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EFFECT OF CARBOHYDRATE SOURCE ON *IN VITRO* PROPAGATION AND ROOTING OF TWO CULTIVARS OF PETUNIA (*PETUNIA* × *ATKINSIANA* D. DON)

WPŁYW RODZAJU CUKRU NA MIKROROZMNAŻANIE I UKORZENIANIE DWÓCH ODMIAN PETUNII (*PETUNIA* × *ATKINSIANA* D. DON) W KULTURACH *IN VITRO*

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Streszczenie. W pracy określono wpływ rodzaju i stężenia cukru na namnażanie i ukorzenianie pędów dwóch odmian petunii *Petunia* × *atkinsiana* D. Don 'Prism White' i 'Prism Rose'. Do pożywek dodano sacharozę, fruktozę, glukozę oraz maltozę w stężeniach 0–50 g · dm^{-3}. Glukoza i maltoza nie wpływała stymulująco na namnażanie pędów petunii. Brak źródła cukru w pożywce lub jego wysokie stężenie (50 g · dm^{-3}) miało negatywny wpływ na ukorzenianie mikrosadzonek obu badanych odmian petunii. Najwyższy współczynnik rozmnażania petunii 'Prism White' obserwowano na pożywce z dodatkiem 30 g · dm^{-3} fruktozy. W przypadku odmiany 'Prism Rose' najlepsza była pożywka uzupełniona 30 g · dm^{-3} sacharozy, dlatego stężenie to uznano za najbardziej optymalne. Najwyższy stopień ukorzeniania pędów petunii 'Prism White' zaobserwowano na pożywce z dodatkiem 40 g · dm^{-3} glukozy, petunii 'Prism Rose' – 30 g · dm^{-3} sacharozy i maltozy. Uzyskane wyniki badań wykazały, że do mikrorozmnażania petunii można zastosować inne źródło węgla niż sacharoza.

Key words: fructose, glucose, maltose, micropropagation, *Petunia* × *atkinsiana* D. Don, sucrose.
Słowa kluczowe: fruktoza, glukoza, maltoza, mikrorozmnażanie, *Petunia* × *atkinsiana* D. Don, sacharoza.

INTRODUCTION

For plant growth in *in vitro* culture, the composition of the medium is a determining factor. Most of the media consists of mineral salts, a carbon source, vitamins, and plant growth regulators. A metabolizable sugar is one of the most important constituents of the culture medium that allow the growth of explants (Panathula et al. 2014). Sugar is an organic carbon source and is used as the energy source and as a substrate in various metabolic pathways of a plant in *in vitro* cultures (Fotopoulos and Sotiropoulos 2004, Klopotek et al. 2012). Sugar is also required as an osmotic agent, which influences the rate of cell division or the degree of morphogenesis of the cells (Paiva Neto and Otoni 2013). Besides, the photosynthetic ability of the cultured tissues is limited because of low irradiance and limited gas exchange (Kozai 1991). The low photosynthetic activity is considered as one of the major limiting

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factors for the improvement of micropropagation efficiency (Jain and Babbar 2003, Yaseen et al. 2013). According to some authors (Mosaleeyanon et al. 2004, Xiao and Kozai 2006, for instance) poor photosynthetic ability may lead to slow growth and low survival rate after acclimatization into *ex vitro* condition. Carbohydrate requirements depend upon the stage of culture and many show differences in respect to species (Yassen et al. 2013).

In *in vitro* cultures, sucrose is the most studied and most widely used sugar and it is found in the phloem sap of many plants (Fuentes et al. 2000, Al-Khateeb 2008, Panathula et al. 2014). Sucrose is highly soluble in water and has no influence on the various biochemical mechanisms. Typically, all media contain sucrose in the range of 1%–3% as a carbon source. Even though sucrose may be successful used in plant tissue culture, it could be satisfactorily replaced by any other sugars as a suitable sources of carbon for *in vitro* culture of many plants. According to Paiva Neto and Otoni (2003) and Schuelter et al. (2009) the nutritional requirements for tissue growth in *in vitro* culture vary among species, among varieties and even within the plant itself and need to be optimized. Therefore, the influence of different types of sugar and its concentration on growth and plant development in *in vitro* culture, is still an important topic of research in micropropagation of many species.

Petunia × *atkinsiana* D. Don belongs to *Solanaceae* family which has rarely been micropropagated, and was used as a model plant. The role of carbohydrate types and concentration on petunia cv. 'Prism White' and 'Prism Rose' on multiplication and rooting stages have not been studied yet. Therefore the objective of this study was to determine the effect and concentration of different carbohydrates on growth and rooting in *in vitro* condition.

MATERIAL AND METHODS

The research material consisted of 15–20 mm shoot tips and lateral buds of *Petunia* × *atkinsiana* D. Don cvs. 'Prism White' and 'Prism Rose' obtained from sterile stabilized *in vitro* culture. Explants resulted from direct organogenesis were transferred to 300 mL flasks filled with 30 mL of multiplication medium based on MS (Murashige and Skoog 1962) formula. Each combination included 48 shoots (6 shoots per flask) in eight series. Proliferated shoots were transferred onto rooting MS medium supplemented with indole-3-butyric acid (IBA) at the concentrations of 1.0 mg · dm^{-3}.

Both multiplication and rooting media were supplemented with 8 g · dm^{-3} agar (Biocorp) and 100 mg · dm^{-3} myo-inositol and with a different type of sugar: sucrose, fructose, glucose or maltose in six different concentrations each (0, 10, 20, 30, 40, 50 g · dm^{-3}). Explants cultured on media without carbohydrate source were the controls. The pH of the media was adjusted to 5.7 prior autoclaving at 121ºC (0.1 MPa) for the time required according to the volume of medium in the vessel. Cultures were incubated in growth room at a temperature of 25ºC under 16-h photoperiod with a photosynthetic photon flux density (PPFD) of 40 µmol · m^{-2} · s^{-1}.

