組織培養で増殖されたラジアータマツのアイソザイム分析による遺伝的安定性の検討

誌名	日本林學會誌 = Journal of the Japanese Forestry Society
ISSN	0021485X
著者名	石井,克明 Moran,G.F. Bell,J.C.
発行元	日本林學會
巻/号	69巻12号
掲載ページ	p. 487-488
発行年月	1987年12月

日林誌 69(12)'87 487～488

短　報

Genetic Stability Examination of Micropropagated Radiata Pine (*Pinus radiata*) Using Isozyme Assays*

Katsuaki Ishii,** Gavin F. Moran,*** J. Charlie Bell,*** and Vic Hartney***

I. Introduction

For clonal forestry and shortening the time of tree improvement, micropropagation is an important method. In the case of radiata pine, micropropagation from mature embryos was established and used for preliminary commecial production in New Zealand (*1, 4*). Three morphologically different types (waxy, wet, and translucent) of shoots were formed during tissue culture of radiata pine (*2*), but there has been no examination of genetic variation within clones. In this report, shoots from mature embryos using the micropropagation technique were checked for enzymatic band changes for the purpose of examining the genetic stability of propagules.

II. Material and Methods

Controlled-pollinated *Pinus radiata* D. Don seeds (Register No. 80087×80007 ; plus trees selected in New Zealand) were surface sterilized with 70% ethyl alcohol for 30 seconds and with 5% hydrogen peroxide for 5 min, and then rinsed five times in sterile water. Whole mature embryos were extracted and cultured on Lepoivre (LP) agar medium (*2*) containing 5 mg/*l* BAP (benzyl aminopurine) (LP1). After three weeks of culture, they were transplanted to hormone-free LP agar medium (LP0) and subcultured every three weeks on the same medium for six months. The average number of shoots from one embryo was 33±13. One-hundred eighty-seven shoots from 10 clones were analyzed for 20 enzymes.

An open-pollinated *P. radiata* seed (Register No. 12038) was surface sterilized as described above ; then the whole embryo was cultured on LP1 for three weeks and subcultured on the same medium every three weeks (*3*).

After the fifth, sixth, and seventh subcultures, each meristematic tissue was subcultured on LP 0, and shoots were induced. After eight subcultures of the meristematic tissue, a hormone-free treatment produced no shoots. The five shoots induced from each meristematic tissues after eight subcultures were checked for 20 enzymes.

Shoots and meristematic tissues were grown in a tissue-culture room at a day time temperature of 25°C, a night time temperature of 20°C, a photoperiod of 20 h, and a light intensity by fluorescent lamps of about 80 $\mu Em^{-2}s^{-1}$.

Isozyme analysis was conducted using starch gel-electrophoresis. The shoots and meristematic tissues were crushed with one to three drops of 0.1 M phosphate buffer (pH 7.0) containing 1 mg per ml of dithiothreitol, and the extracts were run in a horizontal electrophoresis system with 12.5% starch gels. The gel-buffer system and the assays were those used by Moran and other (*5*), and Moran and Bell(*6*). The twenty enzymes were alanine aminopeptidase(AAP), alcohol dehydrogenase(ADH), adenylate kinase(AK), acid phosphatase (AP), fumarase (FUM), glyceraldehyde-3-phosphate dehydrogenase (GAP), glutamate dehydrogenase (GDH), glycerate dehydrogenase (GLY), glutamate oxaloacetate transaminase (GOT), glucose-6-phosphate dehydrogenase (G6PD), isocitrate dehydrogenase (IDH), leucine amino peptidase (LAP), malate dehydrogenase (MDH), menadione reductase (MR), peroxidase (PER), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucose isomerase (PGI), phosphoglucose mutase (PGM), shikimic acid dehydrogenase (SDH), and triose phosphate isomerase (TPI).

