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IN VITRO STORAGE OF *Hosta* Tratt. CULTURES

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A B S T R A C T

Possibility of long-term storage *in vitro* of *Hosta* 'Blue Angel' shoots was evaluated. The effect of temperature (24°C or 6°C), growth regulators (BA, NAA) and sucrose concentration (30 or 60 g·dm^{-3}) in MS medium was studied in respect to a number, length and quality of axillary shoots. All studied factors affected the shoot quality. The best results were obtained during storage at 24°C on the medium containing 2 mg·dm^{-3} BA, 0.05 mg·dm^{-3} NAA, 80 mg·dm^{-3} adenine sulphate and 30 g·dm^{-3} sucrose, whereas storage at 6°C had a negative effect on a number and quality of shoots.

The influence of storage conditions on number and length of the shoots formed during the following 3 culture passages was studied as well. The highest number of axillary shoots was obtained during the first passage after storage, irrespectively of storage length and conditions. Storage conditions didn't affect rooting efficiency and the quality of roots produced by microcuttings.

Key words: *Hosta*, micropropagation, growth regulators, sucrose, rooting

INTRODUCTION

Storage of plant cultures under conditions minimizing their growth allows avoiding frequent passages thus decreasing labour costs needed to grow stock cultures, to maintain virus-free cultures and to keep collections of elite plant material. A further advantage of *in vitro* storage is genetic stability of the stored plant material and a small surface needed for its storage. Shoot cultures can be stored *in vitro* for 3 to 6 months (medium-term storage) and over 6 months (long-term storage) (Lisek, 2001). Conditions minimizing growth during storage must ensure high

plants viability and their poststorage recovery, i.e. ability to regenerate axillary shoots and roots after transfer from the storage conditions. Plant growth can be minimized by manipulations of light, temperature and medium composition (concentration of minerals, sugars, growth regulators and osmotic potential) (Lundergan and Janick, 1979; Dorion et al., 1991; Reed, 1992; Bekheet, 2000; Gollagunta et al., 2005). Cultures of plants from temperate climatic zone can be stored at 2-4°C while species from tropical and subtropical areas need 9-25°C. Cultures of all plants can also be stored for a short time without passaging under temperatures suitable for their growth (Lisek and Orlikowska, 2001a). A condition of stored plants can be evaluated on a basis of their multiplication ratio. It depends on an ability of species, cultivars or genotypes to restart regeneration after transfering to standard culture conditions. For example, in clover (*Trifolium repens* L.) shoots preserved their standard propagation efficiency after having been transferred from storage conditions to the conditions enabling culture growth (Bhojwani, 1981) while in apple shoot cultures multiplication rate even increased after storage at low temperature (Orlikowska, 1992). However, in certain species, for example raspberry, a decrease in regeneration ability is observed, especially during first passages after storage (Lisek and Orlikowska, 2001b).

The aim of this work was to evaluate the influence of temperature, growth regulators and sucrose concentration in the medium on storability and post storage recovery in micropropagated hosta plantlets. Quality of propagated material was evaluated during three successive passages by measuring axillary shoot number and their length as well as the plantlets' rooting ability.

MATERIAL AND METHODS

Shoots of *Hosta* 'Blue Angel' multiplied *in vitro* were used as the experimental material. Apical shoot parts, 1.5 cm long, were excised and placed on the MS medium (Murashige and Skoog, 1962) with 30 or 60 $g \cdot dm^{-3}$ sucrose, either without growth regulators or enriched with 2 $mg \cdot dm^{-3}$ benzyladenine (BA), 0.05 $mg \cdot dm^{-3}$ naphataleneacetic acid (NAA) and 80 $mg \cdot dm^{-3}$ adenine sulphate and solidified with 2 $g \cdot dm^{-3}$ Gerlite. pH of the medium was 5.6. Shoots were cultured in the jars of 300 cm^{-3}, containing 70 cm^{-3} of the medium (5 shoots per jar) at 24°C, in 16 h day under white fluorescent light (PPFD = 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$). After 4 weeks half of the jars from each treatment were placed at 6°C under continuous white fluorescent light (PPFD = 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