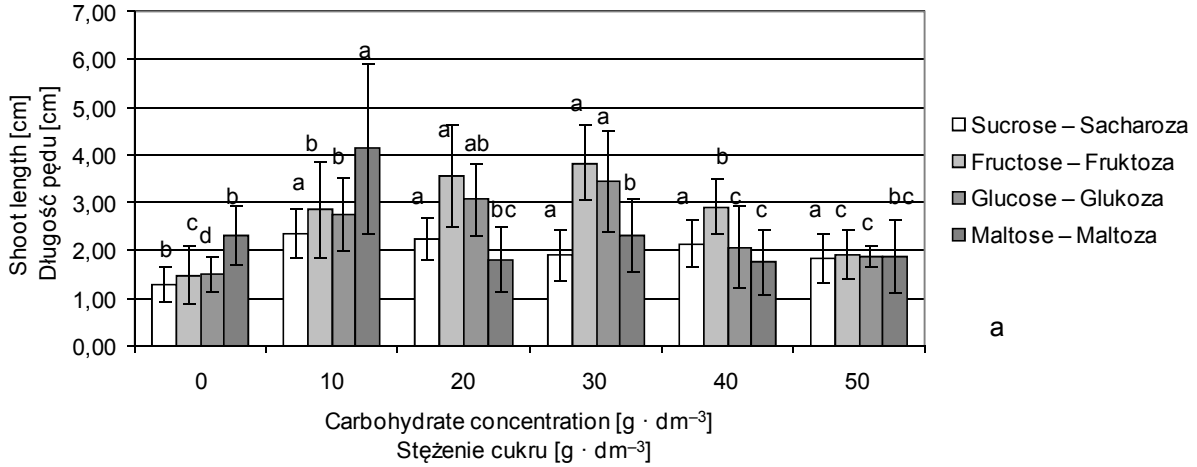
Experiment were conducted in a completely randomized design. The mean values for shoot length, number of shoot per one explant, length and number of root and fresh and dry mass were obtained. The overall effect of treatments was assessed using analysis of variance (ANOVA) and Tukey's honestly significant differences (HSD) test, at the level of significance of $\alpha = 0.05$.

RESULT AND DISCUSSION

According to many authors (Jain and Babbar 2003, Madhulatha et al. 2006, Xiao and Kozai 2006, Shuelter et al. 2009, for instance) carbon source are components of the medium as a source of energy and for maintaining the osmotic potential. The addition of optimal carbohydrate source to the medium induces adventitious shoot or buds. On the contrary, plant cell, tissue and organ growth and morphogenesis are strongly influenced by high osmotic pressure (Paiva Neto and Otoni 2003, Shuelter et al. 2009). The present study indicated the influence of different carbon sources on proliferation and rooting of petunia shoots cvs. 'Prism White' and 'Prism Rose'. The type and concentration of the carbon source in the culture medium had significant effects on the shoot length and number of shoots per explant (Fig. 1a, b). Explants of petunia cv. 'Prism White' which were grown on media supplemented with 10 g · dm^{-3} maltose were characterized by, on average, the highest length of shoots (4.13 cm), (Fig. 1a). In case of petunia cv. 'Prism Rose', the most favorable type of sugar was glucose at concentration of 10 g · dm^{-3}. The average height of the plants on this medium was 5.86 cm (Fig. 1a). The highest number of new petunia shoots (5.5) per explant was observed on medium supplemented with 20 g · dm^{-3} of sucrose (Fig. 1b). Moreover, explants which were grown on medium containing sucrose were characterized by the highest dry weight (1.38 g) (Fig. 1c). Medium supplemented with glucose caused inhibition of shoot growth (length), reduced fresh mass of petunia cv. 'Prism Rose', and reduced the number of new shoots per explant for both cultivars analyzed, 'Prism White' and 'Prism Rose'. A similar response was also observed for maltose. The shoot length growth of petunia 'Prism Rose' was stimulated by increasing the concentration of maltose from 20 to 50 g · dm^{-3}. However, explants 'Prism White' which were grown in medium supplemented with maltose showed low length of shoots and also low number of shoots per proliferated explant in both cultivars. Fructose at 30 g · dm^{-3} produced the best shoot elongation of 'Prism White', likewise sucrose at 20–30 g · dm^{-3} for 'Prism Rose' (Fig. 1). Media without sugar did not produce any new shoots, substantially inhibiting plant growth of both cultivars.

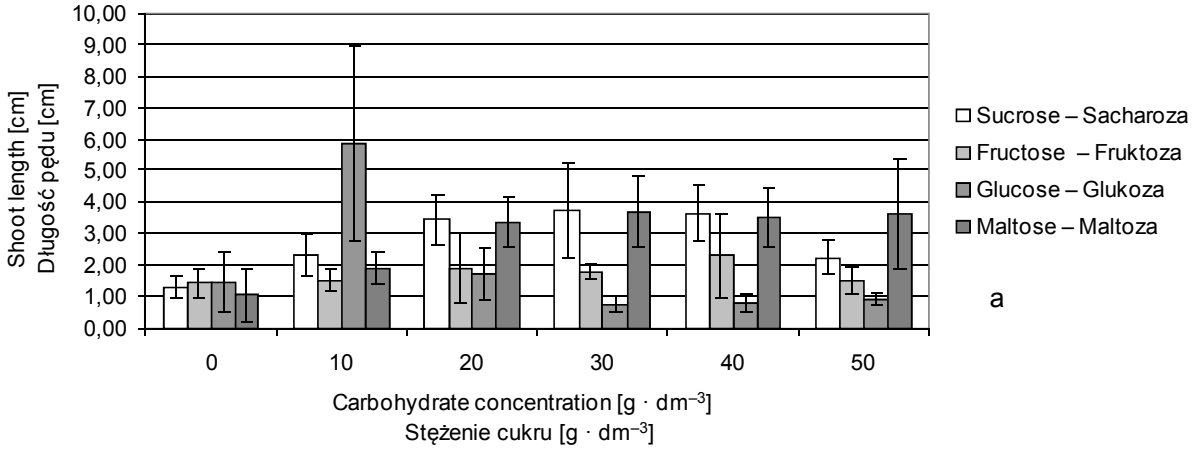
These results were in agreement with Madhulatha et al. (2006), Al-Khateeb (2008), Mustafa (2013) and Panathula et al. (2014) who suggested that sucrose or fructose in comparison with other carbon source (glucose and maltose) was the best for multiple shoot proliferation of many plants. Besides, sucrose plays an important role, especially in breaking dominancy; but its metabolism is still not clearly understood (Panathula et al. 2014). Nevertheless, according to Chu et al. (1990), the highest frequency of the regenerated explants of *Triticum aestivum* L. was stimulated by glucose. On the contrary, Strickland et al. (1987) indicated that the addition of maltose increased the somatic embryogenesis and regeneration frequency in *Medicago sativa* L. Hossain et al. (2005) reported that maltose is the best carbon source for regeneration of maximum number of shoots in *Centella asiatica* L. Generally, the use of maltose as a carbon source is lower in rank than that of sucrose and fructose. In this study, the poor results obtained with glucose and maltose are similar to those obtained by Panathula et al. (2014). They suggested that glucose and maltose are not efficiently metabolized but regulate osmotic potential.

