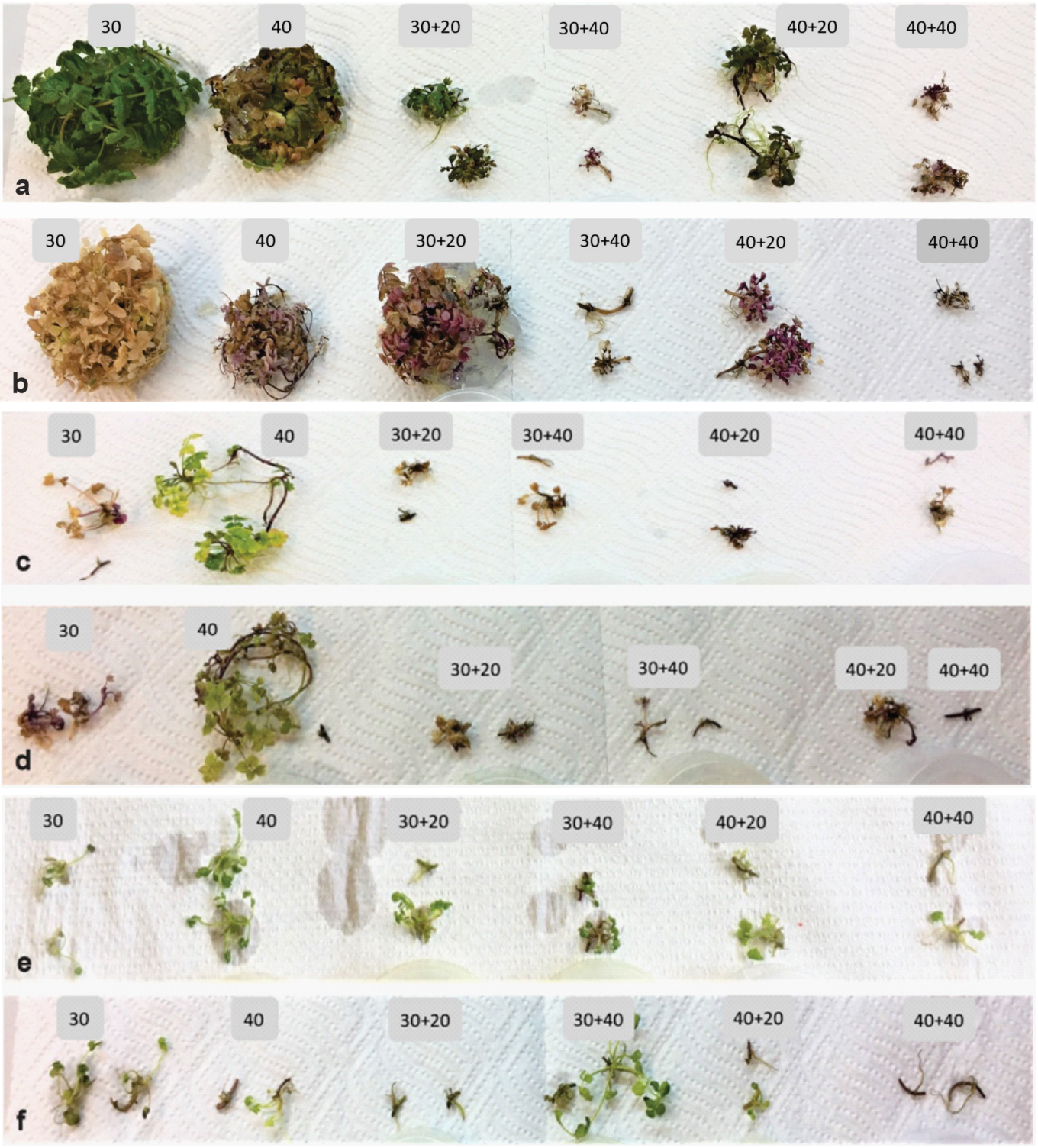

Figure 1: Plants of *B. erecta* on MS media with different concentrations of sucrose and sorbitol: (a) at 23°C after two months, (b) at 23°C after four months, (c) at 13°C after four months, (d) at 13°C after six months, (e) at 4°C after two months, (f) at 4°C after six months.

