FOLIA
HORTICULTURAE
Ann. 17/2, 2005, 81-87

Growth *in vitro* cultures of strawberry (*Fragaria* × *ananassa* Duch.) depending on different photoperiods

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Key words: strawberry, micropropagation, light/dark cycles

ABSTRACT

The influence of three photoperiods: a) 16/8 (d/n) - control, b) 4/2 (d/n) (4 cycles per 24 h), and c) 22/2 (d/n) on the growth of *in vitro* cultures of strawberry (*Fragaria* x *ananassa* Duch.) cultivars 'Senga Sengana' and 'Elsanta' was investigated. The application of 22/2 (d/n) photoperiod was not advantageous as it did not increase culture efficiency, stimulated the growth of callus at the explant base and caused chlorosis of leaves. Photoperiod 4/2 (d/n) significantly enhanced shoot proliferation of 'Senga Sengana' cultures when compared to control 16/8 (d/n). The reaction of 'Elsanta' cultures was not so distinct. As the change of photoperiod from 16/8 (d/n) to 4/2 (d/n) needs neither special instrumentation nor creates any additional costs it could be recommended in strawberry micropropagation, especially in the case of cultivar 'Senga Sengana'.

INTRODUCTION

The method of micropropagation of strawberry was elaborated about thirty years ago by Boxus (1974) and numerous studies regarding that technique have been published so far. However, the articles describing the reaction of strawberry *in vitro* cultures on different photoperiods were not found. The application of shortened light/dark cycles was proved to be beneficial in micropropagation of some woody plants species (Morini et al. 1991, 1992, Zimmerman and Scorza 1994, Litwińczuk 2000). It is well known that many strawberry cultivars are sensitive to photoperiod. The purpose of this study was to examine the reaction of strawberry *in vitro* cultures on different light/dark cycles.

MATERIAL AND METHODS

The experiment was carried out on *in vitro* cultures of two strawberry cultivars (*Fragaria×ananassa* Duch.) 'Senga Sengana' and 'Elsanta'. The influence of three photoperiods: a) 16/8 (d/n) - 16 hours of day and 8 hours of night (control), b) 4/2 (d/n) (4 cycles per 24 h), and c) 22/2 (d/n) was investigated through three subsequent 4-week long subcultures. Cultures were grown under fluorescent light (Fluora, OSRAM) at 51.6 µmol m^{-2} s^{-1} PPFD in glass jars closed with plastic transparent cup. The basic medium recommended by Boxus (1999) supplemented with 6-benzylaminopurine (BA, 0.5 mg dm^{-3}), gibberellic acid (GA_3, 0.1 mg dm^{-3}), indole-3-butyric acid (IBA, 0.1 mg dm^{-3}), glucose (40.0 g dm^{-3}) and Bacto-Difco agar (6.4 g dm^{-3}) was applied. Additionally, an other medium was used in the third subculture also. It was prepared by replacing glucose with mannitol (40.44 g dm^{-3}). A small glass vessel containing 12 cm^3 of medium with mannitol was placed inside a glass jar filled with 60 cm^3 of the basic medium. Each combination (photoperiod) was represented by 10 jars in first subculture and 6 jars in second and third ones. The replication was one jar containing 7 cultures grown on basic medium (and additionally 2 cultures on mannitol medium in the third subculture). Thus, each combination was represented by at least 42 cultures grown on basic medium in each subculture and 12 cultures for mannitol medium. The following data were collected at the end of each subculture: number of shoots (rosettes), number of stolons, fresh weight of shoots, fresh weight of callus, number of rooted cultures, length of the longest petiole, length of the biggest terminal leaflet, width of the biggest terminal leaflet, and then subjected to ANOVA and LSD mean separation test at α=0.05 significance level using Statgraphics 4.2 computer software. Percent data (number of rooted cultures) were transformed using arcsin of square root for analysis of variance. Original means are presented in Tables 1 and 2.