Petunia 'Prism White'



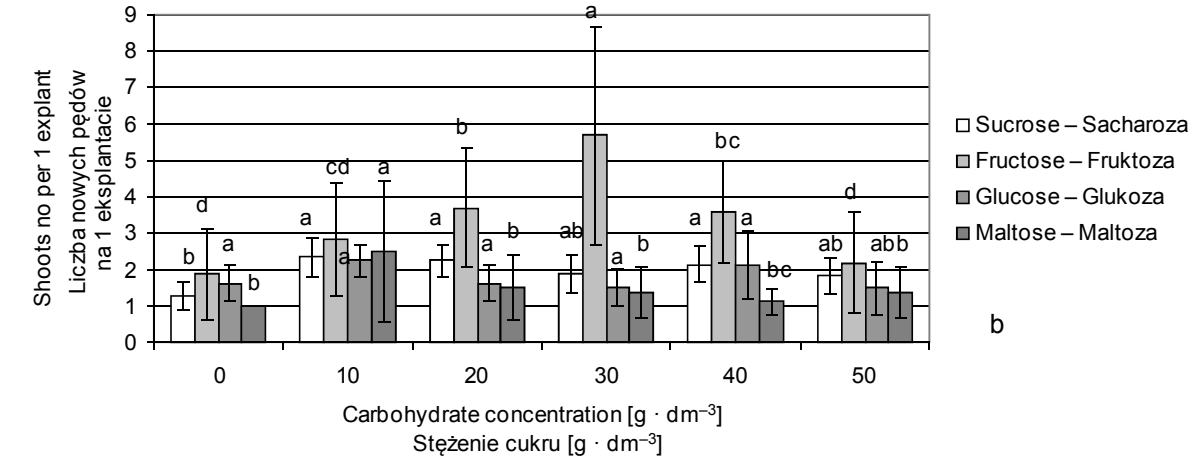
a

Petunia 'Prism Rose'



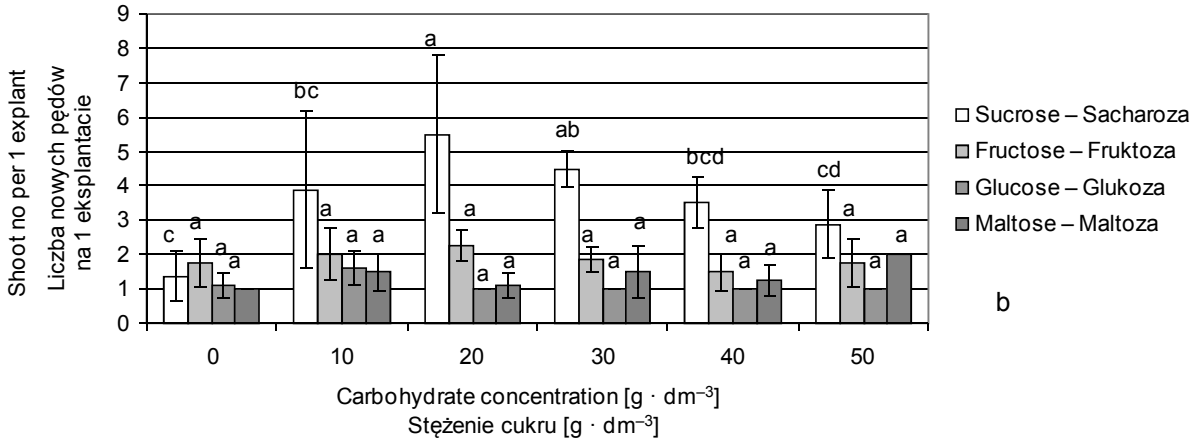
a

Petunia 'Prism White'



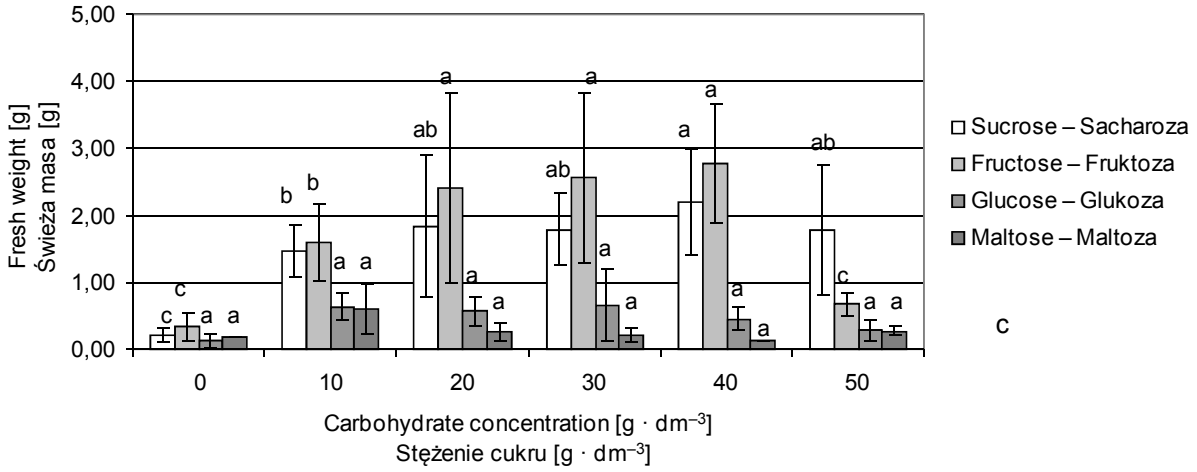
b

Petunia 'Prism Rose'



