Agricultural Science & Technology,2008,9(2):30 -32, 115

Effects of Different Culture Conditions on Vitrification of *Lycium barbarum* L. Plantlets in Tissue Culture

CAO You-long*, LUO Qing, ZHANG Xi-yan, LI Xiao-ying

Engineering and Research Center for Chinese wolfberry, Ningxia Academy of Agricultural and Forestry Sciences, Yinchuan 750002

Abstract The tender stems from new *Lycium barbarum* L. cultivar "Ningqi 3" released by Ningxia Academy of Agricultural and Forestry Sciences were regarded as explants to investigate the vitrification of *Lycium barbarum* plantlets in tissue culture under different concentrations of 6-BA, sucrose, agrose, culture temperature, and illumination duration with MS as basic medium. The results show that the conditions for maximal proliferation coefficient and minimal vitrification are as following: the basic medium with 0.2 mg/L 6-BA, 3% sucrose and 0.65% agarose; culture at 25 ℃; 12 h/d(daylight lamp, 2 000 lx) illumination.

Key words *Lycium barbarum* L.; Tissue culture; Vitrification; Influencing factor

Chinese wolfberry(*Lycium barbarum* L.), a rare Chinese medicinal material and tonic healthy food, has been widely concerned. In proliferation process of virus-free culture or breeding new cultivars through tissue culture, vitrification is often a hindrance that influences subsequent differentiation potential, proliferation, rooting and survival in transplanting. To provide reference for preventing the occurrence of vitrification plantlet and enhancing the quality of tissue culture plantlet, we investigated the factors influencing vitrification in tissue culture.

Materials and Methods

Material

The experimental material is a new *Lycium barbarum* L. cultivar "Ningqi 3" released by Ningxia Academy of Agricultural and Forestry Sciences.

Methods

The tender stem fragments were sampled from field and washed with running water for 5 min after the big leaves were discarded, then disinfected with 75% ethanol for 10 s followed by 0.1% $HgCl_2$ for 6 - 8 min, finally washed with sterilized water for 3 times. The disinfected stems were cut into 1.5 - 2.0 cm fragments, inoculated onto MS medium (supplemented with 0.2 mg/L 6-BA, 0.01 mg/L NAA, 3% sucrose and 0.65% agarose) and incubated at 25 ℃ with 12 h/d(daylight lamp, 2 000 lx) illumination for 4 weeks. The healthy and sterile clustered shoots were then selected for subculture.

The tissue culture plantlets subcultured for 4 weeks (with plant height of 1.5 - 2.0 cm) were inoculated onto MS medium for test. Different concentrations of 6-BA, sucrose, agarose, culture temperature and illumination duration were designed to optimize the conditions for tissue culture of Chinese wolfberry. Each treatment contained 20 plantlets and was replicated for 3 times. The vitrification situation of tissue culture plantlets were observed after 45 d.

Results and Analysis

Effect of different 6-BA concentrations on proliferation and vitrification in tissue culture of Chinese wolfberry

The media with 0.01 mg/L NAA, 3% sucrose and 0.65% agarose and different concentrations of 6-BA(0.2, 0.5 and 1.0 mg/L) were designed to inoculate subcultured plantlets. It can be seen from Table 1 that maximal proliferation coefficient and lower vitrification occurred under 0.2 mg/L 6-BA; higher proliferation coefficient but meanwhile higher vitrification appeared under 0.5 mg/L 6-BA; lower proliferation coefficient and highest vitrification appeared when 6-BA concentration reached 0.5 mg/L, suggesting the poor proliferation potential and high vitrification under this condition.

Table 1 Effect of different 6-BA concentrations on proliferation and vitrification in tissue culture of Chinese wolfberry

6-BA concentration mg/L	Inoculated plantlets	Total proliferated plantlets	Bud proliferation coefficient	Vitrification plantlets	Ratio of vitrification plantlets %
0.2	20	720	36	11	1.5
0.5	20	620	31	48	7.7
1.0	20	100	5	73	73.0

Effect of different sucrose concentrations on vitrification in tissue culture of Chinese wolfberry

The media with 0.2 mg/L 6-BA, 0.01 mg/L NAA, 0.65% agarose and different concentrations of sucrose (2%, 3% and 4%) were designed to inoculate subcultured plantlets. It can be seen from Table 2 that most severe vitrification of tissue culture plantlets occurred under 2% sucrose; strong plantlets and more cluster buds meanwhile lower vitrification appeared under 3% sucrose; bud differentiation was inhibited when sucrose concentration reached 3%.

