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RAPD Marker를 이용한 택솔 고함유 울릉도 자생주목의 탐색[*1]

최명석[*2] · 양재경[*2] · 문현식[*2]

Characterization of Taxol High-Containing Ullung Islands Yew(*Taxus cuspidata* var. *latifolia)* using RAPD Markers[*1]

Myung-Suk Choi[*2], Jae-Kyung Yang[*2] and Hyun-Shik Moon[*2]

ABSTRACT

항암제 택솔을 고함유하는 울릉도 주목을 탐색하기 위한 marker 개발을 위해 RAPD 분석을 행하였다. 사용된 20종의 10-mer primer 중 10종의 operon primer는 4지역의 한국산주목 모두에서 polymorphism이 관찰되었다. 각 primer 마다 4개이상의 밴드가 확인되었고, 5개 primer에서 각 지역간 특징적인 밴드가 관찰되었다. Primer들간에 나타난 polymorphism을 cluster 분석한 결과 울릉도주목은 타 지역에 비해 유사도가 멀게 나타났다. 울릉도주목의 형태조사 결과 타지역 주목에 비해 잎의 길이가 길고, 너비가 넓으며, 건물중이 무겁게 나타났다. RAPD 분석 marker는 택솔 고함유 울릉도 주목의 선발과 동시에 우수 germplasm 관리에 유용한 도구가 될 수 있을 것이다.

Keywords: *Korean native yew (Taxus cuspidata), taxol, polymorphism, RAPD marker, and Ullung Islands*

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[*2] 경상대학교 농과대학 산림과학부 Faculty of Forest Science, Gyeongsang Nat'l Univ., Jinju 660-771, Korea.

I. Introduction

Phenotyphic identification of breeding lines and cultivars is a critical activity for breeding program. It allows them control the propagation and marketing of their novel germplasm, as well as perform quality control of their products(Hu and Quiros 1991).

There is an urgent need for the development of genetic markers in plant species for application in gene resource management and for genetic improvement of isoenzyme, restriction fragment length polymorphism(RFLPs), and random amplified polymorphic DNA(RAPDs)(Gocmen et al. 1993). Isoenzymes are excellent genetic markers and have been successfully used to detect applications in plant species. However, small number of isozyme markers has been a limiting factor. RFLP would be useful markers to develop, but because large F1 families do not exist to determine the inheritance of markers, it would take many years to develop these marker(Mulcahy et al., 1993). RAPD have generated great interest among plant geneticists because these fragments are very useful for constructing genetic linkage maps(Williams et al., 1990), tagging chromosomes(Martin et al. 1991). Additionally, RAPD methodology is technically simple, can be performed quickly, requires only small amounts of DNA, and involves no radioactivity(Munthali et al. 1992; Xu et al. 1993). In plant the technique has been successfully used for linkage map studies in *Prunus*(Levi et al. 1993), and also cultivar identification in broccoli(Hu and Quiros 1991).

Taxol, a diterpene taxane, originally isolated from Pacific yew bark is currently considered the most promising chemotherapeutic agent for the treatment of ovarian cancer(Wani et al., 1971) It has a unique mode of action on the microtubulin protein responsible for the formation of the spindle during cell division. However, the limited supply of this compound hinders the treatment of patients with ovarian and other cancer. However, *Taxus* spp. is slow growing and the removal of the bark results in death of the tree. Among alternatively supply program of taxol, uses of needle and stem sources which contained much higher taxol concentrations most practical in a short run. Choi et al. (1995) reported that needles of Ullung Island and Cheju Island yews in Korea of tested location as a renewable sources contained high level of taxol. The needles on Ullung Island yew contained relatively high concentrations of 0.0545%. There results suggest that foliage from yew trees growing in their natural

habitats on Ullung Island may provide a renewable sources of taxol. In this investigation we reported to determine of RAPD markers for characterization of Ullung Islands yew among native Korean yew trees and to the genetic variation of among locations of native Korean yew.

II. Materials and methods

1. Plant material

Twigs (average diameter, 2-4 cm) at 1.5 m height were collected from yew trees in their habitats on Ullung Island (*T. cuspidata* var. *latifolia*), and Mt. Jiri, Mt. Sobaek, and Cheju Islands (*Taxus cuspidata* Sieb et Zucc). Taxonomic identification was verified by Dr Byung-Yun Sun(Director of Herbarium, Chonbuk National University, Chonju, Korea) and Voucher specimens are deposited in the Herbarium. Five samples from each locations were collected, mixed, and then stored at -70℃ until analysis.

