
ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. VIII

SECTIO EEE

2000

Department of Ornamental Plants, Agricultural University Lublin, Poland

DANUTA KOZAK

**The Influence of Sucrose Concentration
on Microtuber Formation of *Gloriosa rothschildiana* O'Brien**

Wpływ stężenia sacharozy na formowanie mikrotubulw *Gloriosa rothschildiana* O'Brien

Abstract. The effect of sucrose concentration on the tuber formation of *Gloriosa rothschildiana* O'Brien 'Red Dark' *in vitro* was investigated. Individual shoots were excised from aseptically grown shoot clusters and cultivated on the modified Murashige and Skoog (MS) regulator free medium supplemented with different sucrose levels (3, 6, 9, 12%). The cultures were maintained at 22°C, light intensity of 35 $\mu\text{M m}^{-2} \cdot \text{s}^{-1}$ and 16-h photoperiod during 12 weeks. The fresh weight and length of microtubers were the highest in the presence of 6% sucrose. The majority of primary tubers sprouted and formed new tubers. This process was promoted by 6-9% sucrose concentration. The best regeneration of roots was observed on the medium containing 3% of sucrose.

INTRODUCTION

Propagation of *Gloriosa* through tissue cultures has been studied by several researchers (Chandra and Tarar, 1988; Finnie and Van Staden, 1989; Samarajeewa et al., 1993; Custers and Bergervoet, 1994; Guo and Goi, 1998). The majority of experiments concerned the effect of different growth regu-

lators on the initiation and multiplication of *Gloriosa* shoots. Transplantation of rooted shoots to the soil is still causing several difficulties. Custers and Bergervoet (1994) reported that plantlets of *Gloriosa* wilted very quickly after they were planted to the soil in glasshouse. Microtubers, corms and bulbils adapt easily after transference out of culture and therefore they are a final product of *in vitro* propagation of many plants: *Dioscorea* (Forsyth and Van Staden, 1984), *Gladiolus* (Dantu and Bhojwani, 1995), *Narcissus* (Squires et al., 1991), *Solanum* (Forti et al., 1991), *Tulipa* (Custers et al., 1992; Hulscher et al., 1992). Very important factors in tuberization *in vitro* are medium constituents such as sucrose, mineral salts, growth regulators. In culture media the concentration of sucrose normally ranges between 2 and 3% but for the induction of tuberization it is usually recommended to use the concentration of 6 to 9%. High sucrose concentration stimulated formation and growth of *Gladiolus* corms (Dantu and Bhojwani, 1987, 1995; Steinitz et al., 1991; De Bruyn and Ferreira, 1992), tubers of *Solanum* (Hussey and Stacey, 1984; Forti et al., 1991), bulbils of *Hyacinthus* (Bach and Pawłowska, 1993), *Lilium* (Niimi and Onozawa, 1979; Takayama and Misawa, 1979) and *Narcissus* (Chow et al., 1992; Santos et. al., 1998).

The aim of the study was to investigate the formation of *Gloriosa rothschildiana* O'Brien 'Red Dark' microtubers in relation to sucrose level in the medium.

MATERIAL AND METHODS

Sprouts emerging from tubers of *Gloriosa rothschildiana* O'Brien 'Red Dark', after the dormant period, were used as a primary material in the experiment. The explants, after disinfection for 45 minutes in the solution of NaOCl containing 1% active chlorine, were cultured on the basic medium consisting of Murashige and Skoog (1962) (MS) mineral salts and NaH_2PO_4 — $170 \text{ mg} \cdot \text{l}^{-1}$, thiamine — $0.4 \text{ mg} \cdot \text{l}^{-1}$, pyridoxine — $0.5 \text{ mg} \cdot \text{l}^{-1}$, nicotinic acid — $0.5 \text{ mg} \cdot \text{l}^{-1}$, glycine — $2 \text{ mg} \cdot \text{l}^{-1}$, myo-inositol — $100 \text{ mg} \cdot \text{l}^{-1}$, sucrose — $30 \text{ g} \cdot \text{l}^{-1}$. The shoots were initiated and multiplied on the basic medium supplemented with $5 \text{ mg} \cdot \text{l}^{-1}$ of BA and $0.1 \text{ mg} \cdot \text{l}^{-1}$ of IAA and solidified with agar — $6.5 \text{ g} \cdot \text{l}^{-1}$. The pH of the medium was adjusted to 5.7. After 3 passages of multiplication the individual shoots (4-5 cm long) were dissected from shoot clusters and transferred to the regulator free medium consisting of MS basal components and the following concentration of sucrose: 3, 6, 9, 12%. Shoots grown on the basic medium without sucrose were used as a control. The explants were cultured in 300 ml Erlenmeyer flask. Five shoots were incubated in each flask. The experiment was repeated twice with 20 replications in each treatment.