Development of roots, stolons and shoots

Plants of *B. erecta* developed particularly long roots on MS 30 and MS 40 medium in the 23°C chamber. The average root length increased with the duration of the experiments. In MS 30 + 40 medium, the root length of plants grown at 4°C was significantly longer than that of plants grown at 23°C after 6 months. Roots of plants grown at 13°C were significantly longer than those of plants grown at 23°C. Plants in the 23°C chamber developed the highest number of roots (data not shown), on the control medium MS 30 and the medium MS 40, followed by the medium MS 30 + 20. There were no differences between the other treatments and temperatures.

Only the plants in the 23°C chamber developed stolons

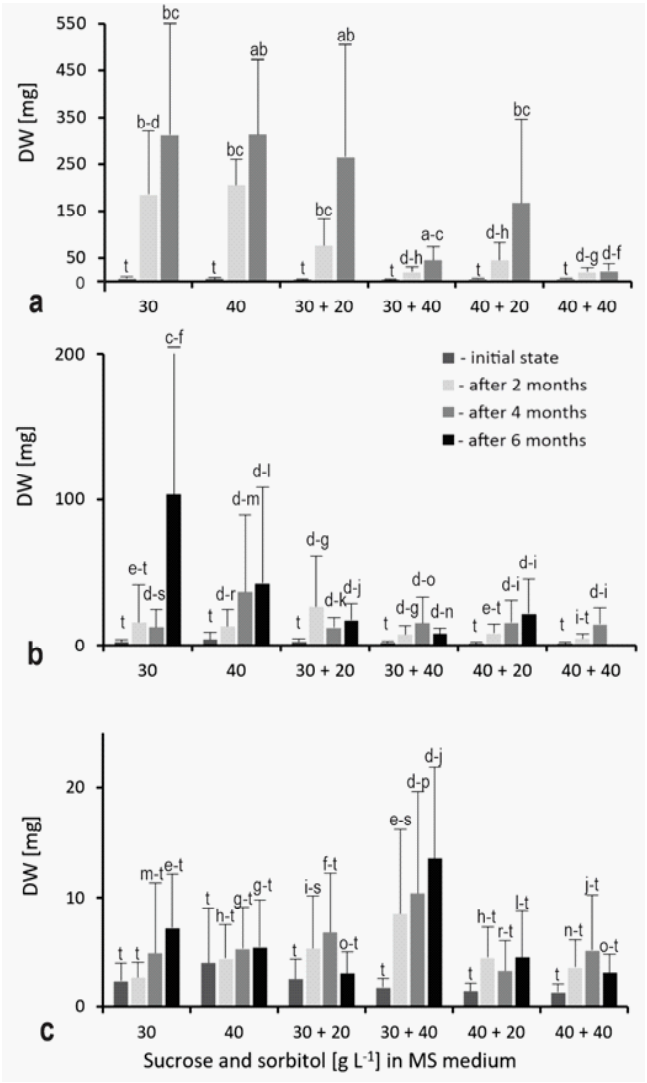

Figure 2: Dry weights of *B. erecta* on MS medium with different concentrations of sucrose and sorbitol [g L^{-1}] in a growth chamber at (a) 23°C, (b) 13°C, (c) 4°C after 2, 4, and 6 months. The average weights [mg ± SD] are shown. Individual treatments were compared only simultaneously. Different letters indicate significant differences ($P < 0.05$) between means (Kruskal-Wallis test).