RESULTS

The investigated photoperiods influenced growth of strawberry clones *in vitro* on media supplemented with glucose. The application of 22/2 (d/n) photoperiod was not advantageous as it did not improve shoot proliferation of both 'Elsanta' and 'Senga Sengana' *in vitro* cultures, whereas stimulated the growth of callus at the explant base and caused chlorosis of leaves when compared to control [16/4 (d/n)] (Tables 1 and 2). Photoperiod 4/2 (d/n) significantly enhanced shoot proliferation of 'Senga Sengana' cultures (Table 1). However, in principle such reaction of 'Elsanta' cultures was not distinct as the significantly better proliferation of shoots under 4/2 (d/n) photoperiod was only recorded in the third passage (Table 2). The leaf blades of both clones grown under 4/2 (d/n) cycle were significantly reduced (Tables 1 and 2). On the other hand, the leaf petioles of 'Elsanta' were visibly elongated under mentioned photoperiod (Table 2). In general, other studied traits of *in vitro* cultures of both clones remained unchanged under examined photoperiods (Tables 1 and 2). The growth of cultures on the medium supplemented with mannitol was poor and was not influenced by studied photoperiods (Table 3).

DISCUSSION

The fact that many strawberry cultivars are sensitive to photoperiod and plants develop stolons under short night conditions is well known. However, none of studied photoperiods stimulated stoloning *in vitro* although all of them belong to 'short night' regime. Possible other factors which exist *in vitro* suppressed such process. Nevertheless, improved shoots proliferation was achieved while shortened photoperiod - 4/2 (d/n) was used, especially in the case of 'Senga Sengana'. The same effect for *in vitro* cultures of peach, plum, and sour cherry was reported by Morini et al. (1991, 1992), as well as Zimmerman and Scorza (1994). However, they did not find the reason of such phenomenon. It is also not possible in the present study. In comparison to other examined photoperiods, the 4/2 (d/n) cycle should improve photosynthesis through more stable level of CO_2 and its better utilisation by cultures *in vitro*. However, the fresh weight of cultures grown on mannitol medium (which was contingent only on CO_2 level in the jar) under 16/4 (d/n) and 4/2 (d/n) cycles remained similar or was even smaller in the last case (Table 3). Too small sample size (only 12 cultures) do not permit to state whether the differences were significant or not. The dry weight of cultures was not determined as well. Thus it is impossible to find out whether better shoot proliferation was caused by improved photosynthesis of cultures. On the other hand, examined photoperiods differed in the march of temperature on the shelves (Figure 1 and Table 4).

Table 1. Effect of studied photoperiods on the growth of strawberry 'Senga Sengana' *in vitro* cultures on medium supplemented with glucose

Subculture	Photoperiod	Number of shoots (rosettes)	Number of stolons	Fresh weight of shoots (mg)	Fresh weight of callus (mg)	Number of rooted cultures (%)	Length of the longest petiole (cm)	Length of the biggest terminal leaflet (cm)	Width of the biggest terminal leaflet (cm)
I	22/2 (d/n)	3.9 a*	0 a	nd	nd	90.0 a	nd	nd	nd
	4/2 (d/n)	5.9 b	0 a	nd	nd	92.0 a	nd	nd	nd
	16/8 (d/n) control	4.5 a	0 a	nd	nd	88.0 a	nd	nd	nd
II	22/2 (d/n)	2.7 a	0 a	nd	nd	65.0 a	nd	nd	nd
	4/2 (d/n)	3.1 b	0 a	nd	nd	64.3 a	nd	nd	nd
	16/8 (d/n) control	2.6 a	0 a	nd	nd	63.4 a	nd	nd	nd
III	22/2 (d/n)	5.2 ab	0 a	395 a	600 b	100.0 a	2.3 a	0.7 b	0.6 b
	4/2 (d/n)	5.8 b	0 a	363 a	437 a	100.0 a	2.3 a	0.6 a	0.5 a
	16/8 (d/n) control	4.9 a	0 a	365 a	426 a	100.0 a	2.4 a	0.7 b	0.6 b

* the means followed by the various letters within a column are significantly different at $\alpha = 0.05$; nd – not determined;

