RJH Vol. V, 2024: 9-14
10.51258/RJH.2024.01

Effects of nutrient solution concentration during the nursery period on the growth of *Stevia* cuttings in hydroponics

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ABSTRACT

The effects of nutrient solution concentration on the growth of stevia cuttings during the nursery period in hydroponics were investigated. Cuttings were inserted in continuously aerated water, nutrient solution of 0.3 dS m^{-1}, and nutrient solution of 0.7 dS m^{-1}. Rooting of cuttings began on the 10 days after cutting. The rooting rate of cuttings under water and EC 0.3 dS m^{-1} reached 100% on day 13. The nutrient solution concentration during the nursery period did not affects rooting rate. At transplanting, best stem length and number of leaves were observed 0.7 dS m^{-1}. The maximum root length was not affected nutrient solution concentration. At harvest, the shoot dry weight of 0.7 dS m^{-1} was significantly heavier than that of water. Survival rates after transplanting of 0.3 dS m^{-1} and 0.7 dS m^{-1} were lower than that of water. Despite some issues with the survival rate, the use of a nutrient solution during the nursery period in hydroponics of stevia could lead to more efficient production.

Keywords*:* electric conductivity, medicinal plant, rooting, survival rate, vegetative propagation

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a perennial plant of the Asteraceae family. It has been introduced in Japan, Brazil, Korea, Mexico, USA, Indonesia, Tanzania, and Canada (Megeji *et al*, 2005). The leaves of this plant are mainly used as a sweetener, because it is with compounds that 200-300 times sweeter than sugar (Hossain *et al*., 2017). Sweetness of stevia comes from steviol glycosides. The four major steviol glycosides in stevia are stevioside, rebaudioside A, rebaudioside C, and dulside A (Singh and Rao, 2005). Stevia plants included wide ranges of genetic variation in content and in composition of the glycosides (Nakamura and Tamura, 1985). Hata and Fujita (1996) bred tetraploid plants of stevia. Hata *et al*. (2001) bred a triploid plant of stevia with high rebaudioside A content. Morita Kagaku Kogyo Co., Ltd. selected and bred stevia that produces a higher ratio of rebaudioside A (Ohta *et al*., 2010). In this way, the breeding of stevia is gradually being reported. However, transplants of stevia are propagated often using wild stevia as a propagation source. The main propagation methods of stevia are seeds, cutting (Miyazaki and Watanabe, 1974), and tissue culture (Miyagawa *et al*., 1984). Since stevia is allogamous plant, various phenotypes appear in seed propagation (Nakamura and Tamura, 1985). Vegetative propagation such as cutting propagation and tissue culture can produce transplants of the same phenotype. Especially, cutting propagation are a feasible technique in the field. Effects of cutting position (Abdullateef and Osman, 2012; Kassahun and Mekonnen, 2012; Kassahun *et al*., 2013; Rakibuzzaman *et al*., 2018), node number of cuttings (Kassahun *et al*., 2013), plant growth regulator (Abdullateef and Osman, 2012; Kassahun and Mekonnen, 2012; Rakibuzzaman *et al*., 2018; Manohar *et al*., 2022), and growing media (Miyazaki and Watanabe, 1974; Ogao-Ogao *et al*., 2017; Manohar *et al*., 2022) on rooting of cuttings were investigated in stevia. Thus, cutting propagation are a major propagation method in stevia. Stevia was cultivated in soil, but in recent years it has also been cultivated in hydroponics. Yoneda (2021) reported on the cultivation of stevia in hydroponics at a closed-type plant factory. In cutting propagation, cuttings are inserted in the media such as vermiculite, sand, and rock wool. However, when cultivating by hydroponics, the media in the root zone of the transplants needs to be removed. In hydroponics of tomato, cuttings were inserted in the nutrient solution when raising transplants (Namiki *et al*., 1977). Mizushima (2016) reported that nutrient solution concentration to insert cuttings during the nursery period by hydroponics affected the growth of cuttings in *Elatostema involucratum* Franch. & Sav. With this method, removal of the media in the root zone of the transplants not required. However, in stevia, the effect of nutrient solution concentration by hydroponics is unclear. Therefore, this paper aims to clarify the effects of nutrient solution concentration on stevia cuttings during the nursery period in hydroponics.

