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EFFECTS OF SUCROSE CONCENTRATIONS ON INCREASE IN BULB SIZE OF *IN VITRO* REGENERATED HYACINTH (*Hyacinthus orientalis* L.) BULBLETS

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Abstract

In vitro regenerated bulblets of Hyacinthus orientalis L.*, improved their size using MS medium containing different sucrose concentrations. The sucrose concentrations showed higher growth and better development of the bulblets on MS medium stored at 24 °C. Sucrose showed the highest performance rate at 9 % concentration for both small and large bulblets. Sucrose treatments showed positive effects of induction of bulb diameter, number of shoots per bulb, shoot length and bulb weight in the culture medium.*

Key words: *Hyacinthus orientalis, bulb size, carbohydrate, growth, micropropagation.*

INTRODUCTION

Hyacinthus species, mostly natives of the mild climate of the Mediterranean region, are cultivated for their strong fragrant flowers (Nowak and Rudnicki, 1993).

Among the three species of Hyacinthus, *H. orientalis* is the only commercially important one (Rees 1992, Nowak and Rudnicki (1993) but the natural production rates of their bulblets are very low and the number of the bulblets developed in the scale segments of mother bulbs are also very small (Bach et al., 1992).

Under natural conditions, axillary bulbs develop at the base of the mother bulb; small numbers and could start to bloom only after 2–3 years of cultivation under ideal conditions of no biotic and abiotic stress. (Smigielska and Jerzy, 2013).

The techniques and methodologies used in plant tissue-culture are playing a very important role in basic and applied scientific studies (Brown and Thorpe, 1995).

Therefore, there is need to use these techniques in effective way for increased and rapid multiplication of plants in parallel to the traditional multiplication techniques.

This could offer alluring options for commercial multiplication of numerous geophytes especially bulbous plants (Bach and Sochacki, 2013).

Plant tissue additionally offers an exceptionally useful pathway for rapid clonal multiplication of slow multiplying elite plant species like hyacinth.

Explants under *in vitro* culture conditions require exogenous carbon as energy source for their growth and differentiation because they lack complete autotrophism. Although sucrose is the most generally used carbon source with the concentration of 2-3% under *in vitro* studies, other types of carbon sources are also used (Huang and Okubo, 2005).

Sucrose is often assumed to be the best choice of carbon source in cell and tissue culture media because it is the main sugar translocated in the phloem of many plants (Peterson et al., 1999). However, there are a number of plants that can grow on other sources of carbons for plant regeneration. Regeneration via organogenesis or somatic embryogenesis was stimulated in a number of plants on the media containing glucose, fructose, maltose, mannose or sorbitol (Bach, 1992, Hossain et al., 2013). There are some reports that compared the response to carbon sources for growth and development of some bulbous plant species like Bach et al. (1992), who reported that glucose and sucrose containing medium was superior to fructose containing medium in induction of shoots and bulblets.

The aim of this study was to understand, the role of different sucrose concentrations in increasing bulblet development in hyacinth.

MATERIALS AND METHODS

Plant materials and experiments

The study made use of *in vitro* regenerated *H. orientalis* bulblets obtained in the previous study (Kizil et al., 2016). (Figure 1a) regenerated on MS medium containing 0.05 mg/l TDZ and 0.10 mg/l NAA or 0.10 mg/l TDZ and 0.10 mg/l NAA under *in vitro* conditions (primary medium).

To obtain desired increase in bulblet size, they were cultured on MS basal medium (Murashige and Skoog, 1962) containing with 0.00, 30, 60 or 90 g/l sucrose (w/v) and solidified with 6.2 g/l agar (w/v) in Magenta GA^7 vessels. The pH of all cultures medium was adjusted to 5.6 - 5.8 with 0.1 M KOH or 0.1 M HCl before autoclaving at 121ºC, 104.5 kPa for 20 min.

Hardening and acclimatization

Well-developed bulbs were washed thoroughly in running tap water transferred to 250 ml plastic pots containing sterilised peat moss under controlled greenhouse conditions at temperature (24º ± 1ºC) and light 3000 lux (16/8 h photoperiod) conditions for sprouting and growth.