Received: May 26, 2008 Accepted: June 6, 2008

Supported by Fund for the Transformation of Scientific and Technological Achievements in China (2006GB2G300311) and National Natural Science Foundation of China (30760127).

* Corresponding author. E-mail: youlongchk@163.com

Table 2 Effect of different sucrose concentrations on vitrification in tissue culture of Chinese wolfberry

Sucrose concentration %	Inoculated plantlets	Total proliferated plantlets	Bud proliferation coefficient	Vitrification plantlets	Ratio of vitrification plantlets %
2	20	140	7	32	22.9
3	20	720	36	11	1.5
4	20	100	5	0	0

Effect of different sucrose concentrations on vitrification in tissue culture of Chinese wolfberry

The media with 0.2 mg/L 6-BA, 0.01 mg/L NAA, 3% sucrose and different concentrations of agarose (0.50%, 0.65% and 0.80%) were designed to inoculate subcultured plantlets. It can be seen from Table 3 that ratio of vitrification plantlets, as well as proliferation coefficient, reduced remarkably with the increase of agarose concentration. Most severe vitrification of tissue culture plantlets occurred under 0.50% agarose; strong plantlets and more cluster buds meanwhile slighter vitrification appeared under 0.65% agarose; no vitrification plantlet but wilting plantlets appeared when agarose concentration reached 0.80 %.

Table 3 Effect of different agarose concentrations on vitrification in tissue culture of Chinese wolfberry

Agarose concentration %	Inoculated plantlets	Total proliferated plantlets	Bud proliferation coefficient	Vitrification plantlets	Ratio of vitrification plantlets %
0.50	20	80	7	25	31.2
0.65	20	720	36	11	1.5
0.80	20	100	5	0	0

Effect of different temperatures on vitrification in tissue culture of Chinese wolfberry

Three different temperatures (20, 25 and 30 ℃) were designed to inoculate subcultured plantlets. It can be seen from Table 4 that slightest vitrification and higher proliferation coefficient appeared under the temperature of 25 ℃; vitrification increased with the temperature exceeding 30 ℃; lower growth as well as lower proliferation coefficient with the temperature lower than 20 ℃, meanwhile a higher vitrification than that of 25 ℃.

Table 4 Effect of different temperatures on vitrification in tissue culture of Chinese wolfberry

Temperature ℃	Inoculated plantlets	Total proliferated plantlets	Bud proliferation coefficient	Vitrification plantlets	Ratio of vitrification plantlets %
20	20	100	5	25	4.0
25	20	720	36	11	1.5
30	20	140	7	27	19.3

Effect of illumination durations on vitrification in tissue culture of Chinese wolfberry

Three different illumination durations (10, 12 and 14 h/d) were designed to inoculate subcultured plantlets. It can be seen from Table 5 that more severe vitrification and lower growth appeared under the illumination time of 10 h/d; slightest vitrification and highest proliferation coefficient obtained under the illumination time of 12 h/d; highest vitrification appeared when illumination time reached 14 h/d.

Table 5 Effect of different durations on vitrification in tissue culture of Chinese wolfberry

Illumination duration h/d	Inoculated plantlets	Total proliferated plantlets	Bud proliferation coefficient	Vitrification plantlets	Ratio of vitrification plantlets %
10	20	120	6	4	3.3
12	20	720	36	11	1.5
14	20	340	17	17	5.0

Conclusion and Discussion

Vitrification in tissue culture of Chinese wolfberry results from many influencing factors. In the present study, the optimized conditions for tissue culture of Chinese wolfberry are as following: MS basic medium supplemented with 0.2 mg/L 6-BA, 0.01 mg/L NAA, 3% sucrose, 0.65% agarose, pH 5.8 – 6.0; incubation at 25 ℃, illumination duration 12 h/d (daylight lamp, 2 000 lx). Under the conditions, higher proliferation coefficient and lower vitrification were synchronously achieved.