After 2, 6 and 12 months axillary shoots were counted, their length measured and quality scored according to the following evaluation scale: 1 – shoots with evident senescence symptoms, leaves dead or almost dead; 2 – shoots green, with only a few dead leaves; 3 – shoots in good condition, leaves green. From each treatment 4 jars (20 shoots) were taken at random for the above evaluation. Then the shoots were

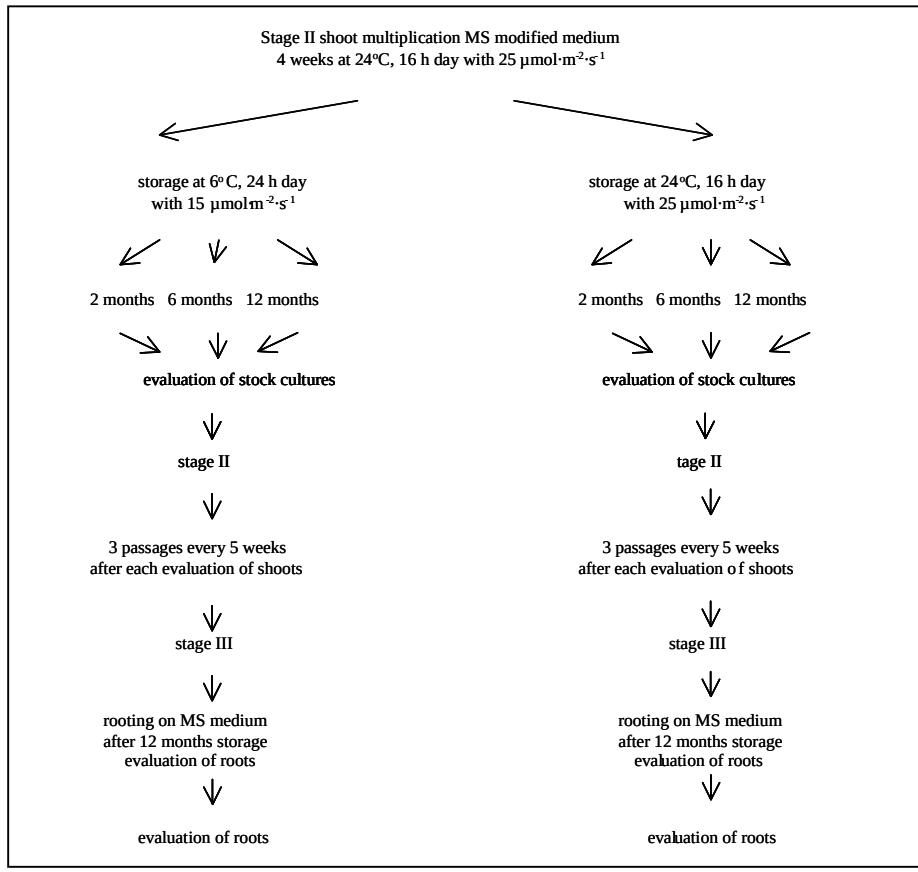

Figure 1. Schematic diagram of experiment

transferred on the MS medium enriched with 2 mg·dm^{-3} BA, 0.05 mg·dm^{-3} NAA and 80 mg·dm^{-3} adenine sulphate and placed in growth chamber at 24°C under the above light conditions. Every 5 weeks they were transferred on the new medium (3 passages in total). In each treatment 5 jars (25 shoots) were taken at random for culture evaluation where axillary shoots were counted and their length was measured.

After the 12 months of storage on MS medium enriched with 2 mg·dm^{-3} BA, 0.05 mg·dm^{-3} NAA, 80 mg·dm^{-3} adenine sulphate and with either 30 or 60 g·dm^{-3} sucrose, the shoots were transferred 3 times to MS medium with 5 mg·dm^{-3} BA, 80 mg·dm^{-3} adenine sulphate and 30 g·dm^{-3} sucrose. Then they were placed in 300 cm^{-3} jars containing 40 cm^{-3} MS medium supplemented with 0.1 mg·dm^{-3} NAA (5 shoots per a jar, 25 shoots in each treatment) and culture in conditions as above. After 7 weeks the percentage of rooted shoots was determined and the root system quality was scored. Figure 1 shows the treatment plan in each step.

