Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(12):1446-1450

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Effects of different photoperiods on *in vitro* plantlet regeneration of paulownia plants

Xiaojuan Yang[1], Yanqin Huang[1] and Guoqiang Fan[2]

[1]*College of Chemistry and Chemical Engineering, Xinxiang University, Xinxiang, Henan, P. R. China*
[2]*Institute of Paulownia of Henan Agricultural University, Zhengzhou, Henan, P. R. China*

ABSTRACT

We examined the effects of different photoperiods on in vitro plantlet regenerations of Paulownia plants. The results indicated that the rates of shoot induction from leaves treated with different photoperiods directly or 5 d and 10 d continuous illumination and dark pretreatments respectively were the highest under 24 h light photoperiod condition, but the time varied with leaves of different pretreatments when the induction rate was the largest. For root induction, the influences of different photoperiods on the rooting from shoots of 3 kinds of Paulownia plants were various. Much longer or shorter light illumination in the photoperiods might inhibit the rootings of the plants. The root inductions from the shoots of P. tomentosa and P. fortunei were inhibited much more than those of P. elongate.

Key words: Paulownia, Pphotoperiod, Explant, Medium, *In vitro* plant regeneration

---

INTRODUCTION

In vitro plantlet regeneration is an important step in plant cell engineering and gene engineering, and is one of the principal approaches to the rapid propagations of new species of plants. The establishment of an effective system for *in vitr*o plant regeneration is of undoubtedly significance to the study of biotechnology. There are various factors exerting effects on *in vitro* plant regeneration, one of which is photoperiod. Photoperiod plays an important role in the life cycle of plants. The photoperiod of 16 h light and 8 h darkness is considered to be the appropriate photoperiod for *in vitro* different plant regenerations [1-5]. However, the effects of different photoperiods on *in vitro* plant regeneration had been little studied [6]. Paulownia, a photophilous plant, is an important fast-growth tree for timbers and afforestation in China. Although much work had been done about in vitro plant regeneration [7-11], the paper about the effects of photoperiods on *in vitro* plant regeneration of Paulownia plants has not been found at home and abroad. The purpose of this paper is to study effects of different photoperiods on *in vitro* plant regeneration of Paulownia plants for further development of their gene engineering and optimization of the system of the *in vitro* plant regeneration.

EXPERIMENTAL SECTION

2.1. Seed materials. The seeds of *Paulownia tomentosa* (Thunb.) Steud, *P. fortunei* (Seem) Hemsl and *P. elongata* S. Y. Hu were picked in Zhengzhou, Henan province in Sep. 2004.

2.2. Culture of Paulownia seedlings. Sterilize the above-mentioned seeds in 70% alcohol for 30 s respectively, and then in 0.1% $HgCl_2$ for 5 min, wash them with sterilized water five times, and at last put on hormone-free PC medium supplemented with 20 $g \cdot L^{-1}$sucrose, 0.8% agar powder in an incubator where temperature is (25 ± 2) ^oC, light density is 1500 lx. After 80 d, seedlings of the Paulownia plants with 6-8 leaves can be acquired for the following experiments.

2.3. Shoot induction from leaves of Paulownia plants. Cut the three kinds of Paulownia leaves mentioned above into 0.5×1 cm^2 pieces (explants)，and put them on the optimal direct organogenesis media which are MS+0.1 mg·L^{-1} NAA+15 mg·L^{-1}BA、MS+0.1 mg·L^{-1} NAA+15 mg·L^{-1} BA and MS+0.1 mg·L^{-1} NAA+18 mg·L^{-1}BA respectively. 300 explants are treated in 100 bottles for each phytohormone combination. The shoot induction can be conducted directly or after being treated with continuous illumination or darkness for 5 d and 10 d under different photoperiods (24 h darkness, 8 h light and 16 h darkness, 12 h light and 12 h darkness, 16 h light and 8 h darkness, and 24 h light, namely, L_0D_{24}, L_8D_{16}, $L_{12}D_{12}$, $L_{16}D_8$ and $L_{24}D_0$). The temperature and light density for shoot induction are the same as those mentioned above, and the times for the induction with continuous illumination and darkness for 5 d and 10 d include that for their pre-treatments. The soot induction of explants within 20 bottles is observed at every 10 d. Finally, the shoot induction ratios are calculated at 40 d. The shoot induced is visible to the naked eyes.

$$R_s = N_s / N_e \times 100\% \quad (1)$$

Where, *R*s is Shoot induction ratio; *N*s is Number of explants of shoots induced; *N*e is Number of explants.

$$R_r = Nr / N_s \times 100\% \quad (2)$$

Where, *R*r is Root induction ratio; *N*r is Number of shoots induced roots; *N*s is Number of shoots.