Table 2. Effect of studied photoperiods on the growth of strawberry 'Elsanta' *in vitro* cultures on medium supplemented with glucose

Subculture	Photoperiod	Number of shoots (rosettes)	Number of stolons	Fresh weight of shoots (mg)	Fresh weight of callus (mg)	Number of rooted cultures (%)	Length of the longest petiole (cm)	Length of the biggest terminal leaflet (cm)	Width of the biggest terminal leaflet (cm)
I	22/2 (d/n)	2.5 a*	0.0 a	nd	nd	80.4 a	nd	nd	nd
	4/2 (d/n)	2.4 a	0.2 b	nd	nd	89.3 a	nd	nd	nd
	16/8 (d/n) control	2.3 a	0.1 ab	nd	nd	94.3 a	nd	nd	nd
II	22/2 (d/n)	3.0 a	0 a	nd	nd	42.1 a	nd	nd	nd
	4/2 (d/n)	3.2 a	0 a	nd	nd	48.0 a	nd	nd	nd
	16/8 (d/n) control	3.0 a	0 a	nd	nd	33.3 a	nd	nd	nd
III	22/2 (d/n)	4.0 a	0 a	348 a	344 b	100.0 a	1.2 a	0.8 b	0.6 b
	4/2 (d/n)	5.0 b	0 a	343 a	219 a	100.0 a	2.4 c	0.6 a	0.5 a
	16/8 (d/n) control	3.8 a	0 a	317 a	265 a	100.0 a	1.8 b	0.8 b	0.7 b

* explanation of abbreviations, see Table 1.

Table 3. Effect of studied photoperiods on the growth of strawberry *in vitro* cultures on medium supplemented with mannitol

Cultivar	Photoperiod	Number of shoots (rosettes)	Fresh weight of shoots (mg)
'Senga	22/2 (d/n)	1.5 a*	179 a
Sengana'	4/2 (d/n)	1.2 a	71 a
	16/8 (d/n) control	1.0 a	93 a
'Elsanta'	22/2 (d/n)	1.0 a	71 a
	4/2 (d/n)	1.0 a	66 a
	16/8 (d/n) control	1.0 a	75 a

* explanation of abbreviations, see Table 1.

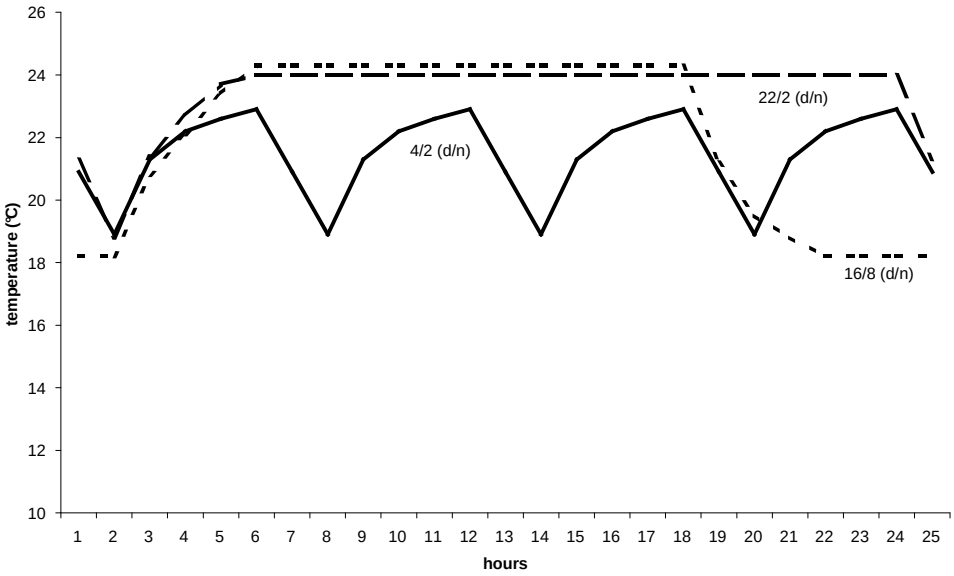

Figure 1. Temperature changes on the shelves depending on photoperiod

Table 4. Temperature characteristics of studied photoperiods

Photoperiod	Temperature (°C)			
	Maximum	Minimum	Amplitude	Mean
22/2 (d/n)	24.0	18.8	5.2	23.5
4/2 (d/n)	22.9	18.9	4.0	21.5
16/8 (d/n) (control)	24.3	18.2	6.1	22.2