6-BA influences vitrification of plantlets in tissue culture. The higher of 6-BA concentration, the more vitrification plantlets are, which is consistent with our previous reports[1]. 0.2 mg/L 6-BA is suitable for tissue culture of Chinese wolfberry. Sucrose is decisive for osmotic pressure of medium[2]. The vitrification phenomenon often occurs due to the unadaptable of plantlet to medium and culture environment. Proper increase of sucrose concentration could decrease the osmotic pressure of medium, causing water stress of medium and further reducing the vitrification. Chinese wolfberry plantlets could grow well with addition of 3% sucrose. Agarose influences the available water in medium. Under lower agarose concentration, high moisture in medium promotes vitrification and lodging of tissue culture plantlets. Medium with high concentration of agarose is too hard for the plantlet to absorb the nutriment[3], resulting in the decrease of bud differentiation rate. In the present study, the suitable concentration was proved to be 6.5 g/L. The temperature required for plant tissue culture is commonly (25 ± 3) ℃, vitrification often appeared beyond the temperature range. In the present study, lowest vitrification existed under the temperature of 22 – 25 ℃, which is consistent with the view of WANG Xiao-min[4]. In the process of plant tissue culture, materials are often illuminated 10 – 14 h/d with 2 or 3 daylight lamps (40 w) for each shelf[5]. Illumination duration lower than 10 h/d or higher than 14 h/d may lead to vitrification of tissue culture plantlets. Additionally, vitrification may disappear gradually when transferred to natural condition. Illumination duration of 12 h/d is the optimal condition for tissue culture of Chinese wolfberry. In short, comprehensive regulation of tissue culture condition could effectively prevent and decrease the occurrence of vitrification.

References

[1] CAO YL, CHEN F, LUO Q, *et al.* In vitro culture of pith tissue and high-

frequency plant regeneration of *Lycium barbarum* L. [J]. Guihaia, 1999, 19(3):239-242. (in Chinese).
[2] HUANG HB,DAN M,GUO AP, *et al*. Main problems and their countermeasures in plant tissue culture [J]. Plant Physiology Communications, 1992,27(4):288. (in Chinese).
[3] WEI Q,ZHOU L,XUAN P, *et al*. Study on the vitrification of *Cinnamomum longepaniculatum* (Gamble) N. Chao in tissue culture [J]. Journal of Sichuan Normal University: Natural Science, 2006, 29(5): 606-608. (in Chinese).
[4] WANG XM,LI WL,ZHAO ZQ, *et al*. Effects of different conditions of tissue culture on vitrification of *Mentha haplocalyx* Briq. plantlets [J]. Journal of Plant Resources and Environment, 2006, 15(3):51-54. (in Chinese).
[5] GAO WY,JIA W. Large-scale tissue culture of medicinal plant [M]. Beijing: Chemical Industry Press, 2005. (in Chinese).

不同培养条件对枸杞组培苗玻璃化的影响

曹有龙*,罗 青,张曦燕,李晓莺 (宁夏农林科学院枸杞工程技术研究中心,宁夏银川 750002)

笔者对枸杞玻璃化现象的影响因素进行了研究,以期为预防枸杞玻璃化苗的产生和提高枸杞组培苗的质量提供有效措施。

1 材料与方法

1.1 材料 试材为宁夏农科院培育的枸杞新品种"宁杞3号"。

1.2 方法 从大田里取枸杞幼嫩茎段,去掉较大的叶子,先用自来水冲洗5 min,再用75%酒精溶液消毒10 s,然后用0.1% $HgCl_2$ 溶液消毒6~8 min,最后用无菌水冲洗3遍。吸干水分后切成1.5~2.0 cm的茎段,接种在MS+0.2 mg/L 6-BA+0.01 mg/L NAA+30 g/L 蔗糖+0.65 g/L 琼脂的培养基上,pH值5.8~6.0,于25 ℃、光照时间为12 h/d(日光灯,光照强度2 000 lx)的条件下培养4周后,选取健康无菌的丛生芽进行继代培养。

取继代培养4周后的组培苗(株高约1.5~2.0 cm)接种于MS基本培养基,以6-BA浓度、蔗糖浓度、琼脂浓度、培养温度和光照时间为试验因子,设置不同处理进行试验。每个处理接种20株苗,重复3次。培养45 d后,观察组培苗出现玻璃化的情况。