2.4. Induction of roots from the shoots. Clip the shoots with 5 cm height from the base of their stems cultured for 40 d with different photoperiods, and then transfer them on the optimal rooting media, that is 1/2 MS + 0.1 mg/ LNAA medium for root induction under different photoperiods (L_0D_{24}, L_8D_{16}, $L_{12}D_{12}$, $L_{16}D_8$ and $L_{24}D_0$). In this experiment, 60 shoots are put in 20 bottles. The rooting (under the same conditions as above) is observed at 5, 7, 9, and 11 d, and the root induction ratios are calculated at the same time.

RESULTS AND DISCUSSION

3.1. The effects of photoperiod treatment on the shoot induction from Paulownia leaves. It could be seen from the results of photoperiod treatment of *P. tomentosa*, *P. fortunei* and *P. elongata* plant leaves (Table 1) that the treatment had an obvious effect on the shoot induction from Paulownia leaves, and the effect was different on different kinds of Paulownia. With a fixed induction time (except 10 d), as the lighting time during different photoperiods increases, the shoot induction ratio of the Paulownia leaves with different types of gene gradually increases all the way to 100%. However, when the shoot induction time of the leaves was 10 d, and the lighting time during the photoperiod was or shorter than 16 h, the induction rate is 0; when the lighting time is 24 h, the shoot induction ratios from the leaves of *P. tomentosa*, *P. fortunei*, *P. elongata* were respectively 3.3%, 1.7% and 15.0%. Furthermore, when the lighting time during the photoperiod was increased from 12 h to 16 h, and the induction time was gradually 20 d, 30 d, and 40 d, the shoot induction ratio of the leaves of the three Paulownia plants significantly increased. When the photoperiod was fixed and the cultivating time increased, the shoot induction ratio of the different types of Paulownia also gradually increased. When the photoperiod was 24 h darkness,and the cultivating time was 40 d, the highest induction ratio of the shoots from the three Paulownia plant leaves was 16.7%, which is the shooting induction ratio of *P. tomentosa*; when the lighting time were 8 h and 12 h respectively, those having the highest induction ratio were the leaves of *P. tomentosa* and *P. fortunei* .when the lighting time was 16 h and 24 h,with 30 d of cultivation ,the shoot induction ratio of the three Paulownia leaves all reached up to 100%; However, in terms of the shoot induction ratio of the leaves and the time needed for the highest induction ratio, the photoperiod treatment of continuous 24 h lighting was the best for the induction of the three Paulownia leaves. This indicated that different photoperiod treatments had different effects on the induction of Paulownia leaves with different genotypes.

Table 1: The effect of different photoperiods on shoot inductions of those plant leaves

Photoperiods	*P .tomentosa*				*P. elongata*				*P. fortunei*			
	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d
L_0D_{24}	0	1.7	13.3	16.7	0	3.3	10.0	11.7	0	0	0	1.7
L_8D_{16}	0	15.0	35.0	51.7	0	20.0	43.3	50.0	0	3.3	11.7	18.3
$L_{12}D_{12}$	0	23.3	41.7	61.7	0	25.0	65.0	88.3	0	6.7	15.0	26.7
$L_{16}D_8$	0	85.0	100	100	0	88.3	100	100	0	95.0	100	100
$L_{24}D_0$	3.3	93.3	100	100	1.7	95.0	100	100	15.0	98.3	100	100

3.2. The effect of photoperiod on the shoot induction from Paulownia leaves with darkness treatment. The effects of photoperiod on the shoot induction from Paulownia leaves with 5 d continuous darkness treatments. It could be seen from the effects of the different photoperiods on shoot inductions of those plant leaves with 5 d continuous darkness treatment (Table 2) that continuous darkness treatment had exerted an inhibitory effect on the shoot induction ratio