The results were statistically evaluated using ANOVA 1 or ANOVA

2 and the significance of differences between the means was tested by Duncan's test at $p = 0.05$.

RESULTS

It was possible to store hosta cultures for twelve months, regardless of the medium. Better effects gave the storage at 24°C (Tab. 1) where after 6 months 100% or 90-95% of shoots were viable on the medium without or with growth regulators, respectively. After 12 months 60-65% shoots were in a good condition. At 6°C a number of viable shoots dropped to 70-75% after 2 month, while after 12 months 5-55% shoots survived, depending on a medium. The best for a long storage was MS medium enriched with 2 $mg \cdot dm^{-3}$ BA, 0.05 $mg \cdot dm^{-3}$ NAA, 80 $mg \cdot dm^{-3}$ adenine sulphate and 30 $g \cdot dm^{-3}$ sucrose on which 65% and 55% shoots were viable after 12 months at 24°C and 6°C, respectively (Tab. 1).

Storage temperature affected number, length and quality of axillary shoots (Tab. 2). More shoots, which were longer and of better quality, was obtained at 24°C as compared to 6°C. After 2 and 6 months number of shoots produced under both temperatures was similar, falling significantly after 12 months of storage while shoot quality kept decreasing steadily between 2nd and 12th month.

Sucrose concentration in the medium during storage also affected number, length and quality of axillary shoots (Tab. 3). After 12 months of storage more shoots were produced on MS medium with 2 $mg \cdot dm^{-3}$ BA, 0.05 $mg \cdot dm^{-3}$ NAA, 80 $mg \cdot dm^{-3}$ adenine sulphate and 3% sucrose. These shoots were longer and of a better quality than those cultured in presence of 6% sucrose.

There were differences in a number and length of shoots obtained in the successive passages. Regardless of the storage time and conditions the best results were obtained in the first passage. In case of cultures stored for 2 months a negative effect of the storage at 6°C on the average shoot number produced during 3 passages was observed (Tab. 4). Shoot number after second and third passage did not differ significantly. More axillary shoots were produced in cultures originated from shoots stored without growth regulators. There was no effect of sucrose concentration in storage medium on shoot proliferation rate in the successive passages. Shoots from 3 passages differed in their length. On the average, longer axillary shoots were in population originated from shoots stored at 24°C (data not presented).

Temperature during 6 month of storage had no effect on number of shoots produced during 3 passages (Tab. 5). In all the treatments a decrease in shoot number was observed in the successive passages. After the first passage the least number of shoots was found in cultures stored on the medium with growth regulators and lower sucrose concentration while the other treatments did not differ significantly in this respect.

Table 1. Effect of medium composition and storage duration on stored shoots viability

MS medium supplementation		Storage time (months)	Percentage of alive shoots	
Growth regulators [mg·dm^{-3}]	Sucrose [g·dm^{-3}]		24°C	6°C
0	30	2	100*	70
		6	100	70
		12	60	35
0	60	2	100	70
		6	100	70
		12	60	45
2 BA + 0.05 NAA + 80 adenine sulphate	30	2	100	70
		6	90	70
		12	65	55
2 BA + 0.05 NAA + 80 adenine sulphate	60	2	100	75
		6	95	75
		12	65	5

*Number of shoots per treatment 20 = 100%

Table 2. Influence of storage length and temperature on the number, quality and length of axillary shoots

Storage time (months)	Shoot number		Shoot quality (scale 1-3)		Shoot length [cm]	
	24°C	6°C	24°C	6°C	24°C	6°C
2	3.03 d*	2.41 c	3.00 e	1.68 b	6.29 c	3.75 b
6	2.70 cd	2.61 c	2.53 d	1.93 c	7.48 d	5.68 c
12	1.64 b	0.78 a	1.66 b	1.35 a	5.99 c	1.94 a

