SOMATIC EMBRYOGENESIS IN LOBLOLLY PINE (PINUS TAEDA L.)

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Abstract:--Factors affecting embryogenic culture initiation for loblolly pine have been determined, and a maturation medium that efficiently produces cotyledonary embryos has been developed. Basal medium, the combination of plant growth regulators, Phytagel™ concentration, potassium chloride, silver nitrate and myo-inositol showed significant effects on embryogenic culture initiation. For embryo maturation, polyethylene glycol and maltose synergistically promoted production of cotyledonary embryos. About a hundred cotyledonary embryos from a gram of embryogenic tissue can be potentially produced on this newly-developed maturation medium. The mature embryos had well-developed cotyledons and root cap.

Keywords: embryogenic culture, initiation, maltose, maturation, polyetthylene glycol, somatic embryos.

INTRODUCTION

Forest productivity needs to be raised to satisfy the increasing demands for wood worldwide. The conventional method for reforestation of conifer species is primarily artificial regeneration with seedlings or by natural regeneration. Tree improvement is based on selection, establishment of seed orchard, and progeny testing. The conventional method for genetic improvement is a very slow process because of long generation cycles (Gupta and Durzan 1991), and is limited in genetic gain due to the variation among sibling seeds. Large-scale production of control-pollinated seeds is very expensive. Asexual propagation has been recognized as having great potential for capturing genetic advantage in tree improvement programs (Hall 1980). In loblolly pine, asexual propagation can be accomplished by grafting, rooting of cuttings and tissue culture. Grafting is used only to propagate superior individuals in a limited quantity. Vegetative propagation by cuttings is problematic in most coniferous species, including loblolly pine, due to rooting difficulty. Tissue culture methods, such as somatic embryogenesis, provide the potential for rapidly multiplying valuable genotypes for reforestation and will help in the race to increase forest productivity (Gupta et al. 1993). Tissue culture methods may also interface with genetic engineering techniques to regenerate transgenic plants.

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Somatic embryogenesis has been very successful in several coniferous species, especially in Picea species, whereas Pinus species are highly recalcitrant to this technique and success has been limited (Tautorus et al. 1991). Successful somatic embryogenesis in loblolly pine will be highly significant for reforestation and tree improvement programs.

Loblolly pine (Pinus taeda L.) is the most important timber tree in the southern United States and may be the second most dominant plantation timber species in the world (Gupta and Durzan 1991). During the past 4 years, we have used an Arkansas source of loblolly pine to initiate embryogenic cell lines, which were then used to develop an embryo maturation medium. A relatively efficient embryo maturation protocol has been developed. We present here a summary of research results.

INITIATION OF EMBRYOGENIC CULTURE

In loblolly pine, the initiation of embryogenic cultures has been solely from immature zygotic embryos, and the initiation frequency has been low, generally less than 10% (Becwar et al. 1990; Li and Huang 1996). Several factors affecting the initiation frequency were identified, including genotype (Becwar et al. 1990), maturity of zygotic embryos (Gupta and Pullman 1991), initiation medium (Becwar et al. 1990), plant growth regulators (Becwar et al. 1988), and concentration of gelling agents (Becwar et al. 1995). Several other factors, which potentially promoted initiation of embryogenic culture, have been identified in our laboratory, including potassium chloride, silver nitrate, myo-inositol or their combination at various concentrations (Li and Huang 1996).

DCR_1 (Becwar et al. 1995) and BM_1 (Gupta and Pullman 1991) are two standard culture media to initiate embryogenic cultures in Pinus species. To determine which is better for initiation for an Arkansas source of loblolly pine, the culture medium was divided into three parts: basal medium including organic and inorganic nutrients, plant growth regulators (PGRs) and gelling agent. We determined superiority of each part in both culture media.

The BM_1 basal medium was superior to DCR_1, with less browning and higher extrusion and proliferation frequencies. However, combination of PGRs used in DCR_1 standard medium showed better initiation results than those used in BM_1 standard medium. Phytagel level at 2 g/L, which is commonly used in tissue culture medium, achieved better initiation results than that at 1 g/L. Although 4 g/L Phytagel also showed promising results, a difficulty with further maintenance was observed later at this concentration. Therefore, 2 g/L Phytagel recommended for initiation.

This study showed that both standard initiation media were not optimum for the selected loblolly pine genotypes. Since each medium has its advantages and drawbacks, they need to be further improved or should be employed with selection. Our study in exploring factors affecting initiation may also provide important information to develop more effective initiation media for other Pinus species.