of Paulownia leaves. When the induction time was 10 d, the shoot induction ratio of the three Paulownia leaves, with different photoperiods, are all 0. In addition, with fixed shoot induction time (except 10 d) of those plant leaves, as the lighting time during the photoperiods increased, the shoot induction ratio of the three plant leaves tended to increase. This indicated that prolonging the lighting time during the photoperiods could increase the shoot induction ratio of the three Paulownia leaves with 5 d continuous darkness treatments. With a fixed photoperiod, as the cultivating time increased gradually, the shoot induction ratios of the three plant leaves tended to increase. However, the effects of different photoperiods on the shoot induction ratio of Paulownia leaves with different cultivating time were different. When the lighting time during the photoperiod was respectively 0, 8 and 12 h, of the three Paulownia leaves cultivated for 40 d, the *P. tomentosa* had the highest shoot induction ratio, which were 16.7%, 61.7%, 63.3% respectively. When the lighting time was 16 h and 24 h, the shoot induction ratios of the three plant leaves, with 40 d of cultivation were 76.7%, 100%, 63.3% and 80.0%, 100%, 100% respectively. In terms of the shoot induction ratio, the photoperiod of 24 h continuous lighting contributed the most to the shoot induction of the three plant leaves with 5 d darkness treatments.

Table 2: The effect of different photoperiods on shoot inductions of those plant leaves with 5d continuous darkness treatments

Photoperiods	*P. tomentosa*				*P. elongata*				*P. fortunei*			
	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d
L_0D_{24}	0	1.7	13.3	16.7	0	3.3	8.3	10.0	0	0	0	1.7
L_8D_{16}	0	11.7	28.3	61.7	0	8.3	11.7	13.3	0	0	6.7	18.3
$L_{12}D_{12}$	0	15.0	38.3	63.3	0	15.0	16.7	33.3	0	0	10.0	55.0
$L_{16}D_8$	0	21.6	43.3	76.7	0	45.0	80.0	100	0	0	28.3	63.3
$L_{24}D_0$	0	31.7	53.3	80.0	0	48.3	81.7	100	0	15.0	100	100

The effect of the photoperiods on shoot inductions of the Paulownia leaves with 10 d continuous darkness treatment. From the effect of different photoperiods on shoot induction of the Paulownia leaves with 10d continuous darkness treatments (Table 3), it could be seen that, with fixed photoperiod, as the cultivating time increased, the shoot induction ratios of the three plant leaves gradually increased; when the shoot induction time of *P. tomentosa*, *P. fortunei* and *P.elongate* leaves was 20 d, 30 d, and 40 d, and that of *P. fortunei* leaves was 30 d and 40 d, with the increase of lighting time during different photoperiods, the shoot induction ratio of the three plant leaves gradually increased. When the Paulownia leaves were treated by 10 d continuous darkness , the highest shoot induction ratios of *P. tomentosa*, *P. fortunei* and *P. elongata*, leaves were all those with 40 d and 24 h lighting time, which were 76.7%, 81.7% and 88.3% respectively. This indicated that the treatment of different photoperiods had exerted different effects on the shoot inductions of the three Paulownia leaves. In terms of the shoot induction ratio of the plant leaves, 24 h lighting treatment was the best photoperiod for the shoot induction of *P. tomentosa*, *P. fortunei* and *P. elongata* leaves with 10 d continuous darkness treatment.

Table 3: The effect of different photoperiods on shoot inductions of those plant leaves with 10 d continuous darkness treatments

Photoperiods	*P. tomentosa*				*P. elongata*				*P. fortunei*			
	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d
L_0D_{24}	0	1.7	13.3	18.3	0	3.3	10.0	11.7	0	0	0	1.7
L_8D_{16}	0	3.3	15.0	23.3	0	6.7	11.6	13.3	0	0	6.7	10.0
$L_{12}D_{12}$	0	5.0	21.7	38.3	0	11.7	15.0	23.3	0	0	8.3	15.0
$L_{16}D_8$	0	6.7	23.3	63.3	0	23.3	60.0	75.0	0	0	24.2	31.7
$L_{24}D_0$	0	8.3	50.0	76.7	0	28.3	76.7	81.7	0	0	77.8	88.3

3.3. The effect of photoperiod on shoot induction of the Paulownia leaves with lighting treatments. The effect of photoperiods on shoot inductions of the Paulownia leaves with 5 d lighting treatments. From the shoot induction result of different photoperiod to the Paulownia leaves with 5 d continuous lighting treatment (Table 4), it could be seen that,with a fixed shoot induction of the plant leaves, as the lighting time during photoperiod increased, the shoot induction ratios of the three Paulownia leaves gradually increased (except 10 d), particularly, when the shoot induction time is 40 d and the photoperiod is 12 h lighting, the shoot induction ratios of *P. tomentosa*, and *P. elongata* leaves could reach 100%,, and the shoot induction ratio of *P. fortunei* leaves could reach 83.3%. These results indicated that increasing the lighting time during a photoperiod contributed to the increase of shoot induction ratios of the three plant leaves with 5 h continuous illumination treatments. As the periods of fixed illumination of the plant leaves increased, the shoot induction ratios also increased gradually, but different photoperiods had various facilitating effects on the shoot induction ratio of the plant leaves with 5 d lighting treatments. Furthermore, in terms of shoot induction, the leaves of different species of Paulownia had different sensitivity to photoperiods.