b

Petunia 'Prism White'



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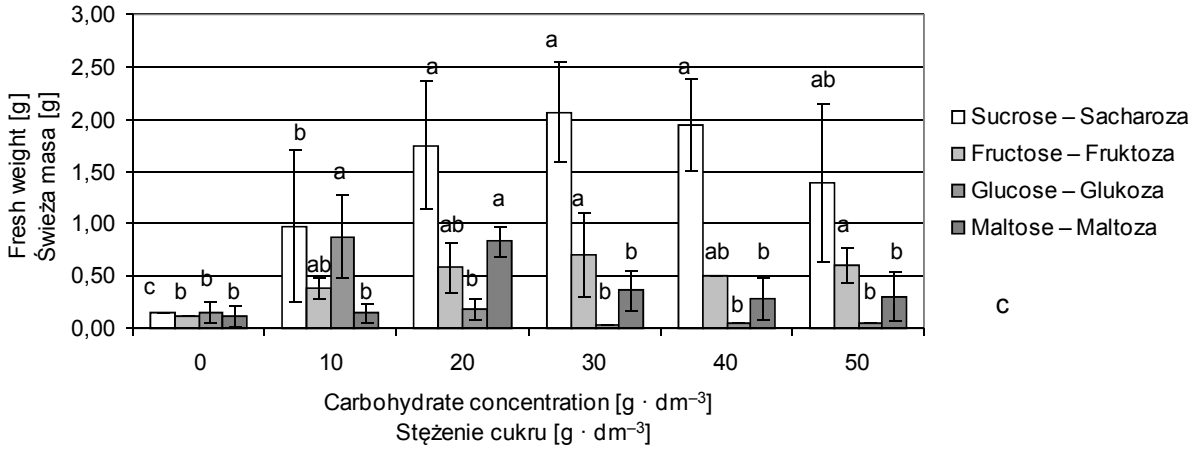
Petunia 'Prism Rose'



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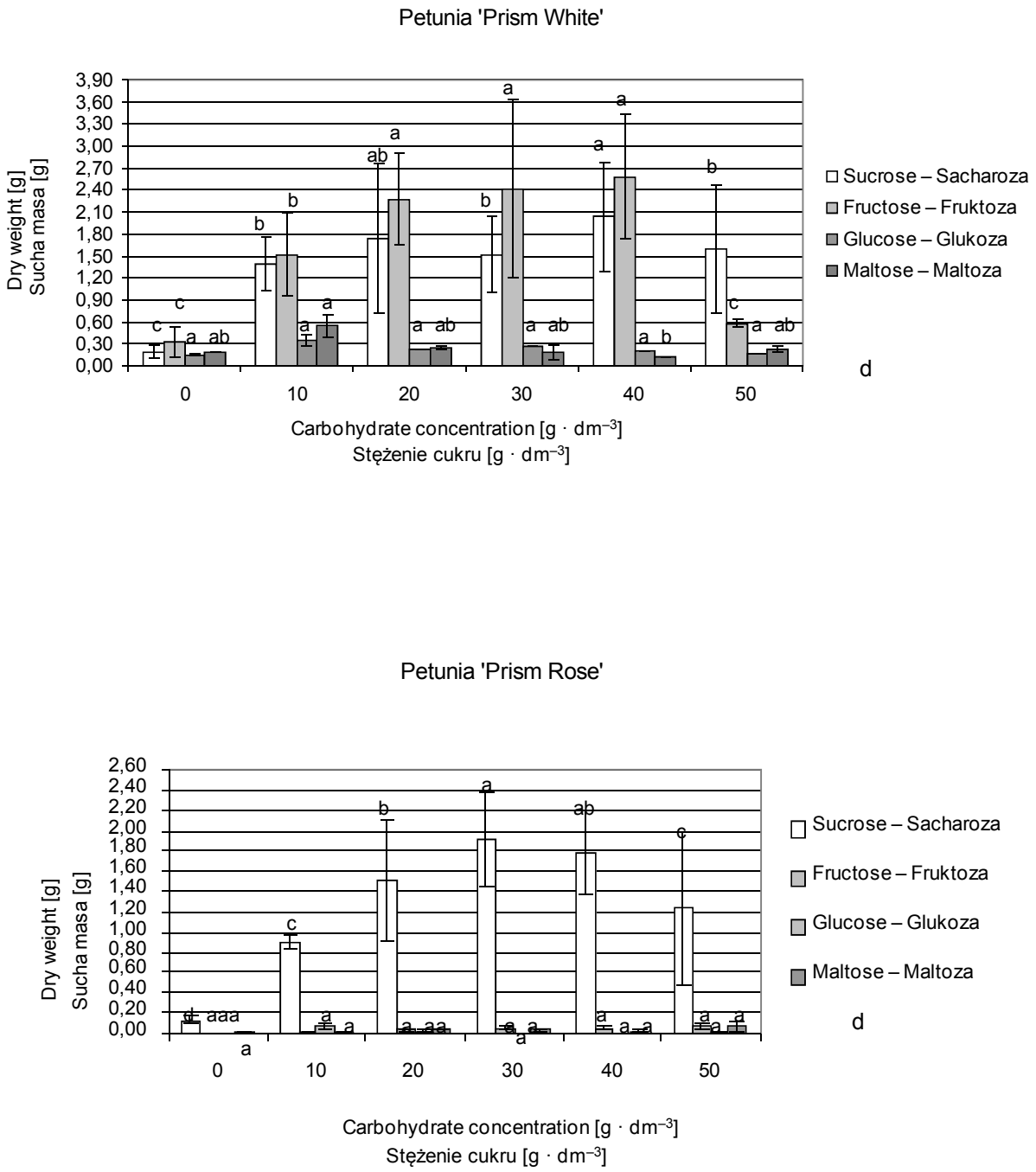
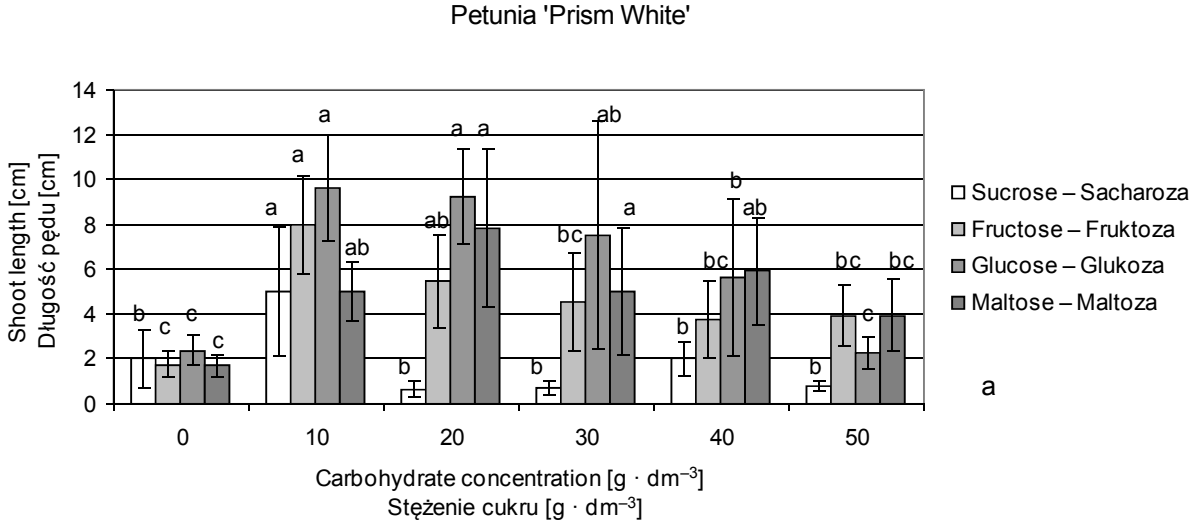