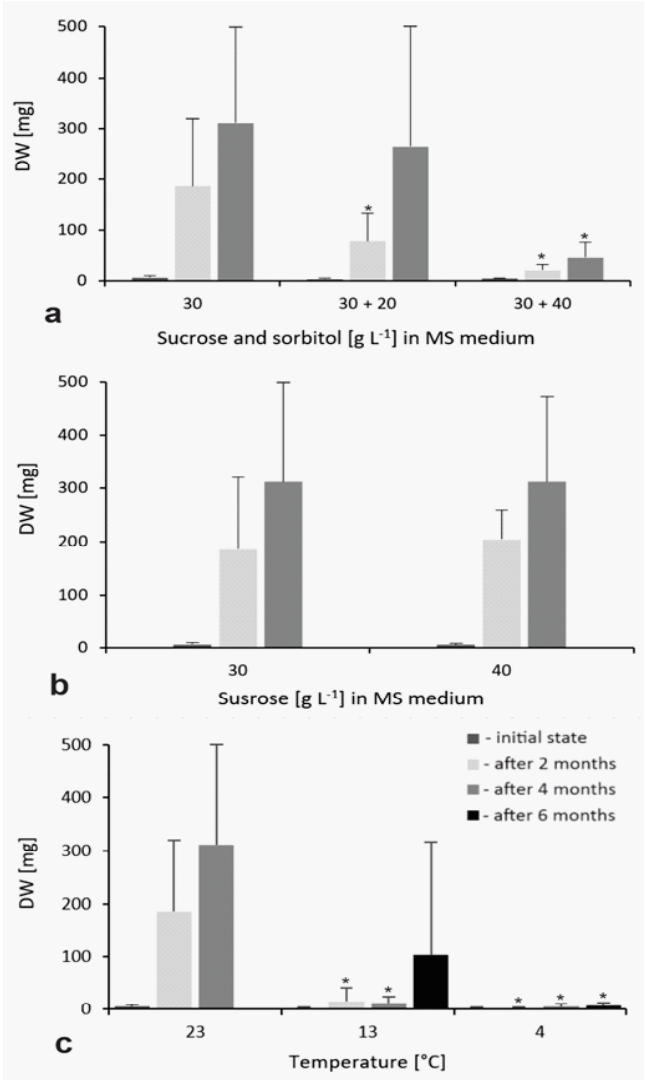

Figure 3: Dry weight of *B.erecta* shoots on MS 30 medium with different concentrations of (a) sorbitol [g L^{-1}], (b) sucrose [g L^{-1}], and (c) temperature [23, 13, 4°C] after 2, 4, and 6 months. Average weights [mg ± SD] are shown. Individual treatments were compared simultaneously. The sign * indicates significant differences ($P < 0.05$) between the means (Kruskal-Wallis test).

(data not shown). The highest number was found in the MS 30 and MS 40 media. In the case of other chambers, a trend of number of stolons increasing in time was observed. At 13°C, the number of stolons increased only slightly (1-2 stolons per specimen), while no stolons were formed at 4°C.

The plants developed the highest number of shoots in the control medium in the chamber at 23°C. This was followed by the MS 40 treatment and then in the case where 20 g L^{-1} sorbitol (MS 30 + 20 and MS 40 + 20) was added. In the 23°C chamber, the number of shoots had a tendency to increase in the course of the experiment. Only the control MS 30 and MS 40 had significantly more shoots than the other treatments (Figure 4). At 13°C, MS 30 and MS 40 showed an increasing trend in the number of shoots as the experiment took place, while the number of buds did not change in the other treatments. At 4°C, no new buds were formed.

Discussion

Experiments in the 23°C growth chamber were terminated after four months because the media had almost completely dried up. It was possible that the culture was terminated due to lack of sucrose in the media or lack of water in the dried media. Similar findings were made in other studies in which *in vitro* senescence was attributed to depletion of nutrients from the media, evaporation of water from the jars, or accumulation of inhibitory products of cell metabolism (Alvim et al., 2020). The experiment in the 13°C and 4°C growth chambers was continued after 6 months of culturing.