*Means with the same letter don't differ at the level of significance p = 0.05

Table 3. Influence of medium on number, length and quality of shoots after 12 months of storage

MS medium supplementation		Shoot number	Shoot quality (scale 1-3)	Shoot length [cm]
Growth regulators [mg·dm^{-3}]	Sucrose [g·dm^{-3}]			
0	30	0.90 a*	1.48 ab	4.46 b
0	60	1.00 a	1.58 ab	5.18 b
2 BA + 0.05 NAA + 80 adenine sulphate	30	2.08 b	1.63 b	4.18 b
2 BA + 0.05 NAA + 80 adenine sulphate	60	0.85 a	1.35 a	2.05 a

* Explanations, see Table 2

Table 4. Influence of 2 months storage on number of axillary shoots produced during 3 subsequent passages

MS medium supplementation		Passage	Shoot number		Mean
Growth regulators [mg·dm^{-3}]	Sucrose [g·dm^{-3}]		24°C	6°C	
0	30	1	4.0 f*	4.1 f	4.03 d
		2	2.1 bc	1.9abc	1.98 b
		3	1.5 ab	1.6 ab	1.53 ab
0	60	1	5.1 g	3.5 def	4.30 d
		2	2.2 bc	1.4 a	1.75 ab
		3	2.4 c	1.5 ab	1.95 ab
2 BA + 0.05 NAA + 80 adenine sulphate	30	1	3.3 de	3.1 d	3.20 c
		2	1.5 ab	1.5 ab	1.48 a
		3	1.5 ab	1.5 ab	1.50 ab
2 BA + 0.05 NAA + 80 adenine sulphate	60	1	3.8 ef	3.2 de	3.48 c
		2	1.7 ab	1.7 ab	1.50 ab
		3	1.5 ab	1.5 ab	1.48 a
Mean			2.53 b	2.19 a	

*Explanations, see Table 2

Table 5. Influence of 6 months storage on number of axillary shoots produced during 3 subsequent passages

MS medium supplementation		Passage	Shoot number		Mean
Growth regulators [mg·dm^{-3}]	Sucrose [g·dm^{-3}]		24°C	6°C	
0	30	1	2.7 de*	3.9 f	3.28 e
		2	1.9 abc	1.6 ab	1.73 a
		3	1.4 a	1.3 a	1.33 a
0	60	1	2.9 e	4.2 f	3.55 e
		2	1.8 abc	1.9 abc	1.80 bc
		3	1.4 a	1.4 a	1.35 a
2 BA + 0.05 NAA + 80 adenine sulphate	30	1	2.6 de	2.1 bcd	2.35 d
		2	1.4 a	1.8 abc	1.60 ab
		3	1.4 a	1.3 a	1.33 a
2 BA + 0.05 NAA + 80 adenine sulphate	60	1	3.8 f	2.7 e	3.25 e
		2	2.4 cde	1.9 abc	2.13 cd
		3	1.7 ab	1.6 ab	1.60 ab
Mean			2.08 a	2.13 a	

*Explanations, see Table 2

After 12 months of storage a significant effect of temperature was observed on the mean shoot number produced in all 3 passages (Tab. 6). More shoots appeared after storage at 24°C. The highest shoot number after the first passage was found on medium without growth regulators with 6% sucrose and on medium with growth regulators and 3% of sugar. No differences were noted in shoots originated from other treatments between the first and the second passage.

Storage temperature affected as well the mean shoot length in the successive passages (data not presented). Longer axillary shoots were obtained from stock explants stored under 24°C as compared to 6°C.

Generally, there was no effect of storage conditions during 12 month storage on successive rooting of shoots which rooted in 100%, except of these from stock shoots stored at 6°C on medium with growth regulators which rooted in 92%. Neither medium composition nor storage temperature affected quality of the root system in hosta (data not presented).