Potted plantlets were covered with transparent plastic bags to ensure high humidity and each bulb was given 100 ml water every day for 15 days during acclimatization.

All cultures were placed in Fitotron growth chamber (Fitotron SGC 120; Epinal Way, Loughborough, UK) with 16 h of cool white fluorescent light (Philips lamps TLD 36 W/54, Hungary) at a photon flux density of 35 µmol/ m^2/ s per day.

Statistical analysis

All experiments made use of 60 explants equally divided into 10 replications. Statistical analysis was performed using IBM SPSS 22 program for windows by comparing means using One Way ANOVA. All values expressed in percentage were arcsine transformed before statistical analysis (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Results

It was made sure to measure initial bulblet diameter before culturing using different concentrations of sucrose as mentioned in Materials and Methods (Table 1 – Figure 1a). Thereafter, 9 weeks on the culture medium the final increase in bulb diameter and weight was made (Figure 1b, c). The results showed that cultured small and large bulblets of hyacinth on MS medium supplemented with different concentration of sucrose (control, 30, 60 and 90 g l^{-1}) showed increase in all growth parameters that were significantly ($p \leq 0.05$) different except for shoot length (Table 1).

Table 1. Bulbils diameter and weight at the beginning and end of the study

Sucrose amount (g l^{-1})	Initial				Final							
	Bulb diameter (cm)		Bulb weight (g)		Bulb diameter (cm)		Number of shoots per bulb		Shoot length (cm)		Bulb weight (g)	
	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large	Small	Large
Control (0)	0.46	0.60	0.32	0.95	0.46 c	0.59 d	2.48 c	4.33 b	0.67	1.14	0.43 c	1.16 d
30	0.43	0.58	0.25	0.93	0.47 c	0.76 b	3.25 c	4.83 b	0.68	1.17	0.44 c	1.52 c
60	0.43	0.64	0.26	1.01	0.75 b	0.69 c	5.00 b	4.86 b	0.75	1.18	0.50 b	1.73 b
90	0.50	0.68	0.32	1.08	0.87 a	1.21 a	18.16 a	6.83 a	1.06	1.26	0.54 a	2.02 a
Mean	**0.46**	**0.63**	**0.29**	**0.99**	**0.63**	**0.81**	**7.22**	**5.21**	**0.79**	**1.19**	**0.48**	**1.61**

Means within a column followed by the same letter are not significantly different according LSD test at $p \leq 0.05$.

Bulblets were categorized depending on their size, those ≥0.5 cm (0.58 -0.68 cm) were called as large bulblets and those ≤ 0.5 cm (0.43-0.50 cm) were called small bulblets. The weight of small bulblets changed between 0.25 and 0.32 g and those of large ones changed between 0.93 - 1.08 g.

Irrespective of sucrose concentrations used in the study, Sucrose at 90 g l^{-1} concentration exhibited maximum gain in bulb diameter

compared to the other concentrations tested for enhancing diameter of bulblets of *H. orientalis*. Increase in bulb diameter for small bulblets was determined between 0.46 -0.87 cm, while for large bulblets, the bulb diameter was determined between 0.59 - 1.21 cm. Bulb diameter for both sizes increased depending on sucrose concentration in the culture medium.

Moreover, shoot length for small size bulbils varied between 0.67 - 1.06 cm, and for large size bulbils between 1.14 - 1.26 cm.

Bulb weight gain is very important for healthy bulbs production. During two months duration, bulblets, the small bulbs had difficulty in weight gain compared to the large bulbs. The results showed an average gain of 0.19 g weight for small size over initial bulb weight compared to the large sized bulblets that had mean weight gain of 0.62 g bulblets if compared to initial weight at the start of the experiment. This could also be said that the large bulbs were more prone to weight gain compared to small bulbs.

Hardening and acclimatization

These bulbs rooted during hardening on peat moss without treatments with any auxin (Figure 1d). Thakur et al. (2002) reported that for hardening *in vitro* rooted bulblets of Lilium, peat moss gave 100% survival. Gong et al. (1996) obtained 80–90% survival rate of tissue culture plants of Lilium × Connecticut King when transplanted in potting peat moss. The present work was the first attempt to increase bulb diameter and acclimatise them under semi arid conditions with 100% survival rate.