Fig. 1. Influence of carbon source and concentration on microshoots of petunia cvs. 'Prism White' and 'Prism Rose' on multiplication media. Values with the same letters are not significantly different at P ≤ 0.05. Vertical bars represent mean ±SE (Standard error)

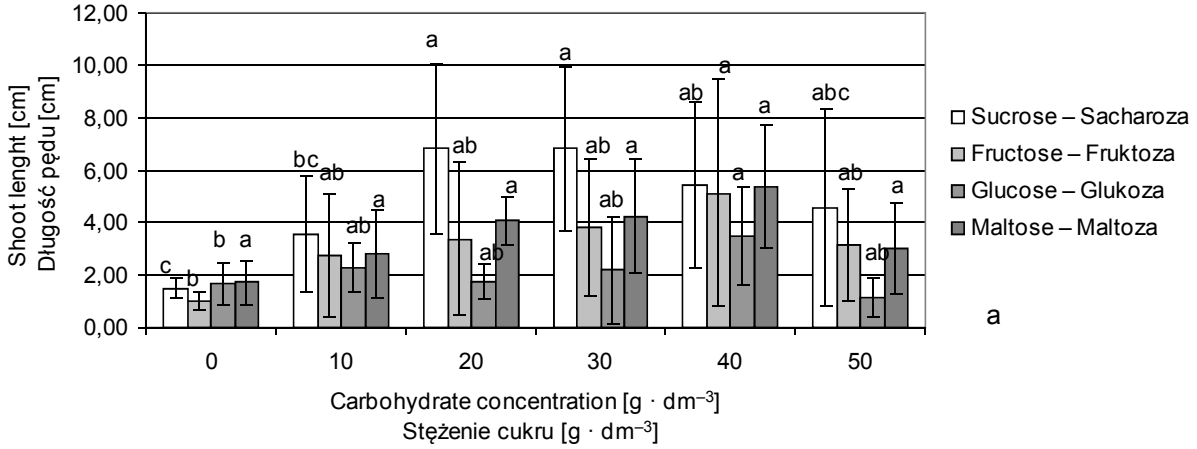
Rys. 1. Wpływ źródła cukru i jego stężenia na cechy morfologiczne petunii odmian 'Prism White' i 'Prism Rose' na pożywce namnażającej. Średnie oznaczone takimi samymi literami nie różnią się statystycznie na poziomie P ≤ 0,05. Linie na słupkach przedstawiają średnie ±SE (odchylenie standardowe)