2 结果与分析

2.1 6-BA浓度对枸杞组培苗增殖和玻璃化的影响 在添加0.01 mg/L NAA、30 g/L 蔗糖、6.5 g/L 琼脂,6-BA的浓度分别为0.2、0.5、1.0 mg/L的3种MS培养基上,接种继代培养后的组培苗。6-BA浓度为0.2 mg/L时,芽增殖系数高,玻璃化率较低;6-BA浓度为0.5 mg/L时,芽增殖系数也较高,但玻璃化率比6-BA浓度为0.2 mg/L时要高;6-BA浓度为1.0 mg/L时,芽增殖系数极低,说明芽扩增能力较差,组培苗的玻璃化率高(表1)。

表1 6-BA浓度对枸杞组培苗增殖和玻璃化的影响

6-BA浓度 mg/L	接种苗数 株	繁殖总苗数 株	芽增殖系数	玻璃化苗数 株	玻璃化率 %
0.2	20	720	36	11	1.5
0.5	20	620	31	48	7.7
1.0	20	100	5	73	73.0

2.2 蔗糖浓度对枸杞组培苗玻璃化的影响 在添加0.2 mg/L 6-BA、0.01 mg/L NAA、0.65 g/L 琼脂,蔗糖浓度分别为20、30、40 g/L的3种MS培养基上,接种继代培养后的组培苗。当蔗糖浓度为20 g/L时,枸杞组培苗玻璃化率高;蔗糖浓度为30 g/L时,枸杞分化植株健壮,丛生芽多,玻璃化率相对较低;蔗糖浓度为40 g/L时,芽的分化受到抑制(表2)。

表2 蔗糖浓度对枸杞组培苗增殖和玻璃化的影响

蔗糖浓度 g/L	接种苗数 株	繁殖总苗数 株	芽增殖系数	玻璃化苗数 株	玻璃化率 %
20	20	140	7	32	22.9
30	20	720	36	11	1.5
40	20	100	5	0	0.0

2.3 琼脂浓度对枸杞组培苗玻璃化的影响 在添加0.2 mg/L 6-BA、0.01 mg/L NAA、30 g/L 蔗糖,琼脂浓度分别为5.0、6.5、8.0 g/L的3种MS培养基上,接种继代培养后的组培苗。随着琼脂浓度的增加,枸杞组培苗的玻璃化率明显下降,但增殖系数也下降。当琼脂浓度为5.0 g/L时,玻璃化现象严重;当琼脂浓度为6.5 g/L时,枸杞分化植株健壮,丛生芽多,玻璃化率相对较低;当琼脂浓度为8.0 g/L时,培养基中无玻璃化苗,但部分组培苗出现萎蔫现象(表3)。

表3 琼脂浓度对枸杞组培苗增殖和玻璃化的影响

琼脂浓度 g/L	接种苗数 株	繁殖总苗数 株	芽增殖系数	玻璃化苗数 株	玻璃化率 %
5.0	20	80	4	25	31.2
6.5	20	720	36	11	1.5
8.0	20	100	5	0	0.0

2.4 培养温度对枸杞组培苗玻璃化的影响 培养温度为25 ℃时,组培苗玻璃化率最低,且增殖系数较高;培养温度超过30 ℃时,玻璃化率有所增加;培养温度低于20 ℃时,枸杞植株生长缓慢,且芽增殖系数较低,玻璃化率比培养温度为25 ℃时高(表4)。

表4 培养温度对枸杞组培苗增殖和玻璃化的影响

培养温度 ℃	接种苗数 株	繁殖总苗数 株	芽增殖系数	玻璃化苗数 株	玻璃化率 %
20	20	100	5	25	4.0
25	20	720	36	11	1.5
30	20	140	7	27	19.3

2.5 光照时间对枸杞组培苗玻璃化的影响 光照时间为10 h/d时,组培苗玻璃化率较高,生长缓慢;光照时间为12 h/d时,玻璃化率最低,芽增殖系数最高;光照时间为14 h/d时,组培苗玻璃化率最高(表5)。

表5 光照时间对枸杞组培苗增殖和玻璃化的影响

光照时间 h/d	接种苗数 株	繁殖总苗数 株	芽增殖系数	玻璃化苗数 株	玻璃化率 %
10	20	120	6	4	3.3
12	20	720	36	11	1.5
14	20	340	17	17	5.0

3 结论与讨论

枸杞组织培养中出现的玻璃化现象是许多因素作用的结果。综合分析,确定以MS+0.2 mg/L 6-BA+0.01 mg/L NAA+30 g/L 蔗糖+6.5 g/L 琼脂(pH值5.8~6.0)的培养基上,并于25 ℃、光照时间为12 h/d(日光灯,光照强度2 000 lx)的条件下进行组织培养,枸杞组培苗的增殖系数较高,玻璃化率较低。