DISCUSSION

Medium composition and storage temperature significantly affected quality of hosta cultures stored *in vitro*. Low temperature limited storability and post storage recovery of hosta stock cultures. Opposite

Table 6. Influence of 12 months storage on number of axillary shoots produced during 3 subsequent passages

MS medium supplementation		Passage	Shoot number		Mean
Growth regulators [mg·dm^{-3}]	Sucrose [g·dm^{-3}]		24°C	6°C	
0	30	1	2.5 fghi*	1.2 b	1.80 de
		2	1.6 bcde	1.6 bcd	1.58 cde
		3	1.2 b	1.4 bc	1.25 bc
0	60	1	2.9 i	1.8 cde	2.35 f
		2	1.8 cde	2.0 defg	1.90 e
		3	1.4 bc	1.5 bcd	1.40 bcd
2 BA + 0.05 NAA + 80 adenine sulphate	30	1	2.2 efgh	2.5 ghi	2.33 f
		2	1.8 cde	1.8 cde	1.75 de
		3	1.5 bcd	1.5 bcd	1.48 cde
2 BA + 0.05 NAA + 80 adenine sulphate	60	1	2.6 hi	0 a	1.28 bc
		2	2.0 def	0 a	0.98 ab
		3	1.4 bc	0 a	0.68 a
Mean			1.88 b	1.25 a	

*Explanations, see Table 2

effect of low temperature was observed in petunia whose stock cultures could be preserved at 6°C for 8-12 months, depending on a medium (Witomska et al., 2006a), while at 24°C it was possible to store for up to 6 months only. Also in asparagus (*Asparagus officinalis*) storage at low temperature was successful: at 5°C, in darkness, 100% plantlets fully recovered after 6 months, in 90% – after 12 months and in 50% after 18 months (Bekheet, 2000). Similar conditions were favourable for 10 months *in vitro* storage of clover (Bhojwani, 1981).

A positive influence of growth regulators, adenine sulphate and sucrose on viability, number and quality of shoots was observed in *in vitro* stored hosta stock cultures. Similarly, in apple presence of 5 mg·dm^{-3} BA and 30 g·dm^{-3} sucrose in the medium had a positive influence on culture storability during 12 months at 1°C or 4°C. Apple shoots maintained vigour and branching ability after transfer from cold to 26°C (Lundergan and Janick, 1979). Presence of BA in a medium ensured as well a good recovery of two apple rootstocks stored over 12 weeks (Orlikowska, 1992). There are, however, reports on a good storability of micropropagated plants on media devoid of auxins or cytokinins. Petunia shoots were differentiating and growing very slowly; thus could be maintained for up to 12 months (Witomska et al., 2006a). On a medium without growth regula-

tors eucalyptus shoots could be stored up to 10 months (Watt et al., 2000) while strawberry shoots even for 24 months (Reed, 1992).

Sucrose in a higher concentration (60 $g \cdot dm^{-3}$) positively affected storability and post storage recovery of petunia stock explants at 6°C due to stimulation of branching and retardation of shoot elongation (Witomska et al., 2006a). Presence of sucrose in concentration 20 $g \cdot dm^{-3}$ improved storability of coffee at 20°C and 27°C while lack of this sugar or its low concentration (5 $g \cdot dm^{-3}$) decreased plantlets' viability (Bertrand-Desbrunais et al., 1992). Light was necessary for maintaining explant growth during hosta storage for 4-12 weeks, especially when storage medium lacked sucrose. In illuminated plantlets dry weight kept increasing at 22°C regardless of sugar concentration in a medium while at 5°C and 10°C dry weight dropped (Wilson et al., 2000). In these trials differences between number and length of axillary shoots produced in successive passages were observed. After 12 months of storage a higher mean number of axillary shoots produced during 3 passages was noted in cultures originated from these kept at 24°C (1.88) than those kept at 6°C (1.25). Shoots stored for 2 or 6 months produced the highest number of axillary shoots in the first passage while in explants stored for 12 months differences in shoot number in successive passages depended on a medium type. Authors propose to increase BA concentration to 3-5 $mg \cdot dm^{-3}$ in a medium for hosta propagation after storage. Decrease in branching ability after storage is common. It was observed in petunia, where branching was better in the second and third passage. Sucrose in a higher concentration increased mean number and length of axillary shoots as well as fresh weight of plantlets obtained in three passages (Witomska et al., 2006b). Similarly, in stored apple rootstock cultures in the second and third passage plantlets branched better than the unstored, control ones (Orlikowska, 1992). Cultures of strawberry and raspberry produce much less shoots in the first passage after storage at 4°C while in the second and third passage proliferation is much more intensive though not always as good as in the unstored material (Lisek and Orlikowska, 2001b). A positive influence of storage under low temperature was also observed in *in vitro* cultures of cherries: the highest number of shoots was produced after storage at 4°C (Borkowska, 1990). Also kiwi shoots stored at 8°C (Monette, 1986) and apple shoots kept at 4°C (Orlikowska, 1992) produced more shoots after cold storage than before. Increase or decrease in branching ability after storage depends on a species as well as on conditions and length of storage.