MATERIALS AND METHODS

This experiment was conducted at Wakasa-higashi High School during the year 2016. Cuttings were taken from mother plants of stevia maintained at a glasshouse in Wakasa-higashi High School experimental farm. Stem were cut from the tip of the plant including apical bud. Stem length and leaves number of each cutting were prepared in the form of 5 cm and 6 to 8, respectively. Cuttings were inserted in continuously aerated water or nutrient solution (SA-prescription; OAT Agrio Co., Ltd, Tokyo, Japan) with different electrical conductivity (EC) levels on 2, May. The experimental plot was following three; water, EC 0.3 dS m^{-1}, and EC 0.7 dS m^{-1}. The cuttings were grown in containers (length; 37.0 cm, width; 25.5 cm, height; 13.5 cm) containing 5 L of each solution in the growth chamber (Preset temperature; 22°C). One container grew 15 cuttings at a spacing of 5 cm×5 cm (Fig. 1 A). An LED unit (Valore Corp., Kyoto, Japan) with alternating red and white LED chips was used as the light source. Cuttings were grown under a 12 h photoperiod and a total photosynthetic photon flux density of 100 µmol m^{-2} s^{-1} on the tip of the cutting. The pH value of each solution was 6.0 to7.0 during the nursery period. The following parameters were recorded: number of rooted cuttings until 15 days after inserted in water or nutrient solution and stem length, number of leaves, and maximum root length at the transplanting. The transplants (rooted cutting) were planted in a hydroponic system at a spacing of 16 cm×16 cm in the glasshouse on 30, May (Fig. 1 B and C). The nutrient solution concentration after transplanting was controlled from 1.3 to 1.7 dS m^{-1} in all experimental plots. Stevia plants were harvested at 39 days after transplanting and recorded shoot dry weight. The shoot dry weight was taken after drying the samples in an electric oven for 72 h at 85°C. The data were analysed using Tukey-Kramer test.

RESULTS AND DISCUSSIONS

The rooted cuttings were not observed in all experimental plots at seven days after cuttings (Fig. 2). The number of rooted cuttings in 10 days after cutting was 11 (rooting rate; 73%) or more in all experimental plots. Miyazaki and Watanabe (1974) reported that rooting of cuttings inserted in Kanuma soil, sand, and sandy soil begins around 10 days and ends around 2 to 3 weeks after cutting. Ogao-Ogao *et al*. (2017) reported that rooting of cuttings in soil culture began between 8 and 12 days after cutting..

Figure 1. Stevia at cutting (A), at transplanting (B), and transplanted rooted stevia cuttings (C).

Similarly, rooting of cuttings in this study began 10 days after cutting. In 13 days after cutting, a number of rooted cuttings was 14 (rooting rate; 93%) or more in all experimental plots. The rooting rate of cuttings under water and EC 0.3 dS m^{-1} reached 100% on day 13 after cutting. The nutrient solution concentration during the nursery period did not affect rooting initiation and rooting rate. In soil culture, the rooting rate of stevia cutting under good conditions was close to 100% (Miyazaki and Watanabe, 1974; Ogao-Ogao *et al.*, 2017).

Since the rooting rate in this study was 93% or more, it was considered that cuttings rooted normally even by inserted into water or nutrient solution. At transplanting, nutrient solution concentration during the nursery period affected stem length and number of leaves (Table 1). The best stem length and number of leaves were observed 0.7 dS m^{-1}.