Well-developed micropropagated shoots of cvs. 'Prism White' and 'Prism Rose' were rooted on MS medium supplemented with 1.0 mg · dm^{-3} IBA, containing the same sugar composition used for micropropagation. According to Yaseen et al. (2013) rhizogenesis *in vitro* is a highly energetic process. It is suggested that carbohydrates caused mild osmotic stress, acting lateral root formation. The results obtained confirmed that in the case of both cvs. 'Prism White' and 'Prism Rose', the absence of sugar in the medium had a negative effect on rhizogenesis process. This affirms the importance of sugar on root formation. Moreover, it was also observed that too high concentration of sugar (50 g · dm^{-3}) negatively influenced the growth of plants, root formation and their number, and fresh and dry weight of petunia (Fig. 2). The longest roots (4.25 cm) and the highest number of roots (13) developed in explants of 'Prism White' which were grown on medium containing 40 g · dm^{-3} glucose (Fig. 2b, c). The presence of glucose also had a positive effect on the average height of the petunia plants (6.10 cm) and their fresh weight (1.63 g) (Fig. 2a, d). Microshoots of 'Prism White' regenerated from medium containing sucrose were reported to produce very low number of roots with good lengths (0.92 cm). In addition, petunia grown on this medium had the lowest height (1.86 cm) and was characterized by the lowest fresh weight (0.64 g). In case of petunia 'Prism Rose', the longest roots (5.56 cm) were developed for explants grown on medium containing 30 g · dm^{-3} sucrose (Fig. 2b), whereas the highest number of roots were observed for explants propagated on medium supplemented with 40 g · dm^{-3} maltose (Fig. 2c). Furthermore, these plants were characterized by the highest fresh weight (1.38 g) (Fig. 2d). A similar effect on *Centella asiatica* L. rhizogenesis process was described by Hossain et al. (2005), where the best results were obtained for the MS medium supplemented with 30 g · dm^{-3} sucrose and 0.2 mg · dm^{-3} IBA, whereas gur (glucunorate) and maltose did not promote production of roots.

Differences in *in vitro* root formation for both cultivars grown on the same type of media containing the same combination of carbon sources may arise from differences in free IBA levels in their basal sections. Thus, carbohydrates exert complex influence on plant growth and development Yaseen et al. (2013).

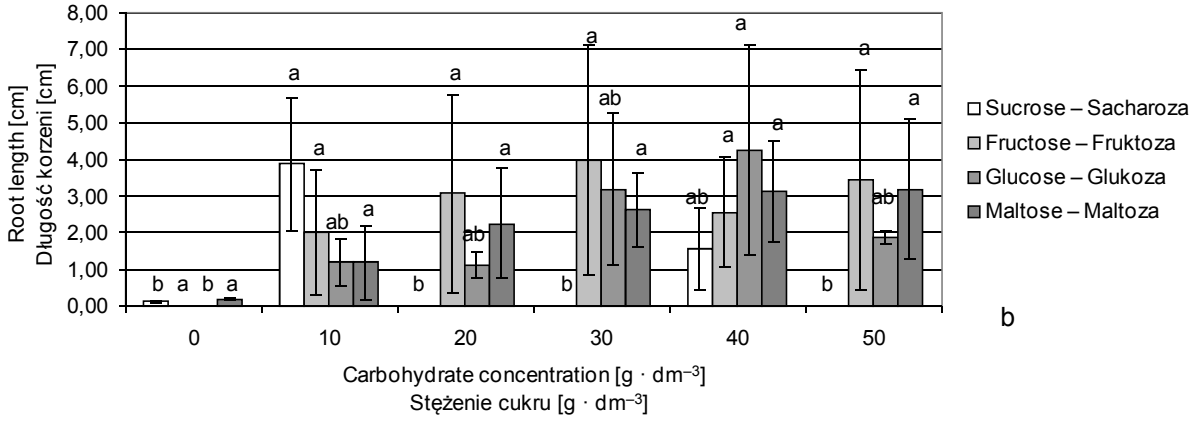


Petunia 'Prism Rose'



a

Petunia 'Prism White'



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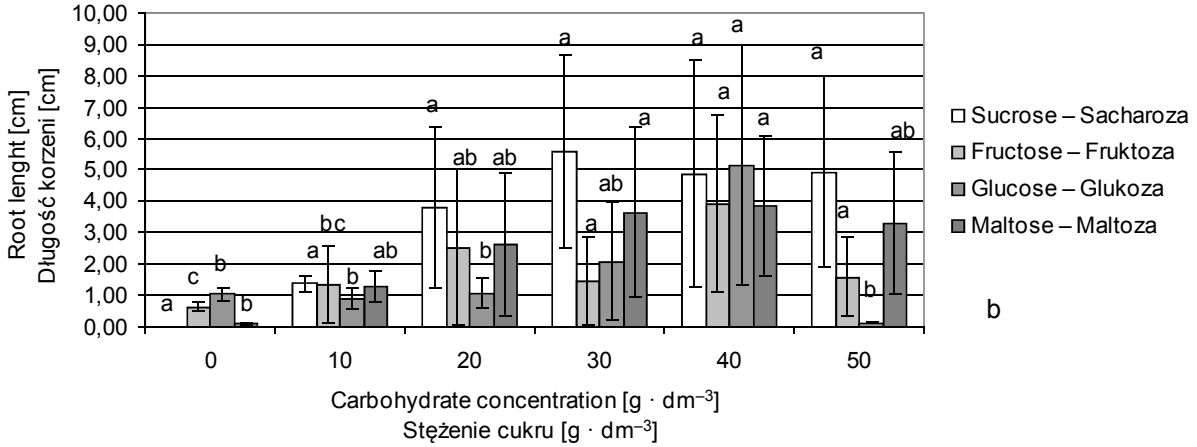
Petunia 'Prism Rose'



