http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (10): 1995-1998, 2006
ISSN 1028-8880

A Primary Investigation on the Eco-environmental Improvement in the Western China by the Use of *in vitro* Propagation Techniques of Taxus (*Taxus media*)

[1-3]Shao Hongbo, [5]Jiang Shuiyuan, [3]Chu Liye, [4]Zhao Changxing,
[1]Wei Jinmin, [1]Li Biao and [3]Guo Qingjie
[1]Key Laboratory of Molecular Biology, Bio-informatics College,
Chongqing University of Posts and Telecommunications, Chongqing 400065,
Pepole's Republic of China
[2]State Key Laboratory of Soil Erosion and Dryland Farming, the Research Centre of Soil and
Water Conservation and Eco-environment, Chinese Academy of Sciences,
Yangling 712100, Pepole's Republic of China
[3]Key Laboratory of Biological Sciences, College of Chemical Engineering,
Qingdao University of Science and Technology, Qingdao 266042, Pepole's Republic of China
[4]College of Plant Science and Technology, Laiyang Agricultural University,
Qingdao 266109, Pepole's Republic of China
[5]Guangxi Institute of Botany, Guangxizhuangzu Autonomous Region and
Chinese Academy of Sciences, Guilin 541006, Pepole's Republic of China

Abstract: In this study, a primary investigation on *in vitro* propagation technique of Taxus (*Taxus media*) for the sake of improving the eco-environment and increasing economic effect in west China zone was conducted. The better result was obtained and a relatively simple and practical procedure of *In vitro* propagating of Taxus (*Taxus media*) was established, which has a broad prospect in improving eco-environment and promotion of economy in west China region. The related results were as followed: The 2 year branches of Taxus media could be selected as explants, obtaining 78% of budding and transplanting survival rate reaching 90%. Use of 0.1% $HgCl_2$ for sterilization for 12 m could reach more than 90% effect of sterilization, which was better than that previous reported. The flowchart for effective *in vitro* propagation of Taxus media under our experimental condition was below: recover and initiation of explants→bud propagation→ subculture→rooting→transplanting in greenhouse→transplanting in fields or going to market.

Key words: *Taxus media*, *in vitro* propagation technology, eco-environmental improvement

INTRODUCTION

Loess Plateau of China plays a great role in sustainable development of Chinese economy because of its special regional unit during the 21st century (Chen and Wang, 2001; Shao, 2001). Eco-environmental improvement is one of the main tasks of the Western Development (Vasil, 2002), during which the most important part is to stop tilling, return to forest and grass. During this Course, the eventual question for us to solve at first is how to select suitable forest species and grass species according to the theory of plant physiology and ecology, pedology and modern biotechnology (Chaves *et al*., 2003; Passioura, 2002; Somerville and Bonetta, 2001; Vasil, 2002). On the basis of multi-disciplinary property, complexity, sustainability of eco-environmental improvement and construction in conjunction with the basis of its economy, ecological and social effect, *Taxus media* is one of the best species. Taxus is the remaining plant species of the 3rd century, which has been ranged as the rarely-endangered forest species for protection over the world; Taxus also has highly-ornamental value and can be seen in the front of the Mansion of UN General Office, in the garden of the White House of USA and the White Gold Palace of England. Taxol is a kind of deterpene compounds only existing in Taxus woody plants, which has special effect on more than ten kinds of cancers such as mammary

Corresponding Author: Zhao Changxing, College of Plant Science and Technology,
Laiyang Agricultural University, Qingdao 266109, Pepole's Republic of China
Shao Hongbo, Key Laboratory of Molecular Biology, Bio-informatics College,
Chongqing University of Posts and Telecommunications, Chongqing 400065, Pepole's Republic of China

cancer, ovary cancer, lung cancer and uterus cancer. This kind of medicine has been considered as the last protective front line for cancer patients, therefore whose price has become more and more expensive and which has become one of the hot topics of medicine and biotechnology abroad Dellapenna, 2001; Chu, 2002; Gan *et al*., 1996, 2000). There are four species and one variety of Taxus in China, Which are ranked as the national rarely-endangered woody species and grow slowly, distribute widely and devoid of resource. Especially since 1990's, their quantity in resource has become extincted nearly because of large amount of illegal felling of trees (Vasil, 2002). Considering the current situation above, scholars at home and abroad have done two main aspects of work of taxol medicinal source pathways involved in chemical synthesis and biotechnology (Ketchum and Gibson, 1996; He *et al*., 2002; Shao, 2001; Shao *et al*., 2005). In biotechnological field, the main work includes cellular engineering (by the use of callus or cell suspension cultures to gain highly-contented Taxus lines or to extract directly taxol) and gene engineering (Yu and Zhang, 1999; Shao, 2002; Chu, 2002). Up to date, some meaningful and important stepping results have taken place in medium selection, culture methodology and conditional regulation, selection of excellent lines, molecular biology of taxol synthesis enzymes and genetic transformation etc (Gan *et al*., 1996; Zhao and Zhu, 1998; Zhou *et al*., 2001; Wang *et al*., 2002), but there is a bigger distance from laboratories to large-scale biological synthesis of taxol (Shao, 2002).