MATURATION OF SOMATIC EMBRYOS

Maturation of somatic embryos is a very critical step in somatic embryogenesis of conifers. Except for Monterey pine (Aitken-Christie et al. 1994; Smith et al. 1994), Pinus species are highly recalcitrant to embryo maturation in comparison to Picea species (Tautorus et al. 1991). Although a total of ten U.S. patents related to somatic embryogenesis in loblolly pine have been claimed, embryo maturation is still very challenging and can not be consistently achieved. Abscisic acid (ABA) has been recognized as an essential component in maturation media to induce cotyledonary (bearing well-developed cotyledons) embryos in conifers (Gupta and Pullman, 1991; Tautorus et al. 1991; Uddin 1993). A high ABA concentration was generally used in Pinus species (Li et al. 1997; Becwar et al. 1995; Gupta and Pullman, 1991). However, in our genotypes, cotyledonary embryos were rarely induced with BM4 medium (Gupta and Pullman, 1991), which contains up to 100 mg/L ABA (Li et al. 1997).

In our laboratory, we have identified two other factors that significantly affect embryo maturation in loblolly pine. One is osmotic potential and the other is the type of carbohydrate. The osmotic potential provided by polyethylene glycol (PEG) plays an important role in somatic embryo maturation (Li et al. 1997). Without PEG, embryo maturation frequently failed to occur on a medium containing sucrose. PEG combined with sucrose increased maturation frequency by at least tenfold. To induce cotyledonary embryos, a high concentration of maltose has been used to replace sucrose as a carbohydrate source and osmoticum (Uddin 1993; Becwar et al. 1995). However, we did not observe that maltose itself could induce a highly efficient production of cotyledonary embryos. Maltose and PEG did act synergistically to promote embryo maturation. Embryo maturation efficiency can be enhanced to about a hundred cotyledonary embryos based on one gram of embryogenic tissue at the proliferating stage when both polyethylene glycol and maltose were present in the maturation medium. When sucrose, maltose and PEG were present in the maturation medium, the maturation frequency was lower than PEG combined with maltose. Further, the optimum concentration of PEG and maltose in the culture medium was determined. We generally recommend that about 6% PEG and 4% maltose should be included in the maturation medium to achieve efficient embryo maturation (Li et al. unpublished data).

Maltose as a carbohydrate source also showed its superiority to sucrose in terms of morphology of cotyledonary embryos. The embryos produced from medium with maltose replacing sucrose generally were longer and had well-defined root caps. Therefore, maltose may be a more favorable carbohydrate than sucrose for embryo maturation, while polyethylene glycol is preferred over additional maltose as an osmoticum.

Currently, protocols efficiently inducing cotyledonary embryos in loblolly pine are still rare and not reliable for most genotypes. Here an alternative embryo maturation protocol is provided for loblolly pine. The basic strategy used in this study may also be employed for further improvement of embryo maturation medium, which hopefully can be used for more genotypes.

GERMINATION OF COTYLEDONARY EMBRYOS

Although embryo germination has been achieved in our laboratory, germination frequency was not high and germinated embryos showed poor vigor for continuous growth. Plant establishment has not been achieved.

Previous research has indicated that partial drying or desiccation treatment enhanced germination frequency in white spruce (Attree and Fowke 1993). We found that most cotyledonary embryos failed to tolerate desiccation treatment, but over 90% did show viability following partial drying (water loss about 50%). Most surviving embryos showed elongation of cotyledons and hypocotyl, and some progressed to epicotyl growth. However, only limited embryos showed radicle emergence, and some did not elongate further after initial radicle emergence.

Several reasons may contribute to the failure of plant establishment. First, the quality of somatic embryos should be considered. Gupta and Pullman (1993) suggested that a lower concentration of ABA should be used at the later embryo development stage than at the early stage. However, other research results showed that plantlet establishment was achievable with steady ABA concentration during embryo maturation. We did not use stepwise-adjusted ABA concentration during maturation in this study, but some deleterious effects of medium with a high ABA level was evident on the further development of early cotyledonary embryos, such as swelling of the hypocotyl region and slowdown of cotyledon elongation. Generally, zygotic embryos at the cotyledonary stage contain a very low level of ABA (Kapik et al. 1995). Further studies need to determine whether maturation medium with a high ABA level affects the quality of somatic embryos and then the germination. Second, germination conditions may also impact the plantlet establishment, including culture medium, temperature, light, and humidity. Information regarding optimum germination conditions is lacking for Pinus species.