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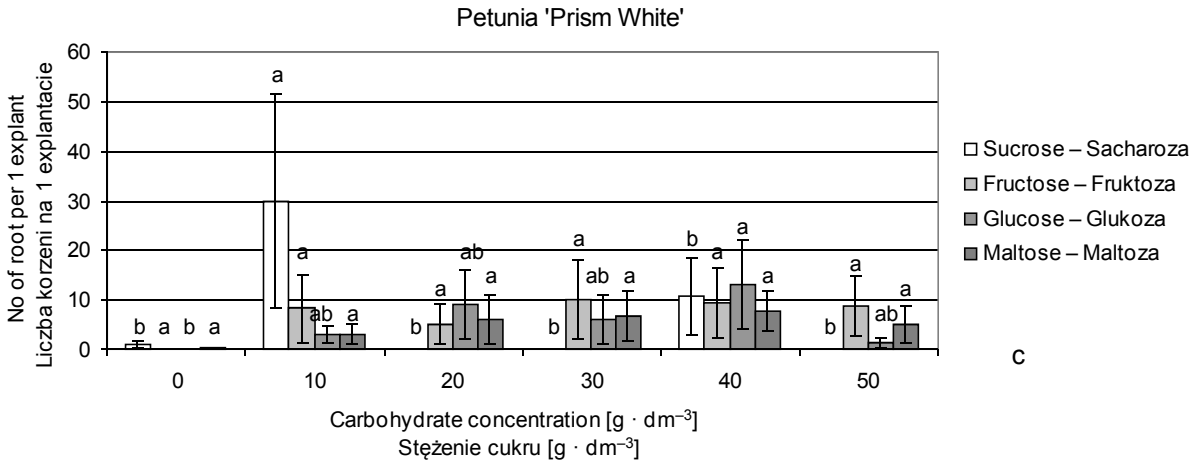
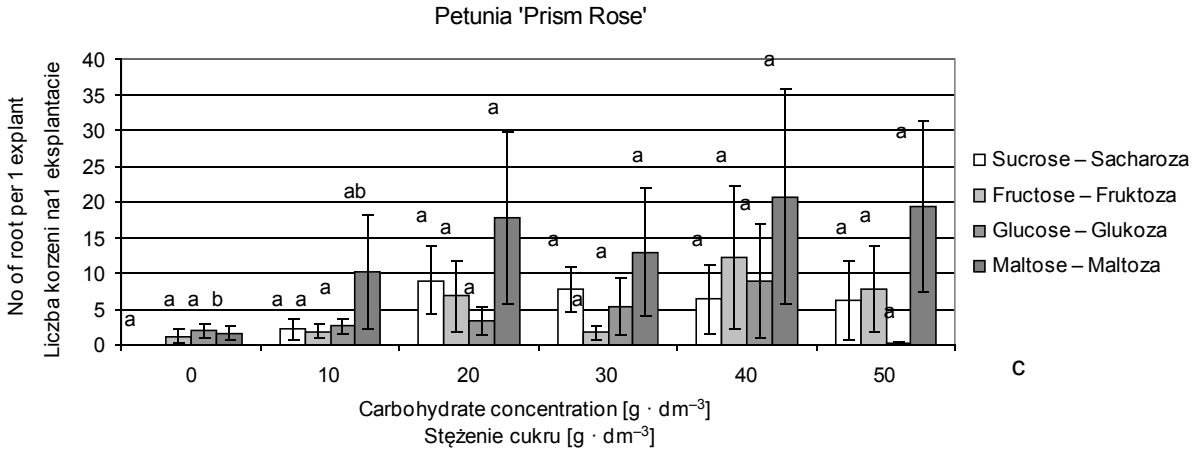
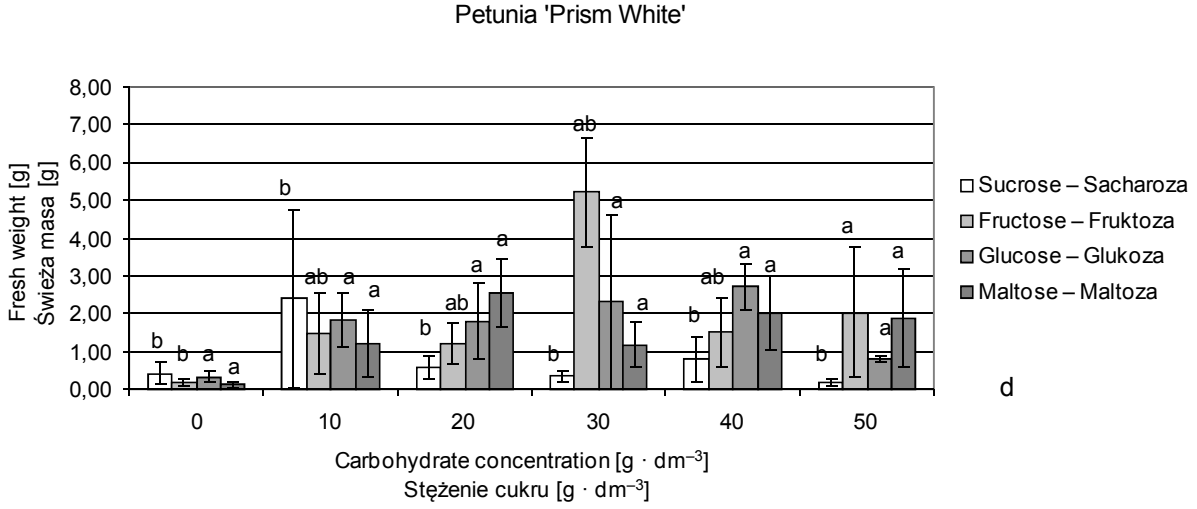

Petunia 'Prism White'
No of root per 1 explant
Liczba korzeni na 1 explantacie
Carbohydrate concentration [g · dm⁻³]
Stężenie cukru [g · dm⁻³]
Sucrose – Sacharoza
Fructose – Fruktoza
Glucose – Glukoza
Maltose – Maltoza

c


Petunia 'Prism Rose'
No of root per 1 explant
Liczba korzeni na1 eksplantacie
Carbohydrate concentration [g · dm⁻³]
Stężenie cukru [g · dm⁻³]
Sucrose – Sacharoza
Fructose – Fruktoza
Glucose – Glukoza
Maltose – Maltoza

c


Petunia 'Prism White'
Fresh weight [g]
Świeża masa [g]
Carbohydrate concentration [g · dm⁻³]
Stężenie cukru [g · dm⁻³]
Sucrose – Sacharoza
Fructose – Fruktoza
Glucose – Glukoza
Maltose – Maltoza