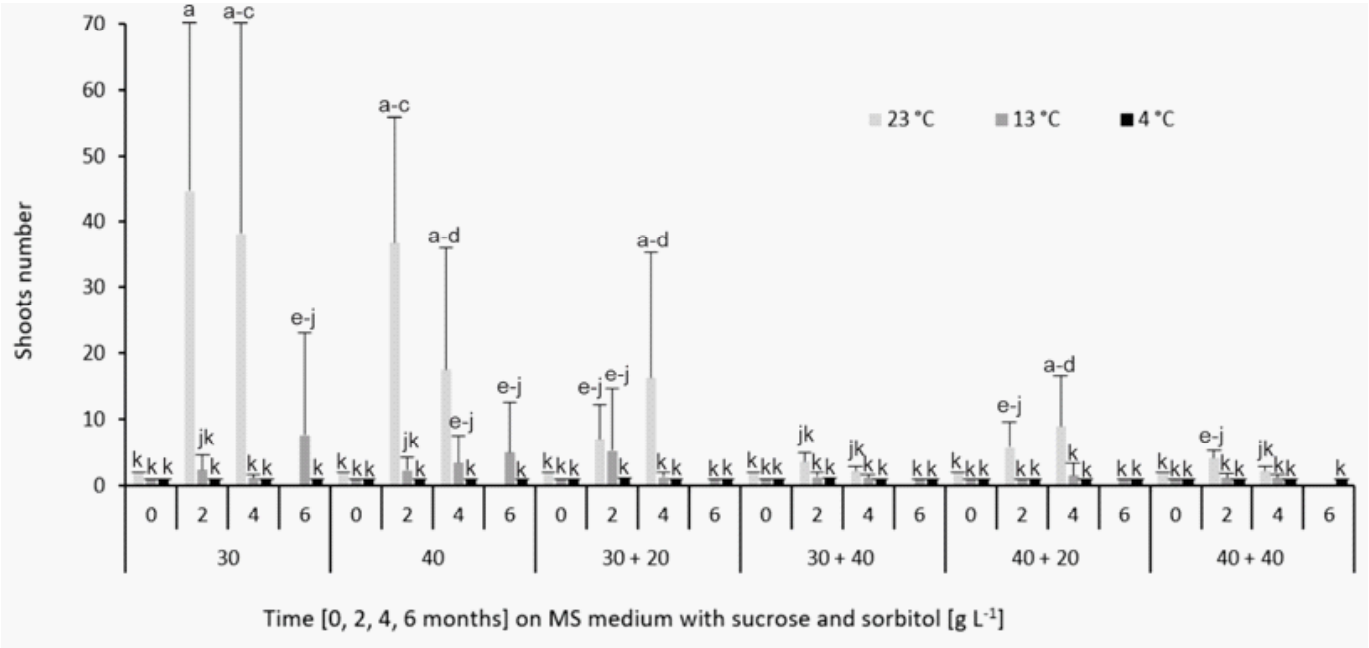

Figure 4: Number of *B. erecta* shoots on MS medium with different concentrations of sucrose and sorbitol [g L^{-1}] at different temperatures [23, 13, 4°C] after 2, 4, and 6 months. average number of shoots [± SD] are shown. Individual treatments were compared simultaneously. Different letters indicate significant differences ($P < 0.05$) between means (Kruskal-Wallis test).

Growth parameters

After two months in a chamber at 23°C, plants were most vigorous on basal control medium MS 30. Leaves were green, stolons were long with many buds, and the plant had outgrown the entire medium. The increased sucrose concentration in the next and further months, however, slightly inhibited plant growth. Plants grew less, the stolons and shoots appeared shorter, and chlorosis occurred. The addition of sorbitol to the medium inhibited growth even more. With the addition of 20 g L^{-1} sorbitol, the plants remained viable but made no growth progress. Thus, this concentration of sorbitol was effective in inhibiting growth and had an osmotic effect. However, the addition of 40 g L^{-1} sorbitol to MS 30 + 40 and MS 40 + 40 media resulted in plant death, indicating that the sorbitol concentration was too high and too stressful for survival. The same effect of sorbitol was observed also in date palms (*Phoenix dactylifera* L.) (El-Bahr et al., 2016).