Tissues were maintained in the growth chamber at 22/20°C day/night with 16-hour photoperiod and light intensity of $35 \mu\text{M m}^{-2} \cdot \text{s}^{-1}$. The observations of growth and develop-

ment of cultured shoots were conducted at weekly intervals during the experiment. After 12 weeks of shoots cultivation the following parameters were determined: number, length, fresh weight of tubers formed at the shoot base (primary tubers) and also of tubers developed from the buds of the primary tubers (daughter tubers) as well as number, length, fresh weight of roots. The results of the experiment were analysed statistically and the Tukey test was used to estimate the differences between the means at a 5% level of significance.

RESULTS

In the present study individual shoots of *Gloriosa rothschildiana* 'Red Dark' multiplied *in vitro*, were induced to form tubers at the shoot base. The number of days required for tuber initiation varied from 20.9 to 35.1, depending on sucrose level. The earliest tuber formation was observed on the medium containing 12% of sucrose. The frequency of tuberization was affected by concentration of sucrose. In sucrose free medium tubers did not appear. On the media containing 6-9% of sucrose, 76.2-95.0 % of cultured shoots formed tubers. Moreover, the fresh weight of tubers obtained on media containing 6-9% of sucrose was significantly bigger than at 3% and 12%. The average fresh weight and length of tubers formed from 1 explant was the highest at 6 % sucrose concentration (401.3 mg and 9.3 mm, respectively) (Tab. 1).

Microtubers of different sizes and shapes were harvested during the experiment. Individual tubers obtained during 12 weeks of culture weighed from 39 mg to 938 mg and had the length from 3 to 22 mm. Most small size tubers (<100 mg) were obtained on the media containing 12% of sucrose (Tab. 2). At the level of 9% of sucrose about 16.1% of tubers achieved more than 500 mg. The shape of tubers was round, oblong or V-shaped. The highest number of bifurcated tubers was obtained on the media with 9% of sucrose.

During the study many microtubers sprouted and new (daughter) microtubers were formed. This process was promoted especially at the sucrose concentration of 6-9%. In this treatment 92.3-94.7% of primary tubers formed daughter tubers, which appeared after 45.8-51.7 days of shoots incubation.

The majority of microtubers developed roots. Explants cultured on the media containing 3% of sucrose produced the biggest number of roots which had the highest fresh weight. However, there were no significant differences between the results obtained in this treatment and treatments with 6 and 9% of sucrose.

Tab. 1. Effect of sucrose content in the medium on *in vitro* formation and growth of tubers and roots of *Gloriosa rothschildiana* 'Red Dark' after 12 weeks of culture

Characteristics	Sucrose (%)				
	0	3	6	9	12
No. of primary tubers/expl.	0	0.7	0.8	0.9	0.7
No. of daughter tubers/expl.	0	0.9a*	1.1a	1.0a	0.6b
Total F.W. ^x of tubers/expl. (mg)	0	201.9b	401.3a	375.5a	207.2b
Length of tubers (mm)	0	6.3	9.3	8.1	7.5
Number of roots/expl.	2.5b	7.7a	7.3a	7.1a	5.5a
Length of roots (mm)	37.8	40.7	42.1	57.6	32.9
F.W. of roots/expl. (mg)	41.1b	178.5a	165.4a	170.5a	82.1b

* Means followed by the same letter are not significantly different

^x Total F.W. — fresh weight of primary tubers + fresh weight of daughter tubers

Tab. 2. Tubers of *Gloriosa rothschildiana* 'Red Dark' in particular weight and length classes in relation to sucrose content in the medium (in %)

Fresh weight and length of tubers	Sucrose (%)				
	0	3	6	9	12
< 100 mg	-	45.5	40.6	32.3	50.0
100-500 mg	-	54.5	50.0	51.6	45.8
500-1000 mg	-	0	9.4	16.1	4.2
< 5 mm	-	45.5	31.2	25.8	33.3
5-10 mm	-	50.0	46.9	38.7	50.0
10-20 mm	-	4.5	18.8	35.5	16.7
>20 mm	-	0	3.1	0	0