The influence of medium composition and storage temperature on subsequent rooting of micropropagated hosta shoots was negligible. Rooting ability of plantlets was high (100%) regardless storage conditions of the stock material. A decrease of

8% in the number of rooted cuttings was noted only in plantlets originated from these stored at 6°C on medium enriched with growth regulators and 6% sucrose; however, root quality was similar in all the treatments. Petunia cuttings also rooted in 100% but the quality of root system differed in progeny of stock explants stored under different temperatures: roots on cuttings from stock explants stored at 24°C were better developed than those on cuttings from material stored at 6°C. In petunia neither sucrose concentration nor presence of ABA in a medium on which stock explants were stored affected root quality (Witomska et al., 2006a).

The results of the research presented as well as the cited data show that various plants respond differently to storage conditions. Therefore, for each species detailed studies of temperature, light and medium composition are needed to elaborate an optimal set of conditions ensuring high quality explants.

CONCLUSION

Storage *in vitro* of the hosta 'Blue Angel' stock cultures was possible for up to 12 months. The best results were obtained at 24°C on MS medium supplemented with 2 mg·dm^{-3} BA, 0.05 mg·dm^{-3} NAA, 80 mg·dm^{-3} adenine sulphate and 30 g·dm^{-3} sucrose

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PRZECHOWYWANIE MATECZNIKA FUNKII (*Hosta* Tratt.) W KULTURACH *IN VITRO*

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S T R E S Z C Z E N I E

Oceniono możliwość długotrwałego przechowywania *in vitro* pędów funkii (*Hosta* Tratt.) 'Blue Angel'. Badano wpływ temperatury (24ºC lub 6ºC), obecności regulatorów wzrostu (BA i NAA) i siarczanu adeniny oraz stężenia sacharozy (30 lub 60 $g \cdot dm^{-3}$) w pożywce MS na liczbę, jakość i długość pędów roślin matecznych. Wszystkie badane czynniki wywarły wpływ na jakość przechowywanych pędów funkii. Możliwe okazało się przechowywanie roślin matecznych przez 12 miesięcy, a najlepsze efekty uzyskano na pożywce MS zawierającej 2 $mg \cdot dm^{-3}$ BA, 0,05 $mg \cdot dm^{-3}$ NAA, 80 $mg \cdot dm^{-3}$ siarczanu adeniny i 30 $g \cdot dm^{-3}$ sacharozy w temperaturze 24ºC. Przechowywanie roślin w temperaturze 6ºC wywarło negatywny wpływ na liczbę i jakość pędów roślin matecznych.

Oceniono także wpływ warunków przechowywania kultur na liczbę i długość pędów roślin potomnych w 3 pasażach po przechowywaniu. Największą liczbę pędów uzyskano w pierwszym pasażu bez względu na warunki i długość okresu przechowywania. Warunki przechowywania nie wpłynęły na procent ukorzenionych sadzonek i jakość systemu korzeniowego roślin potomnych.

Słowa kluczowe: *Hosta*, mikrorozmnażanie, przechowywanie *in vitro*, regulatory wzrostu, sacharoza, ukorzenianie