Since 1996, Forest Academy of Sichuan Province and Northern Taxus Ecological Sci-tech Company of Beijing have introduced good-quality Taxus media lines from USA and done experiments on cuttage propagation study. In 2002, some products of *Taxus media* appeared in market, which illustrates that Taxus media (Table 1), Which have been approved by FDA of USA to be used as the source to extract taxol, is promising to be popularized in China. However, due to the limitation of national fund it is impossible to invest more than 10 millions of fund to establish modern green houses and develop this kind of good-quality Taxus species and besides high cost of products confines to its popularization, especially in the poor region of Loess Plateau. We utilized good-quality Taxus media species as materials and established a primary *in vitro* propagation technology of *Taxus media*, greatly saving space, fund and cost, shortening cycle time and labor, whose purpose is to provide the scientific basis for the new access to improvement and construction of eco-environment of stopping tillage and returning to forest and grass in Loess Plateau. There has been no report on this aspect in relation to such aim up to now. Our primary reports are as follows and can provide insights into improving the environment of Loess Plateau and the Western China.

MATERIALS AND METHODS

Materials: Taxus media materials were bought from Northern Taxus Ecological Sci-tech Company of Beijing and Forest Academy of Sichuan province in August, 2001, respectively. The related phytohormones and regulators with analytical quality were bought from Beijing Chemical Reagents Company. The experiment was conducted on 16 August, 2001 in Guilin (Guangxi Institute of Botany), Guangxi and Yangling (State Key Laboratory of Soil Erosion and Dryland Farming, the Research Centre of Soil and Water Conservation and Eco-environment), Shanxi, China.

Tissue culture and rapid propagation: Take 1 year and 2 year younger branches of *Taxus media* and rinse them routinely. Then the experimental materials were immersed in 70% ethyl alcohol for 50s and sterilized by 0.1% $HgCl_2$ for 12 m and rinsed by sterilized water 6 times. After cutting the stems with buds into the explants with a size of 0.5×0.5 cm, these explants were inoculated in the following medium: MS+6-BA 0.3 mg L^{-1}+others (the medium for bud induction); after 7 weeks' culture the explants with more than 2 buds were sub-cultured (the medium is the same as that above); after 4 weeks' culture the explants from the subculture above were transferred to the medium supplied with MS+6-BA 0.6 mg L^{-1}+others (bud propagation medium); after 6 weeks the cultures above were transferred to the root-growing medium containing MS (half of the macro-elements were added to) +IAA 0.5 mg L^{-1}+NAA0.5 mg L^{-1}+others; after 6 weeks' culture, the explants with roots were transferred (Chen *et al*., 2000) to the nutrition cup with a certain component of nutrition and transplanted. After 8 weeks,

Table 1: Fine characteristics of *Taxus media* (Shao, 2001)

Species	Distribution of taxol	Content of taxol	Growth (%)	Requirement of habitat	Economic income
General taxus	Bark	0.01-0.02	Slow, after 50 years to be harvested for taxol	Special habitat	Peeling barks and one time to harvest
Taxus media	Branch, leaf, root	0.06-0.096	Rapid, after 4 years to be harvested for taxol	No	Harvest branches and leaves for many times, cycling

the younger seedlings can be transplanted to the field or supplied for the market. Culture conditions above are as follows: temperature, 24+2°C; sunlight intensity, 12 h and 1200l ux.

RESULTS AND DISCUSSION

Our experiments indicated that the *in vitro* response of 2 year branches of Taxus media were stronger than that of 1 year branches generally (Table 2), with an emergence bud percentage of 78%. Two year branches were selected as the explants for propagation of buds and good results were obtained (2.7 buds/explant), no rooted buds reaching a percentage of 86% of rooting and survival rate of transplanting getting to 90%. Related field experiments are being executed and documented now. Our experiments also demonstrated that use of 0.1% $HgCl_2$ for sterilization for 12 m could reach more than 90% effect of sterilization, but previous studies applied 0.2% $HgCl_2$ for sterilization of 10 m, gaining only 75% effect of sterilization. It has been thought extensively that Taxus materials are difficult to be sterilized (Gan *et al*., 1996), contaminated highly. Present results provided a simple and convenient protocol to reach satisfying effect, i.e., 12 m's sterilization and rinsing of six times by using sterilized water. Additionally, Taxus explants have a longer time to recover during *in vitro* culture. Generally speaking, the period of time is 4 weeks. We have observed such phenomenon. However, after 6 week's *in vitro* culture, the growth of Taxus explants accelerates, especially undergoing two times of subculture (4-6 weeks per time), the effect being very obvious, greatly cutting down the cycle time of rapid propagation (Somerville and Bonetta, 2001).