DISCUSSION

The results obtained in the experiment indicate that the most beneficial concentration of sucrose in medium used for induction of *Gloriosa* tubers was 6-9%. This is in agreement with the observations made by Forsyth and Van Staden (1984), Chow et al. (1992), Dantu and Bhojwani (1995) that sucrose is an important factor in bulb and tuber induction. However, a lower level of sucrose (5%) was more suitable for *in vitro* tuberization of *Dioscorea rotundata* (Ng, 1988) but Machackova et al. (1997) observed formation of tubers from nodal segments of transformed plants of *Solanum tuberosum* at a sucrose concentration of 2%.

In the study, shoots of *Gloriosa* cultured on the medium without sucrose showed elongation but did not form tubers. Similarly, in the experiments of Niimi and Onozawa (1979) and Takayama and Misawa (1979) bulbilscales segment of *Lilium* did not regenerate of bulblets on media without sucrose. However, in the study of Kim and Han (1993) 37.5% of *Gladiolus* shoots formed cormlets on sucrose free media.

In the presented experiment tubers induced at the base of *Gloriosa* shoots formed new tubers on all the tested media containing 3-12% of sucrose. Seabrook et al. (1993) observed formation of secondary tubers of *Solanum tuberosum* on the apical end of axillary tubers induced *in vitro* on the medium with 8% of sucrose. Dantu and Bhojwani (1987) reported that all *Gladiolus* corms formed *in vitro* were dormant and only 20-25% of corms germinated after being stored at room temperature. Kim and Han (1993) obtained 68.4% cormlets of *Gladiolus* with sprouts after 21.1 days of storage in room temperature while microtubers of *Dioscorea* sprouted after 2-3 months of storage on the aseptic moistened filter paper (Ng, 1988). Microtubers of *Gloriosa* on the medium with the highest sucrose level indicated the lowest ability to sprout. Squires et al. (1991) noted that sucrose at 9% increased the dormancy of *Narcissus* bulblets. A similar tendency was observed by Takayama and Misawa (1980) for bulblets of *Lilium*.

Gloriosa microtubers regenerated roots on all the tested media. The best rooting was observed at the low level of sucrose (3%). At the highest concentration (12%) of sucrose, fresh weight of roots was reduced significantly. Niimi and Onozawa (1979) reported that regeneration of *Lilium* roots was inhibited on the media with 8-12% of sucrose. Takayama and Misawa (1979) observed stimulation of roots of *Lilium* by sucrose concentration up to 9%. Ng (1988) obtained intensive growth of roots of *Dioscorea* rotundata at 8-10% of sucrose. Also, Dantu and Bhojwani (1995) noted development of *Gladiolus* roots on the media containing 6-10% of sucrose.

CONCLUSIONS

1. The fresh weight and length of microtubers obtained from shoots of *Gloriosa* were the highest in the presence of 6% sucrose in MS medium.
2. Sucrose at concentration 6-9% promoted frequent formation of daughter tubers from primary tubers.
3. The best results of roots induction were noticed on the medium with 3% of sucrose.

ACKNOWLEDGEMENTS

This work was supported by the Committee for Scientific Research, under project No. 5 PO6C 030 12.

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STRESZCZENIE

Badano wpływ stężenia sacharozy na formowanie bulw *Gloriosa rothschildiana* O'Brien 'Red Dark' w kulturach *in vitro*. Pojedyncze pędy pobierano z zespołów pędów namnożonych *in vitro*. Zastosowano zmodyfikowaną pożywkę Murashige and Skoog (MS), bez regulatorów wzrostu, z dodatkiem sacharozy w różnych stężeniach (3, 6, 9, 12%). Kultury prowadzono w warunkach 16-godz. oświetlenia, przy natężeniu światła $35 \mu\text{M} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, w temperaturze 22°C , przez 12 tygodni. Bulwy o największej świeżej masie i długości uzyskano na pożywce zawierającej 6% sacharozy. Większość bulw matecznych wydawała bulwy potomne. Proces ten był wzmacniany przy 6-9% stężeniu sacharozy w pożywce. Najlepszą regenerację korzeni obserwowano na pożywce z 3% zawartością sacharozy.