(下转第115页)

金属离子·有机溶剂对黄鳝碱性磷酸酶的影响

黄毅[1*]，唐云明[2]（1. 西南科技大学生命科学与工程学院，四川绵阳 621010；2. 西南大学生命科学学院，重庆 400715）

在生物体内，碱性磷酸酶（Alkaline Phosphate，简称 *ALP*）直接参与磷代谢，维持体内适宜的钙磷比例，同时参与角蛋白的分泌和 DNA、RNA、脂质代谢，是一种非常重要的代谢调控酶。它对水生动物钙质吸收、骨骼形成、磷酸钙沉积都起着重要作用。作为金属酶，*ALP* 的分子结构维持和催化活力表现都需要金属离子。研究金属离子以及其他效应剂对 *ALP* 的影响，对于探讨 *ALP* 的作用机理，促进黄鳝水产养殖等有重要意义。因此，笔者在前期研究的基础上，从动力学角度探讨不同金属离子、有机溶剂等效应剂对黄鳝内脏 *ALP* 活性的影响。

1 材料与方法

1.1 材料和试剂 黄鳝（*Monopterus albus*）购自重庆北碚水产品市场，以其内脏为提取 *ALP* 的原料。DEAE-Sephrose Fast Flow、Sephacryl S-200 为瑞典 Amersham Pharmacia 产品；磷酸苯二钠、4-氨基安替比林、各种金属离子无机盐、有机溶剂及其余试剂均为国产分析纯，所有试剂均用 ddH_2O 配制。

1.2 方法 黄鳝 *ALP* 的分离纯化及部分性质研究按文献[3]进行，所得酶制品比活为 3 021.39 U/mg 蛋白。

1.2.1 基质缓冲液的配制。将 0.05 mol/L 碳酸缓冲液（pH 值 10.2）、0.01 mol/L 磷酸苯二钠、3% 的 4-氨基安替比林及 ddH_2O 按 10:5:1:4 的比例混合（测定 Mg^{2+}、Ca^{2+} 的效应时，将上述碳酸缓冲液用 0.05 mol/L pH 值 9.6 的甘氨酸—氢氧化钠缓冲液替换，其余试剂比例不变）。

1.2.2 金属离子对酶活力影响测定。在酶活力最佳温度及底物浓度条件下，分别向 2 ml 基质缓冲液中加入不同浓度的各种金属离子的无机盐溶液，再加入 0.1 ml 纯酶制品溶液，并用 ddH_2O 补足 5 ml，反应 15 min，用 5% 三氯乙酸终止反应，在 510 nm 处测定光吸收值，并以无金属离子反应系统为对照，计算各种金属离子存在下的相对酶活力。

1.2.3 产物及产物类似物对酶活力的影响测定。分别以 Na_2HPO_4、Na_3WO_4 为效应物，研究磷酸氢盐及其类似物钨酸盐对酶活力的影响。在 2 ml 基质缓冲液中（含 2 mmol/L Mg^{2+}）加入不同浓度的产物（HPO_4^{2-}）及产物类似物（WO_4^{3-}），测定酶促反应的初速度，分析效应物对酶活力的影响，并以 Lineweaver-Burk 双倒数法判断作用机理。

1.2.4 有机溶剂对酶活力的影响测定。在基质缓冲液中加入不同浓度（10%、20%、30%、40%、50%、60%）的甲醇、乙醇、乙二醇、异丙醇 4 种有机溶剂，分析其对酶活力的影响。

2 结果与讨论

2.1 金属离子对酶活力的影响 在不含 Mg^{2+} 的体系中，加入不同浓度的金属离子，于最适 pH 值和温度下反应 15 min，监测酶活力的变化。结果表明（图见第 113 页 Fig. 1～5），Na^+、K^+ 对酶活力影响不大，可能是由于这些离子为细胞内外常见离子，*ALP* 在长期进化中已经能够适应其浓度的变化。Li^+ 对酶活力的抑制作用比较明显；与张洪渊对背角无齿蚌的研究结果不完全一致，这可能与物种不同有关。Mg^{2+}、Ca^{2+} 对 *ALP* 有激活作用，Zn^{2+} 对黄鳝 *ALP* 有一定程度的抑制作用，这与张洪渊等在背角无齿蚌和赵欣平等在白腊虫的试验中所得到的结果相同，表明 *ALP* 的激活机理相同。Cu^{2+} 对酶活力的抑制作用表明重金属对酶是有害的，因此水产养殖中必须注意水质的管理。