After four months in a 23°C chamber, the plants showed many signs of stress. As mentioned earlier, the medium containing 40 g L^{-1} sorbitol caused all plants to die. The other media turned them purple and brown. Thus, after 4 months in a 23°C chamber, the plants were barely viable and were severely stressed regardless of treatment. To summarize, in the 23°C chamber, the addition of 20 g L^{-1} sorbitol slowed growth most effectively (on both the MS 30 + 20 and the MS 40 + 20 media, while sucrose had no effect on growth. Addition of 40 g L^{-1} sorbitol generally caused senescence in all plants. Lower concentrations of sorbitol slow growth, however, slow growth at this temperature is prevented by excessive depletion or/and desiccation of the media.

In the 13°C growth chamber, with 16 hours of light and 8 hours of darkness, all plants in all treatments died after two months, the only exception being the treatment with increased sucrose 40 g L^{-1} (MS 40). The increased sucrose allowed survival at this temperature, although the plants were light green to yellow and the stolons were purple with few buds, indicating that they were under stress. Therefore, storage of *B. erecta* was not relevant and thus is not recommended at 13°C.

Many studies have reported the correlation between two stress factors caused by high light intensity and low temperatures. Light stress can occur as a result of an imbalance between absorption and energy consumption. Absorption of high amounts of light and low metabolic activity due to low temperatures can result in damage to the D1 protein, the binding site of the plastokinone. The inability to transfer energy in photosynthetic reaction systems can lead to the formation of ROS (Kamińska et al., 2020). Under cold conditions, electron transport can be inhibited, leading to changes in cell membrane structure, and a decrease in photosystem II activity can lead to increased stress for the plant (Kamińska et al., 2020). Because of these problems, storage at low temperatures without light or at very low light intensity has been demonstrated for *Taraxacum pieninicum* (Kamińska et al., 2020) and was also successful in our experimental system. In the 4°C growth chamber in constant darkness, plants remained equally developed after two, four, and six months. They did not grow, they became slightly lighter in color, but there was no chlorosis or purple coloration. Thus, they apparently remained fully viable without development, which is the main objective of slow growth. Similar results are reported by Bekheet et al. (2007), in which artichokes (*Cynara scolymus*) remained viable for up to 12 months at 6°C in the dark. In *Asparagus officinalis*, 90% of explants on basal medium MS remained viable for up to 12 months at 5°C in the dark (Bekheet et al., 2002, 2000). As many researchers have already noted, the addition of sorbitol

and the increased concentration of sucrose (which also has an osmotic effect) reduce the water potential of the medium, resulting in more difficult uptake of water and nutrients and poorer growth (Alvim et al., 2020).

Dry weight

Dry weight showed similar results to the visual description. Compared to the control, dry weight increased with time. In the 23°C growth chamber, the increase from baseline after 6 months was highest on MS 30 and MS 40 media and lowest on MS 40 + 40 and MS 30 + 40 media. Weighing only confirmed the observed results that the best growth and development were obtained on MS 30 and MS 40 media, while on MS media containing 40 g L^{-1} sorbitol, plants died. Adding of low levels of sorbitol reduced dry weight or inhibited growth. Similarly, the presence of osmotic sorbitol and high sucrose in the media inhibited the metabolism of *Amburana cearensis* (Alvim et al., 2020). The results from the 13°C chamber showed that the average dry weight also increased with time. Dry weights here were significantly lower than in the 23°C chamber. The dry weight of plants on the MS 40 medium appeared to be slightly higher than in the other treatments, which could be predicted from the results of the observation that only plants on these media remained viable.

Even in the 4°C growth chamber on MS media, there was a trend of increasing plant

dry weight as a function of the duration of the experiments. Exceptions were the MS 30 + 20 and MS 40 + 40 media, where plant dry weight was lower after 6 months than after 4 months. Since the plants hardly grew, there was almost no change in dry weight. The greatest difference between the baseline condition and the condition after 6 months was observed in the medium MS 30 + 40, where plant dry weights were consistently the highest compared to the other treatments. There were no significant differences between the other treatments, i.e., at 4°C, the additional sucrose and sorbitol had no effect. At 4°C, growth was severely inhibited or stopped, but the plants remained fully viable. This suggests that it is useful to keep *B. erecta* at 4°C on the control medium MS 30, as this extends the interval between presentations without adding anything to the basal medium, which is also the most cost-effective solution.