The maximum root length was not affected by nutrient solution concentration. The effect of nutrient solution concentration during the nursery period appeared on the shoot growth. Mizushima (2016) reported the effect of the nutrient solution concentration during the nursery period in *Elatostema involucratum* Franch. & Sav. appeared on the shoot rather than on the roots. This phenomenon was observed in this study too.

The nutrient solution concentration during the nursery period significantly affects shoot dry weight at harvest. The shoot dry weight of 0.7 dS m^{-1} was significantly higher than that of water. Survival rates after transplanting tended to be lower with 0.3 dS m^{-1} and 0.7 dS m^{-1}compared to water.

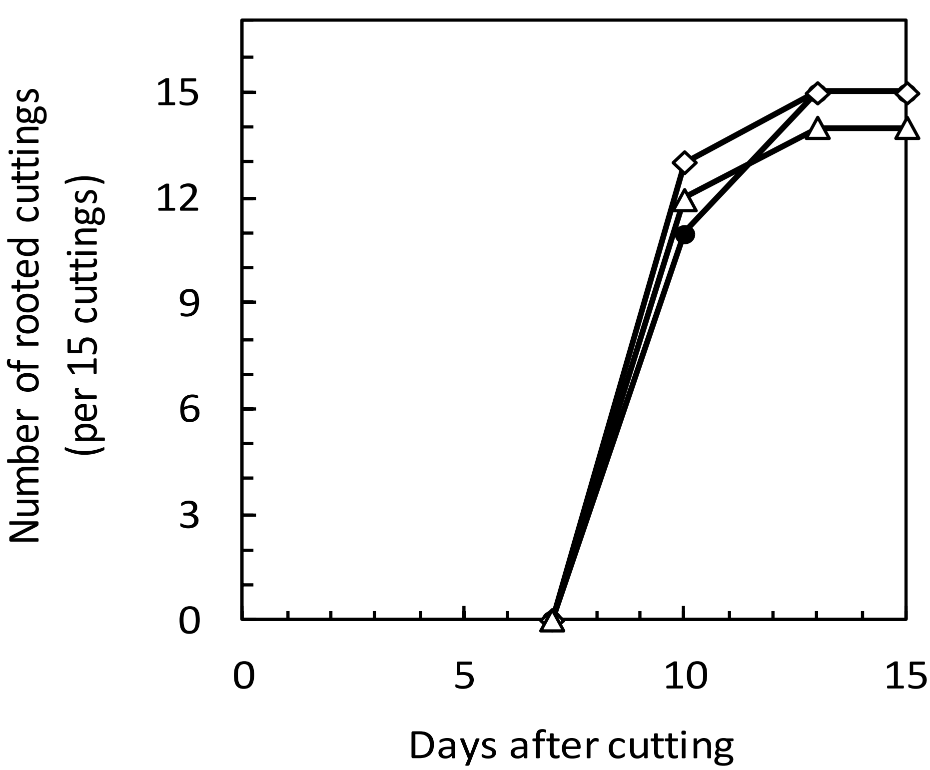

Figure 2. Changes in a number of rooted cuttings of stevia after cutting. Black circle, white rhombus, and white triangle in the figure indicate water, 0.3 dS m^{-1}, and 0.7 dS m^{-1}, respectively.

Table 1. Effects of nutrient solution concentration during the nursery period on the growth at transplanting and at harvest.

Experimental plot	At transplanting			At harvest	
	Stem length (cm)	Number of leaves	Maximum root length (cm)	Shoot dry weight (g plant^{-1})	Survival rate (%)
Water	12.7 b [z]	13.2 b	9.4 a	1.43 b	100
0.3 dS m^{-1}	14.4 ab	15.6 ab	11.3 a	1.69 ab	86
0.7 dS m^{-1}	16.3 a	16.6 a	9.1 a	2.22 a	71

[z] *Means with different letters indicate a significant difference at the 5% level by the Tukey-Kramer test in each column.*

CONCLUSIONS

In this study, despite some issues with the survival rate, the use of a nutrient solution during the nursery period in hydroponic stevia could lead to more efficient production.

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