CONCLUSIONS

For initiation, both standard media for embryogenic culture initiation were not optimum for an Arkansas source of loblolly pine. To modify the formulations, basal medium and PGR combination and level should be considered. Lower concentration of Phytagel did not promote initiation. PEG as an osmoticum played an very important role in embryo maturation. Maltose was a better source of carbohydrate than sucrose in embryo maturation. And most importantly, PEG and maltose acted synergistically to enhance embryo maturation efficiency in loblolly pine. However, it is still difficult to germinate cotyledonary embryos and to establish vigorously growing plants.

LITERATURE CITED

Aitken-Christie, J., K. Gough, D. Maddocks, M. Sigley, F. Burger, and P.C.S. Carter. 1994.

Towards commercialization of conifer embryogenesis. In: Proc. Second International Symposium - Applications of Biotechnology to Tree Culture, Protection, and Utilization. Bloomington, MN, p181-190.

Attree, S.M., and Fowke, L.C. 1993. Embryogeny of gymnosperms: Advances in synthetic seed technology of conifers. Plant Cell, Tiss. Org. Cult. 35:1-35.

Becwar, M.R., E.E. Chesick, L.W. Handley, and M.R. Rutter. 1995. Method for regeneration of coniferous plants by somatic embryogenesis. In: U.S. patent no. 5,413,930.

Becwar, M.R., R. Nagmani, and S.R. Wann. 1990. Initiation of embryogenic cultures and somatic embryo development in loblolly pine (Pinus taeda). Can. J. For. Res. 20:810-817.

Becwar, M.R., S.R. Wann, M.A. Johnson, S.A. Verhagen, R.P. Feirer, and R. Nagmani. 1988. Development and characterization of in vitro embryogenic systems in conifers. In: Ahuja, M.R. (ed) Somatic cell genetics of woody plants. Kluwer, Dordecht Boston London, p1-18.

Gupta, P.K., and D.J. Durzan. 1991. Loblolly pine (Pinus taeda L.). In: Biotechnology in agriculture and forestry. Vol 16. Trees HI Ed. Bajaj, Y.P.S. Berlin: Springer-Velag; p383-407.

Gupta, P.K., and G.S. Pullman. 1991. Method for reproducing coniferous plants by somatic embryogenesis using abscisic acid and somatic potential variation. In: U.S. patent no. 5,036,007.

Gupta, P.K., and G.S. Pullman. 1993. Method for reproducing coniferous plants by somatic embryogenesis using stepwise hormone adjustment. In: U.S. patent no. 5,236,841.

Gupta, P.K., G.S. Pullman, R. Timmis, M. Kretinger, W.C. Carlson, J. Grob, and E. Welty. 1993. Forestry in the 21st century - The biotechnology of somatic embryogenesis. Bio/Technology 11:454-459.

Hall, K. 1980. Biology and genetics. In: Proceedings of conference in paper science and technology - the cutting edge. Inst. Pap. Chem. Appleton, WI, p15-17.

Kapik, R.H., R.J. Dinus, and J.F.D. Dean. 1995. Abscisic acid and zygotic embryogenesis in Pinus taeda. Tree Physiol. 15:485-490.

Li, X.Y., and F.H. Huang. 1996. Induction of somatic embryogenesis in loblolly pine (Pinus taeda L.). In Vitro Cell. Dev. Biol. - Plant. 32:129-135.

Li, X.Y., F.H. Huang, and E.E. Gbur. 1997. Polyethylene glycol-promoted development of somatic embryos in loblolly pine (Pinus taeda L.). In Vitro Cell. Dev. Biol. - Plant. In Press.

Smith, D.R., C. Walter, A. Warr, C.L. Hargreaves, and L.J. Grace. 1994. Somatic embryogenesis joins the plantation forestry revolution in New Zealand. In: TAPPI Proc. 1994 Biological Science Symposium. Minneapolis, MN, p19-29.

Tautorus, T.E., L.C. Fowke, and D.I. Dunstan. 1991. Somatic embryogenesis in conifers. Can. J. Bot. 69:1873-1899.

Uddin, M.R. 1993. Somatic embryogenesis in gymnosperms. In: U.S. patent no. 5,187,092.

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