2.2 产物及其类似物对酶活力的影响 HPO_4^{2-} 及 WO_4^{3-} 对酶活力的影响（图见第 113 页 Fig. 6）表明，它们对酶活力均有一定的抑制作用，其中 9.5 mmol/L HPO_4^{2-}、WO_4^{3-} 分别可使酶活力降低 13%、34%。研究它们对酶活性的抑制类型（图见第 114 页 Fig. 7：HPO_4^{2-} 对酶活力的效应，Fig. 8：WO_4^{3-} 对酶活力的效应，2 图中曲线 1～3 的 HPO_4^{2-} 与 WO_4^{3-} 浓度分别为 9.5、5.7 和 1.9 mmol/L）表明，HPO_4^{2-}、WO_4^{3-} 对 *ALP* 酶活性的抑制类型均为竞争性抑制。同锯缘青蟹、僧帽牡蛎、合浦珠母贝表现一致。比较它们对酶的抑制效果，说明 2 种效应物对大部分水生生物的 *ALP* 具有相同的作用，可能存在相同的抑制机理。因 HPO_4^{2-} 是酶催化的产物，而 WO_4^{3-} 是类似物，也是酶的竞争性抑制剂，由此可推断 HPO_4^{2-}、WO_4^{3-} 能与 *ALP* 的活性中心结合从而阻止底物结合到酶分子上。

2.3 有机溶剂对酶活性的影响 4 种有机溶剂对黄鳝肝脏酶活性均有较强的抑制作用（图见第 114 页 Fig. 9：不同溶剂对 *ALP* 酶活力的影响）。在 0～40% 范围内，随着有机溶剂体积浓度的增大，酶活性呈直线下降；之后，酶活性下降趋缓；当甲醇、乙醇、乙二醇、异丙醇的体积浓度（C）达 60% 时，酶活性分别降低 78%、87%、75% 和 90%，它们对酶抑制作用的大小顺序为异丙醇 > 乙醇 > 甲醇 > 乙二醇。可见，溶剂极性越低，对酶的抑制程度越高，说明酶分子中疏水键对维持酶的三维构象起重要作用。

基金项目 重庆市科委科技基金资助。
作者简介 黄毅（1979－），男，四川成都人，硕士，讲师，从事酶与酶工程方面的研究。*通讯作者。
收稿日期 2008-04-16 修回日期 2008-05-27

（上接第 32 页）

研究表明，6-BA 的浓度越高，玻璃化率越高，这与曹有龙等的研究结果一致。该试验中 6-BA 浓度以 0.2 mg/L 为宜。蔗糖对培养基的渗透压起决定性作用。玻璃化现象的发生是组培苗不适应培养基和培养环境的结果。适当提高蔗糖的浓度，降低培养基中的渗透势，可对培养物造成水分胁迫以降低玻璃化。从该试验结果来看，当蔗糖浓度为 30 g/L 时，枸杞组培苗生长最好。琼脂也影响培养基中可利用水分的量。琼脂浓度低时，培养基中水分过多，培养瓶中湿度过大，组培苗易产生玻璃化，且易倒伏。琼脂浓度过高，培养基太硬，营养物质难以被组培苗吸收利用，芽的分化力降低，在该试验中琼脂浓度以 6.5 g/L 为宜。植物组织培养所需温度一般在（25 ± 3）℃，该研究结果表明，当培养温度为 22～25 ℃时，玻璃化率最低，与王小敏等的研究结果一致。植物组织培养过程中，一般每个培养架使用 2～3 只 40 w 日光灯，要求光照时间为 10～14 h/d。此外，若有可能增加自然光照，玻璃化现象会逐渐消失。该试验结果表明，光照时间为 12 h/d 时，枸杞组培苗的生长最好。总之，通过综合调控枸杞的组织培养条件，可以有效预防和减少玻璃化现象的发生。

基金项目 国家成果转化项目（2006GB2G300311）；国家自然科学基金（30760127）。
作者简介 曹有龙（1963－），男，宁夏银川人，研究员，从事植物遗传育种研究。*通讯作者。
收稿日期 2008-05-26 修回日期 2008-06-06