In summary, by selecting the *Taxus media* branches as explants, we have primarily established the following efficient technique of *in vitro* propagation of *Taxus media* for the purpose of improving eco-environment of Loess Plateau. The flowchart is as follows: recover and initiation of explants→bud propagation→subculture→rooting →transplanting in greenhouse →transplanting in fields or going to market. Using this technique, the cost was reduced to 90% as compared with cuttage propagation. In the case of *Taxus chinensis*, the surviving rate of cuttage only reached 64% (Gan *et al*., 1999). Such flowchart and *in vitro* propagation technology are of importance to large-scale eco-environmental construction of stopping tillage and recovering to forest (grass) on Loess Plateau.Related field experiments are on the way. Such technique also provided the local people with a possible way to get rid of poverty. Detailed data will be published in another paper. According to the updated literature, the investigation related to this study has not been reported at home and abroad.

Table 2: The *in vitro* responses of stems with different age in *Taxus media*

Age	Explants	Death	Buds	Callus	Pollution
One year	100	8	71	79	9
Two year	100	6	78	82	8

ACKNOWLEDGMENTS

Shao HB is grateful for the support from the Doctoral Initiation Foundation of Chongqing University of Post and Telecom(2005A-92), Doctoral Fund of Qingdao University of Science and Technology (QUST) (0022221), State Key Basic Research Development Program of China (G2000018605), Natural Science Foundation of Shandong Province and the Knowledge Innovation Engineering Project of the Chinese Academy of Sciences (KZCX01-6) and Taishan Scholar Construction Project of Shandong (JS200510036) Province.

REFERENCES

Chen, Y.Q., W.H. Zhu and W.Q. Wu *et al*., 2000. Induction of different species of *Taxus* and difference in taxol content. Chinese herbaceous Medicine, 31: 216-218.

Chen, Z.S. and Q. Wang 2001. Impact of polyethanol on growth and taxol production of the cultured cells of *Taxus chinensis*. Acta Bot. Boreal-Occident. Sin., 21: 259-261.

Chaves, M.M., J.M. Maroco and J.S. Pereira, 2003. Understanding plant responses to drought-from genes to the whole plant. Funct. Plant Biol., 30: 239-264

Chu, C.C., 2002. Contributions of Chinese Botanists to plant tissue culture in the 20th century. Acta Bot. Sin., 44: 1075-1084.

Dellapenna, D., 2001. Plant metabolic engineering. Plant Physiol., 125: 160-163.

Gan, F.Y., L.P. Peng and G.Z. Zheng, 1996. The metabolic regulation of taxol in cell culture of *Taxus yunnansis*. Acta Yuannan. Bot. Sin., 18: 451-453.

Gan, F.Y., Y.M. Shen and X.J. Hao, 1999. Progress in the study of *Taxus* biotechnology. Wuhan Bot. Res., 17: 65-74.

Gan, F.Y., Y.M. Shen and X.J. Hao, 2000. Advances in the study of *Taxol* biosynthesis. Biotechnol. Prog., 20: 52-56.

He, S.L., J.G. Zheng and X.F. Wang *et al*., 2002. Plant secondary metabolism: Functional regulation and its gene engineering. J. Applied Environ. Biol., 8: 558-563.

Ketchum, R.F.B. and D.M Gibson, 1996. Paclitaxol production in suspension cultures of *Taxus*. Plant Cell Tiss. Organ. Cul., 46: 9-17.

Passioura, J.B., 2002. Environmental biology and crop improvement. Funct. Plant Biol., 29: 537-546.

Somerville, C.R. and D. Bonetta, 2001. Plants as factories for technical materials. Plant Physiol., 125: 168-177.

Shao, H.B., 2001. Plant Tissue Culture and Rapid, Virus-free Propagation Technology, Zhu, D.W. (Ed.). Beijing. China Sci-Tech Press, pp: 123-127.

Shao, H.B., 2002. The Develepmental research of transgenic plant medicine. Guangxi Sci., 12: 41-46.

Shao, H.B., Z.S. Liang, M.A. Shao and B.C. Wang, 2005. Impacts of PEG-6000 pretreatment for barley (*Hordeum vulgare* L.) seeds on the effect of their mature embryo *in vitro* culture and primary investigation on its physiological mechanism. Colloids and Surfaces B: Biointerfaces, 41: 73-77.

Vasil, I.K., 2002. Turning-point article-the wanderings of a botanist. *In vitro* Cell. Dev. Biol-Plant., 38: 383-395.

Wang, W., Q. Shi and P. Zhu *et al*., 2002. Cloning, expression and identification of taxadiene synthase, a diterpene synthase from *Taxus chinensis*. Acta Bot. Sin., 44: 181-187.

Yu, L.J. and C.H. Zhang, 1999. An primary study on Taxol biosynthesis promoted by inhibitor. Res. Develop. Natl. Prod., 11: 6-10.

Zhao, J. and W.H. Zhu, 1998. Some Taxus plants and their comparative studies on the callus isozymes. Chinese Herbaceous Med., 29: 402-406.

Zhou, Z.Q., X.G. Mei and J.L. Chang *et al*., 2001. Separation and characterization of two taxol compounds from cell cultures of *Taxus chinensis*. Res. Develop. Natl. Prod., 13: 42-43.