Root length and number of roots

In terms of root length, only the MS 30 and MS 40 treatments in the 23°C chamber stood out. While root length tended to increase with time in the other treatments and temperatures, there were no significant differences between the above mentioned treatments. Root growth was not affected by temperature, sorbitol, and/or increased sucrose concentration. The same was true for the number of roots and for length of roots.

Number of stolons and shoots

The stolons and shoots developed only in the 23°C chamber, most intensively at MS 30 and MS 40, with the number of stolons and shoots increasing with time. At 13°C, single stolons with a few buds successfully developed abundantly, while at 4°C no new stolons and shoots developed at all. Thus, the chamber provided better conditions for the plants to develop additional stolons. Since the goal of slow growth is to slow down or stagnate the plants, we do not want any growths or formations of new organs that would further deprive the medium of nutrients and water. Therefore, it makes sense to store the plant at 4°C, where it does not form new organs.

Conclusion

Decrease in temperature slows the growth of *B. erecta*. Decreasing the temperature slowed viability in the 23°C and the 13°C growth chamber, and also caused the culture to die after 4 months. However, lowering the temperature at 4°C in the dark had no effect on viability whatsoever. Lower temperatures increased the time between transfers to fresh media only at 4°C. The addition of sorbitol affected the growth and viability of *B. erecta*. 20 g L^{-1} sorbitol inhibited growth, while 40 g L^{-1} caused plant death. Only the addition of 20 g L^{-1} sorbitol prolonged the time between transfers to fresh media. The addition of sucrose did not affect the growth and viability of *B. erecta* according to the described parameters, although some differences were observed. Increases in sucrose concentrations did not increase the time between transfers to fresh media. The most optimal conditions for slow growth and good viability of *B. erecta* were on medium MS at 4°C in the dark. Here, plants remained fully viable, while growth was stopped. Increasing sucrose concentration from 30 g L^{-1} to 40 g L^{-1} on MS medium alone did not inhibit or slow growth. To slow growth in the 23°C growth chamber, adding 20 g L^{-1} of sorbitol is useful, although for longer storage it would be worthwhile to explore other ways to prevent medium depletion and desiccation. We have found that the most effective way to slow growth is to store the plants in the dark at 4 °C, where they do not grow but remain viable for at least 6 months.

Acknowledgments

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Počasna rast ozkolistnega koščca *(Berula erecta) in vitro* – vpliv saharoze, sorbitola in temperature

IZVLEČEK

In vitro hramba ozkolistnega koščca (*Berula erecta* (Huds.) Coville)) s počasno rastjo bi racionalizirala vzdrževanje tkivne kulture, saj bi zmanjšala število prestavljanj na sveže gojišče. Počasno rast ob hkratnem ohranjanju viabilnost smo poskušali doseči z dodajanjem sladkorjev in sladkornih alkoholov v MS gojišče in nižanjem temperature v rastnih komorah. Preizkuslili smo vpliv nizkih temperatur (13 °C in 4 °C), različnih kombinacij in koncentracij sorbitola (20, 40 g L^{-1}) in saharoze (30, 40 g L^{-1}) ter svetlobe na viabilnost in sposobnost povrnitve rasti *B. erecta*. Povišanje koncentracije saharoze ni zavrlo rasti. Pri 23 ± 2 °C je dodatek 20 g L^{-1} sorbitola uspešno zavrl rast, dodatek 40 g L^{-1} sorbitola je po dveh mesecih kulture vodil v senescenco rastlin. Hramba *B. erecta* pri 13 °C v nobeni obravnavi ni učinkovito zavrla rasti. Kot najbolj učinkovit način za dolgoročno hrambo *B. erecta* s počasno rastjo se je izkazala hramba rastlin pri 4 °C v temi. Ob minimalni stopnji rasti je *B. erecta* ohranila visoko stopnjo viabilnosti, pri čemer so rastline ostale več mesecev nespremenjeno viabilne ter sposobne ponovne regeneracije.

Ključne besede: hramba s počasno rastjo, *Berula erecta*, viabilnost, saharoza, sorbitol, temperatura