III. Results and Discussion

The ten clones are monomorphic at the following loci (*5, 6*): AAP-1, 2, ADH, AK-1, 2, FUM, GDH, GLY, GOT-1, 2, 3, 4, 5, LAP-1, 2, MDH-1, 2, 3, 4, 6PGD-1, 2, PGI-1, and PGM. TPI had no banding. Band patterns of some enzymes were polymorphic resulting from clonal differences. In the case of SDH-1, all the shoots of seven clones were phenotypes of Middle Middle (MM), and other shoots of three clones were phenotypes of Middle Slow (MS). In the case of PER-3 all the shoots derived from six clones were phenotypes of MM, and

* 石井克明・カビン F. モラン・J. チャーリー ベル・ヴィック ハートニー：組織培養で増殖されたラジアータマツのアイソザイム分析による遺伝的安定性の検討

** For. and Forest Prod. Res. Inst., Ibaraki 305 林業試験場

*** Div. of Forest Res., CSIRO, Canberra 2600, Australia

Table 1. Detected phenotypic band changes of micropropagated shoots from ten clones of *Pinus radiata*

Enzymes	MS	M	FM	F	Missing	Total
GAP		164	23			187
G6PD		181	1	5		187
AP	5	182				187
AAP-2		182			5	187
MR-3		175			12	187

MS, middle and slow bands; M, middle band; FM, fast and middle bands; F, fast band

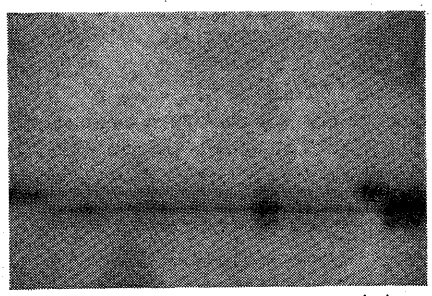

Fig. 1. Isocitrate dehydrogenase (IDH) isozyme of callus and differentiated shoots from callus of *Pinus radiata*

other shoots from four clones were phenotypes of MS. In the case of PGI-2, a S band appeared in five clones out of ten. Some changes occurring in band patterns other than these clonal variations are listed in Table 1. Although we do not know the exact reason for these phenotypical band changes, part of them seem to be the results of developmental and/or physiological states of the shoots or experimental conditions because we used the crude extracts of the young shoots which contain many phenolic substances that might affect the enzymatic detection. We did not detect any missing bands or new bands in single-band enzymes.

In the callus-derived shoots, there were little isozyme variations among the shoots which were cultured for different periods on the medium containing BAP, but there was apparent genetic changes in isozyme loci of IDH in the three cultures of callus (Fig. 1). S band of PGH-2 which suggests the genetic change during the culture also appeared in one of the callus.

We examined 42 loci of 19 enzymes for 187 shoots, and detected the band changes listed in Table 1. Therefore, we can calculate the rate of band changes (not necessarily genetic changes) as $(23+1+5+5+5+12)/(42\times187)=51/7,854=0.6\%$. Also, we examined 42 loci of 19 enzymes for three calluses and detected band changes of 2 loci. Therefore, we can calculate the rate of band change of callus as $2/(42\times3)=1.6\%$. These low rates of band changes suggest the possibility of genetic stability during both the shoot and callus culture of *Pinus radiata* at least for up to six months.

There is much work in progress regarding the production of mutations by using callus culture in crop species but not in tree species. Although the sample size is very small, and point mutation at the single-gene level, instead of chromosome level change, is detected mainly in isozyme analysis, we detected isozyme changes in the callus of *P. radiata*. However, the frequency of change is very small, and these newly-detected isozyme patterns already were present in different clones.

Literature cited

(1) Aitken-Christies, J.: Micropropagation of *Pinus radiata*. The Plant Propagator **30**: 9~11, 1984 a

(2) ——— and Thorpe, T. A.: Clonal propagation of gymnosperms. In: Cell culture the somatic cell genetics of plants, Vol. 1 (Vasil, I. K., ed.), pp. 82~95, Academic Press, New York, 1984 b

(3) ———: A subculturable meristematic callus system for shoot formation in radiata pine. Int. conifer tissue culture work group 3rd meet abstr, 18, 1985

(4) Gleed, J.: The commercial propagation of radiata pine by tissue culture methods: Some aspects and considerations. *ibid.* 5, 1985

(5) Moran, G. F., Bell, J. C. and Matheson, A. C.: The genetic structure and levels of inbreeding in a *Pinus radiata* D. Don seed orchard. Silvae Genet **29**, 190~193, 1980

(6) ——— and ———: The origin and genetic diversity of *Pinus radiata* in Australia. Theor. Appl. Genet. **73**: 616~622, 1987

(Received January 24, 1987)