Table 4: The effect of different photoperiods on shoot inductions of those plant leaves with 5 d continuous illumination treatments

Photoperiods	*P. tomentosa*				*P. elongata*				*P. fortunei*			
	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d
L_0D_{24}	0	13.3	21.1	33.3	0	16.7	30.0	60.0	0	23.3	38.3	40.0
L_8D_{16}	0	23.3	65.0	71.6	0	28.3	45.0	75.0	0	46.7	55.0	73.3
$L_{12}D_{12}$	0	55.0	98.3	100	0	63.3	76.7	100	0	66.7	80.0	83.3
$L_{16}D_8$	0	88.3	100	100	0	88.3	100	100	0	85.0	100	100
$L_{24}D_0$	3.3	93.3	100	100	1.7	95.0	100	100	15.0	100	100	100

The effect of photoperiods on shoot inductions of the Paulownia leaves with 10 d lighting treatments. From the effects of different photoperiods on the shoot inductions of the Paulownia leaves with 10 d continuous light treatment (Table 5), it could be seen that different photoperiods had obvious effect on the shoot induction of the three plant leaves with 10 d continuous lighting treatments. With different photoperiods, as the shoot induction time increased, the induction ratio of the three plant leaves with 10 d continuous lighting treatments tended to increase gradually. With a fixed shoot induction time, as the lighting time during the photoperiod increased, the shoot induction ratio of the three Paulownia leaves increased gradually with more than 20 d lighting treatments. However, the shoot induction ratio of the same Paulownia, with different induction time, increased by different extent. Furthermore, with the same induction time, as the lighting time during the photoperiod increased, the shoot induction ratio of different Paulownia leaves also increased to different extent. These results also indicated that the photoperiod treatment of 24 h continuous lighting with longer induction time did good to the shoot induction of the three Paulownia leaves.

Table 5: The effect of photoperiods on shoot inductions of those plant leaves with 10 d continuous illumination treatments

Photoperiods	*P. tomentosa*				*P. elongata*				*P. fortunei*			
	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d	10 d	20 d	30 d	40 d
L_0D_{24}	2.7	29.4	41.3	68.8	1.4	45.0	65.7	85.7	15.0	46.9	57.7	66.7
L_8D_{16}	2.7	51.6	71.3	84.3	1.4	58.5	76.0	91.1	15.0	55.0	74.6	82.3
$L_{12}D_{12}$	2.7	72.5	100	100	1.4	62.3	100	100	15.0	86.3	89.9	100
$L_{16}D_8$	2.7	88.5	100	100	1.4	75.9	100	100	15.0	95.1	100	100
$L_{24}D_0$	2.7	93.6	100	100	1.4	95.9	100	100	15.0	99.1	100	100

3.4. The effect of photoperiod on the root induction from shoots of Paulownia plants. The effects of photoperiods on rooting of *P. tomentosa*, *P. fortunei* and *P. elongata* shoots (Table 6) reveal that photoperiods extended noticeable effects on root induction of Paulownia shoots. When the root induction time was or shorter than 5 d, the effect of different photoperiods on the rooting ratio of the three Paulownia shoots, and the rooting ratio of these different species of Paulownia shoots were all 0. And when the induction time was or longer than 7 d, the effects of these different species of Paulownia shoots were also different. When the induction time was 7d, only the shoots of *P. tomentosa*, *P. fortunei* and *P. elongata* started to root; and what's more, the *P. tomentosa*, *P. fortune* were induced to root only with a photoperiod of $L_{16}D_8$, and the *P. elongata* with a photoperiod of $L_{12}D_{12}$ and $L_{16}D_8$. When the cultivating time was or longer than 9 d, except for the relatively low root induction ratios of the plant shoots with continuous darkness treatments, the root induction ratio of those shoots with the other four photoperiod treatments all could reach 100%. These results indicated that, when the lighting time during a photoperiod was longer than 16 h, the rooting of the *P. tomentosa*, *P. fortunei* and *P. elongata* shoots was inhibited, while the inhibition to the rooting of *P. elongate* shoots was not so obvious.