d

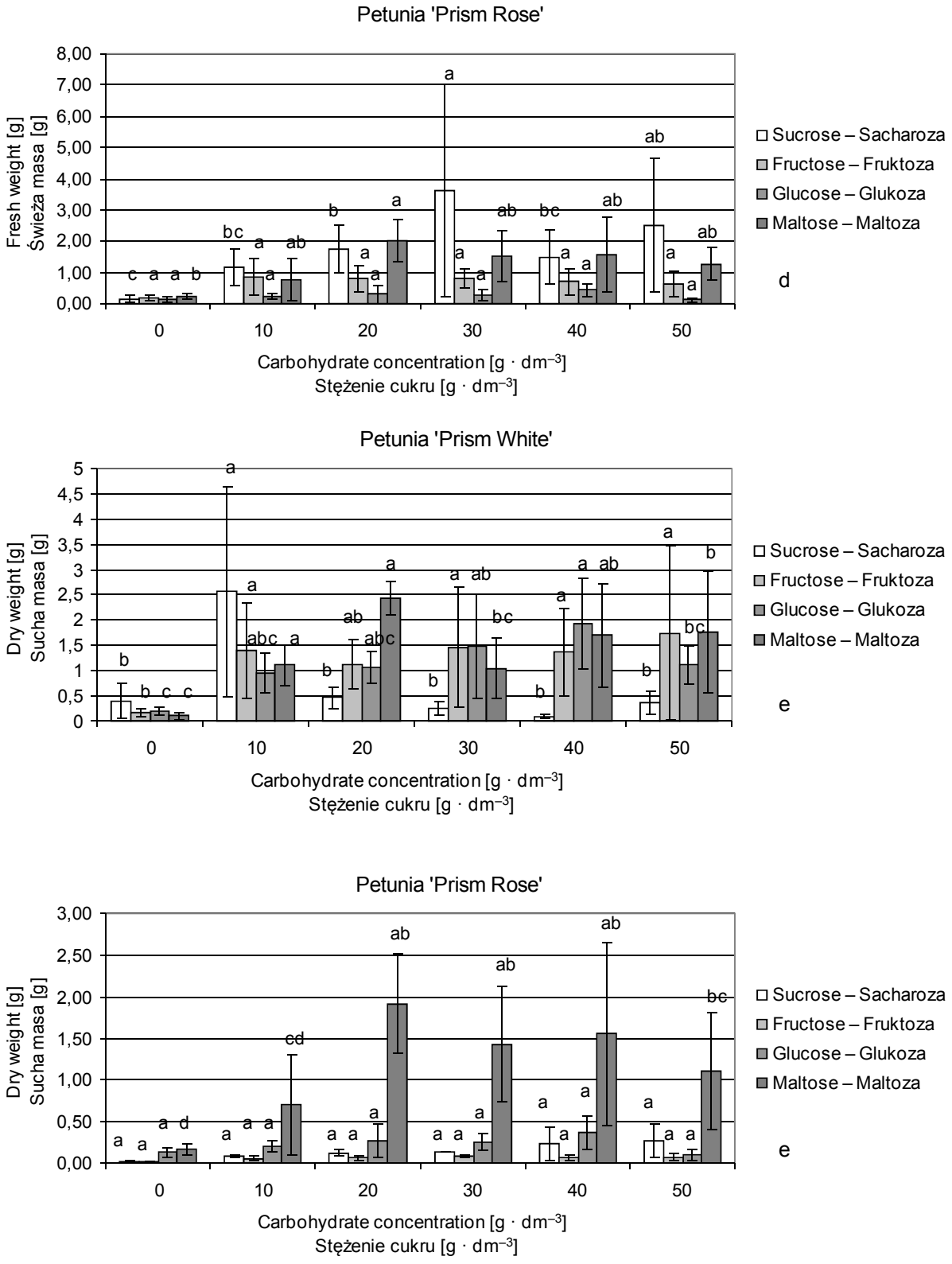


Fig. 2. Influence of carbon source and concentration on microshoots of petunia cvs. 'Prism White' and 'Prism Rose' on rooting media. Values with the same letters are not significantly different at P ≤ 0.05. Vertical bars represent mean ±SE (Standard error)

Rys. 2. Wpływ źródła cukru i jego stężenia na cechy morfologiczne pędów petunii odmian 'Prism White' i 'Prism Rose' na pożywce ukorzeniającej. Średnie oznaczone takimi samymi literami nie różnią się statystycznie na poziomie P ≤ 0,05. Linie na słupkach przedstawiają ±SE

CONCLUSIONS

The results presented in this study demonstrate the usefulness of combination of carbon sources for efficient *in vitro* propagation of petunia cvs. 'Prism White' and 'Prism Rose'. Multiplication and rooting of petunia microshoots depend on the type of sugar and its concentration in the medium, and depend on cultivar. In some cases, sucrose could be replaced totally or partially by other carbon sources (fructose, glucose or maltose).

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Abstract. The effects of carbohydrate type and concentration on shoot multiplication of two cultivars of *Petunia* × *atkinsiana* D. Don 'Prism White' and 'Prism Rose' and on the root formation in *in vitro* culture were investigated. Sucrose, fructose, glucose, and maltose were tested at concentration ranging from 0 to 50 g · dm^{-3}. Glucose and maltose did not stimulate proliferation of petunia shoots. Either the lack of carbon source or its high concentration (50 g · dm^{-3}) was completely ineffective in rooting induction for both cvs. 'Prism White' and 'Prism Rose'. In 'Prism White' the highest mean values for microshoot number together with the highest number of new shoots per one explant was obtained on the medium containing 30 g · dm^{-3} of fructose. Considering 'Prism Rose', the more favorable sugar source and concentration for shoot length was obtained on medium with 30 g · dm^{-3} of sucrose. The highest number of adventitious roots produced per shoot of petunia 'Prism White' was obtained on medium supplemented with 40 g · dm^{-3} of glucose and for petunia 'Prism Rose' – 30 g · dm^{-3} sucrose and maltose. The results presented in this study indicate that sucrose could be replaced by other carbon source in micropropagation of petunia.