Discussion

In the present study bulblets induced on 0.05 and 0.1 mgl^{-1} TDZ and 40 and 80 g sucrose amount in previous studies were used (Kizil et al., 2016).

Sucrose is the most common carbon source as well as an osmotic agent for plant tissue and organ culture. It also supports the maintenance of osmotic potential and the conservation of water in cells. However, high sucrose concentration in the media restricts the photosynthetic efficiency of cultured plants by reducing the levels of chlorophyll, key enzymes for photosynthesis and epicuticular waxes promoting the formation of structurally and physiologically abnormal stomata (Hazarika, 2006).

Variable concentrations of sucrose are used for increase of bulb diameter and weight gains. The reason of this argument should be that sucrose is stored in bulb scales in the form of starch and therefore the availability of this carbohydrate source in the medium may account for the weight increase of bulbs (Santos et al., 2006).

There are many positive results on different bulb increment using different sucrose concentrations. Sun et al. (2012) reported that *Lilium davidii* var. *unicolo* had 100% percent gain in bulblet formation at the sucrose concentration of 100 g l^{-1}, while the diameter and weight increment of bulblets came to the maximum.

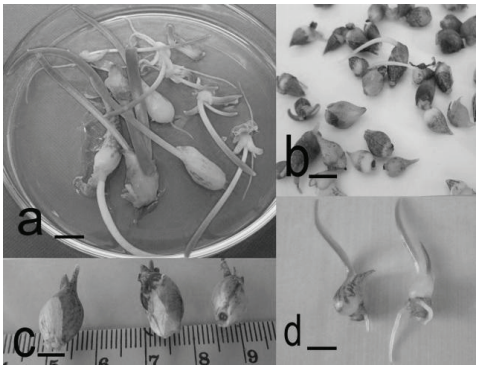

Figure 1. Increasing bulblet diameter of *H. orientalis* under *in vitro* conditions (a) The *in vitro* regenerated *H. orientalis* bulblets regenerated on MS medium containing 0.05 mg/l TDZ (b, c) enhanced bulblet diameter after 9 weeks on the culture medium containing 90 g l^{-1} (d) rooted bulbs after hardening on peat moss without treatments with any auxin. Bar; Fig1 a, c= 0.5 cm, Fig 1b, d = 0.7 cm

Bach et al. (1992) have reported that concentration and variety of sugar affected shoot and bulblet regeneration and glucose- and sucrose-containing medium was superior to fructose-containing medium in induction of shoots and bulblets.

The bulbs belonging to many species develop dormancy to survive under unfavourable surrounding conditions during a period that starts from late autumn to winter and both under *in vitro* and *ex vitro* conditions. This dormancy could be broken by low temperature or vernalisation treatment (Langens-Gerrits et

al., 2003). During this period levels of endogenous abscisic acid (Djilianov et al., 1994; Yamazaki et al., 2002) and sucrose (Hobson and Davies, 1978; Aguettaz et al., 1990) in bulbs are linked with the development of dormancy and its release.

Santos et al. (2006) noted that if bulbs produced on IBA and IBA+BA containing media (irrespective of their size) were transferred onto the same basic medium without growth regulators by increasing sucrose level from 3% to 6%, they promote both rooting and further growth of the bulbs. Furthermore, they found that bulbs attained a mean diameter of 8 mm and medium with 6% sucrose was the most adequate medium to promote bulb enlargement with well-developed root system.

CONCLUSION

Medium without growth regulators and with sucrose increased to 9% (90 g l^{-1}) led to the growth of the bulbs and roots as well in 9 weeks. Thereafter, the bulbs obtained on the MS medium containing different sucrose were planted in a soil mixture in pots. Where the transplanted bulbs showed leaves above the soil, indicating that the bulb dormancy had been broken. Transplantation success was high; with an average survival rate 80-90%.

In conclusion, the data obtained in this study showed that sucrose doses increased from 6-9% could be usefully used to increase the bulb size of this important plant and could be used to overcome the problem of low natural bulb size obtained during multiplication.

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