Table 5: The effect of photoperiods on shoot inductions of those plant leaves with 10 d continuous illumination treatments

Photoperiods	*P. tomentosa*				*P. elongata*				*P. fortunei*			
	0 d	5 d	7 d	9 d	0 d	5 d	7 d	9 d	0 d	5 d	7 d	9 d
L_0D_{24}	0	0	0	60.0	0	0	0	80.0	0	0	0	40.0
L_8D_{16}	0	0	0	100	0	0	0	100	0	0	0	100
$L_{12}D_{12}$	0	0	8.3	100	0	0	40.0	100	0	0	0	100
$L_{16}D_8$	0	0	20.0	100	0	0	68.3	100	0	0	0	100
$L_{24}D_0$	0	0	0	100	0	0	0	100	0	0	0	100

In our experiment, photoperiod with 24 h continuous illumination is optimal to the 3 kinds of Paulownia shoot inductions from leaves treated with direct illumination or after continuous illumination and /or darkness for 5 d, 10 d. It was supposed to relate to Paulownia characteristic of sunlight-favor and to the media in which the mineral and organic substances could meet the need of shoot induction and growth. Moreover, The photoperiods had different effects on rooting from the 3 kinds of Paulownia shoots. This phenomenon was found in *Musa nana*、*C. terminalis* and *J.fallax Dode* [12-14]

CONCLUSION

Different plants had their optimal photoperiods differently for their *in vitro* plantlet regeneration. On the one hand, the difference of the plants reaction to photoperiod was reflected as it growed. On the other hand, it was also reflected as it developed. Also the difference could be inherited steadily in the filial generations. Different kinds of plants had their favorite geographic distribution. Both domestic and international geobotanists did much research on this area [15]. Regarding the effects of photoperiods on *in vitro* plantlet regeneration, The photoperiod of 16 h lighting was commonly considered as the optimal photoperiod for most plants [2-5, 14]. Here the facts that photoperiod with 24 h continuous illumination is the favorite for the shoot inductions from 3 kinds of Paulownia leaves were gotten theoretically, but the economical and practical photoperiods must be considered in production of seedlings for their cost.

Acknowledgement
The author wish to thank Xinxiang University for providing financial support, and highly appreciate the Institute of Paulownia of Henan Agricultural University for providing facilities support.

REFERENCES

[1] X. M. Wu, H. R. Tang, G. Q. Wen, Y. Li, *Acta Hopticulturae Sinica*, **2004**, 31(5), 657-659.
[2] G. Q. Fan, J. P. Jiang, Y. Q. He, F. Li, *Acta Hopticulturae Sinica*, **200**3, 30(2), 236-239.
[3] O. Monteuuis, M. C. Bon, *Plant Cell, Tissue and Organ Culture*, **2000**, 63(3), 173-177.
[4] A. Matt, J. A. Jehle, *Plant Cell Reports*, **2005**, 24, 468-476.
[5] H. Y. Mao, Z. Y. Gu, P. F. Zhu, *Acta Boorealt-Occidentalia Sinica*, **2010**, 30(10), 2074-2080.
[6] A. T.Wang, X. J. Yang, X. Q. Zhai, X, Y. Ma, M. Li, G. Q. Fan, *Journal of Henan Agricultural University*, **2005**, 39(1), 46- 50.
[7] Z. Ipekci, N. Gozukirmizi, *Plant Cell Reports*, **2003**, 22, 16-24.
[8] C. D. Rao, C. J. Goh, P. P. Kumar, *Plant Cell Reports*, **1996**, 16(3-4), 204-209.
[9] B. A. Bergmann and H. K. Moon, "I*n vitro* adventitious shoot production in *Paulownia*," *Plant Cell Reports*, Vol. 16, no. 5, pp. 315-319, **1997**.
[10] X. Q. Zhai, X. Y. Ma, M. Li, *Acta Agricultural Nucleatae Sinica*, **2005**, 19(4), 274-278.
[11] Z. L. Zhao, J. He,, X. Q. Zhai, G. Q. Fan, *Journal of Henan Agricultural University*, **2011**, 45(1), 59-65.
[12] A. M. Wang, G*uihaia*, **2003**, 23(1), 61-63.
[13] Kodym, F. G. Zapata-Arias, *Plant Cell, Tissue and Organ Culture.* **1999**, 55(2), 141-145.
[14] B. Zeng, T. M. He, Y. X. Wu, X. Wang, T. Li, **2011**, 48(7), 61-63.
[15] X. J. Wei, L. Jiang, J. F. Xu, X. Liu, S. J. Liu, H. Q. Zhai, J. M. Wan, *Journal of Integrative Plant Biology*, **2009**, 51(10), 922-932.