The average shelf temperature was the smallest for 4/2 (d/n) cycle. Maybe the worse growth of 22/2 and 16/2 (d/n) cultures was caused by their overheating as temperature inside the jar should be higher than on the shelf. The changes in temperature were more often and rapid and its amplitude was smaller under 4/2 (d/n) cycle. Both vegetative and reproductive development in strawberry are highly sensitive to photoperiod and temperature (Le Miere et al. 1998). Thus obtained result (improved shoots proliferation) may be a combined effect of photo- and thermoperiodism, as well as thermal inhibition and/or better photosynthesis.

In general, propagation of plants through *in vitro* cultures is expensive. Thus each solution which increases culture efficiency should be advantageous. In the case of strawberry *in vitro* cultures the propagation ratio can be increased by application higher doses of BA (1-2 mg dm^{-3}). However, Boxus (1999) recommended lower BA concentration to reduce development of adventitious shoots on leaf stipules, as plants of adventitious origin tend to overproduce runners and flowers, which leads to decrease the fruit quality. Therefore, other methods improving culture multiplication should be elaborated. In the present study the enhanced shoot proliferation was observed under 4/2 (d/n) cycle while compared to control - 16/4 (d/n). The 4/2 (d/n) photoperiod reduced also the leaf size, which should make the culture/shoot manipulation easier. It is worth mentioning that the change of photoperiod from 16/4 (d/n) to 4/2 (d/n) needs neither special instrumentation nor creates any additional costs. Thus it could be recommended in strawberry micropropagation, especially in the case of 'Senga Sengana' on the condition it will not worsen field performance of such obtained plants.

CONCLUSIONS

1. The application of 22/2 (d/n) photoperiod was not advantageous in the proliferation stage of strawberry micropropagation.
2. Photoperiod 4/2 (d/n) significantly enhanced shoot proliferation of 'Senga Sengana' cultures when compared to control [16/4 (d/n)], whereas the reaction of 'Elsanta' cultures was not so distinct.
3. The change of photoperiod from 16/4 (d/n) to 4/2 (d/n) could be recommended in strawberry micropropagation, especially in the case of 'Senga Sengana'.

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WZROST KULTUR *IN VITRO* TRUSKAWKI (*FRAGARIA×ANANASSA* DUCH.) W ZALEŻNOŚCI OD RÓŻNYCH FOTOPERIODÓW

Streszczenie: Badano wpływ trzech fotoperiodów: a) 16/8 (d/n) – 16 h światła i 8 h ciemności (kontrola), b) 4/2 (d/n) (4 cykle na dobę), i c) 22/2 (d/n) na wzrost kultur *in vitro* truskawki (*Fragaria* x *ananassa* Duch.) odmian ‘Senga Sengana’ i ‘Elsanta’. Zastosowanie fotoperiodu 22/2 (d/n) nie było korzystne, ponieważ nie poprawiał on wydajności kultur, stymulował wzrost kalusa u nasady eksplantatu i wywoływał chlorozę liści. Fotoperiod 4/2 (d/n) istotnie zwiększał proliferację pędów kultur ‘Senga Sengana’ w porównaniu z kontrolą. Reakcja kultur ‘Elsanta’ nie była tak wyraźna. Ponieważ zmiana fotoperiodu z 16/8 (d/n) na 4/2 (d/n) nie wymaga stosowania specjalnych urządzeń i nie kreuje dodatkowych kosztów, może być polecana w mikropropagacji truskawki, zwłaszcza odmiany ‘Senga Sengana’.

Received January 24, 2005; accepted December 14, 2005