2. Extraction and amplification of DNA

DNA was extracted using the method described by Murray and Thomson(1980) with the following modifications. 0.5 g of mixed five needle tissue from each location were mixed, freeze-dried, and macerated with a homogenizer at room temperature in 400 μl of CTAB buffer(2% CTAB, 100 mM Tris- HCl pH 8.0, 1.4 M NaCl, and 1% PVP). Then sample was vortexed for 5 seconds. Supernatant(350 μl) was transferred to a fresh Eppendorf tube. 4 μl of RNase was added and the supernatant was incubated at 37°C for 30 min. 350 μl isopropanol was used to precipitate the DNA. After centrifugation, the pallet was dissolved in 100 μl sterile distilled water. One μl of this sample was used for a 25 μl volume PCR reaction.

3. RAPD analysis

Ten commercial 10-mer primers(Operon Tech. Alameda, USA) were used for PCR amplification(Table 1). Amplification reactions were performed in reaction mixture(1X reaction buffer, 0.2 μM of dNTPs, 1 μM primer, 0.5 unit of Taq polymerase(Promega), and 25 ng of genomic DNA in sterile distilled water). The final volume for each amplification reaction was 25 μl. The PCR machine(Perkin Elmer Cetus) was programmed for 50 cycles at 94°C for 30 sec., 35°C for 30 sec., and 72°C for 90 sec. Denaturing, annealing and primer

Table 1. The primer code and sequences tested in the study

No. of Primer	Sequence (5′ to 3′)	M.W.
B 10	CTGCTGGGAC	3035
B 11	GTAGACCCGT	3019
B 12	CCTTGACGCA	2979
B 13	TTCCCCCGCT	2906
B 14	TCCGCTCTGG	2986
B 15	GGAGGGTGTT	3130
B 16	TTTGCCCGGA	3010
B 17	AGGGAACGAG	3117
B 18	CCACAGCAGT	2988
B 19	ACCCCCGAAG	2973
B 20	GGACCCTTAG	2979

extension, and final cycle were at 94°C for 3 min., 35°C for 3 min, and 72°C for 3 min., respectively. On completion, 10 μl of each sample was loaded onto a 2.0% polyacrylamide gel and run in 1X TBE buffer at 100 V/cm for 3 hr. DNA molecular maker 1 kb DNA ladder was used as moleculor weight marker. The gel was stained with 10 ppm of ethidium bromide solution for 30 min, destained with tap water for 10 min and photographed under UV light with Polaroid film 667. This electrogram was used for the analysis of the amplification products. Each amplification product was identified based on its size in base pairs following the primer used in the reaction

4. Data analysis

A pairwise different maker among locations was determined with the microcomputor program.

5. Characterization of needle morphology

For the characterization of needles, 5 cm shoots were cut in breast height of collected yew tree and then we counted needle number and measured, width, and dry weight.

III. Results and Discussion

The profiles of the amplified products

from Ullung Islands and Korean native yews were compared each other for identification of location specific markers. Ten Operon primers were randomly selected to test the RAPD markers produced in the Ullung Islands and other native Korean yews. All of the primers produced above 4 bands in each location, of the ten primers tested, five showed significantly different banding patterns among locations which can be used in identification of needle source characterization between locations. The size of bands in the profiles varied, depending on the primers and locations tested. In general, the size of amplified DNA fragments by ten primers ranged from about 500 to 2000 base pairs.

Primer B11 produced four bands, two of which were different banding profile polymorphic in location, and used to distinguish Ullung Island location from other locations(Figure 1A).

Primer B13 produced a few scorable bands in each location and four of them were observed with different banding profile among all locations(Figure 1B). Ullung Island revealed the defferent banding pattern while Mt. Sobaek and cheju Island location showed the same banding patterns but different from these of the former.

Primer B14 produced two to seven scorable bands in each location and four of them were observed with different banding profile among all locations(Figure 1C). Ullung Island and Mt. Sobaek revealed the defferent banding patterns while Mt. Jiri and cheju Island location showed the same banding patterns.

However, Primer B15 produced 4 bands in 4 location, all of which revealed same banding pattern in each location(Figure 1D).

With regard to these observations several factors must be taken into account when using RAPD markers to determine locational variations. It is important to find suitable primers which can produce additive banding patterns between each locations. Using RAPD marker described here, will be identify all markers that have been useful for mapping the yew genome. Reactions with primers regardless of GC content consistently produced above 5 amplified DNA fragments(above 5). However, polymorphisms in 60-70% GC content was observed UBC primers as well as Operon Tech. primers. Different patterns of PCR amplification products will be resulted from deletions or insertions in the amplified regions or base changes altering primer binding sites(Valles et al. 1993).

The pairwise distance matrix among all the location computed by the Basic program

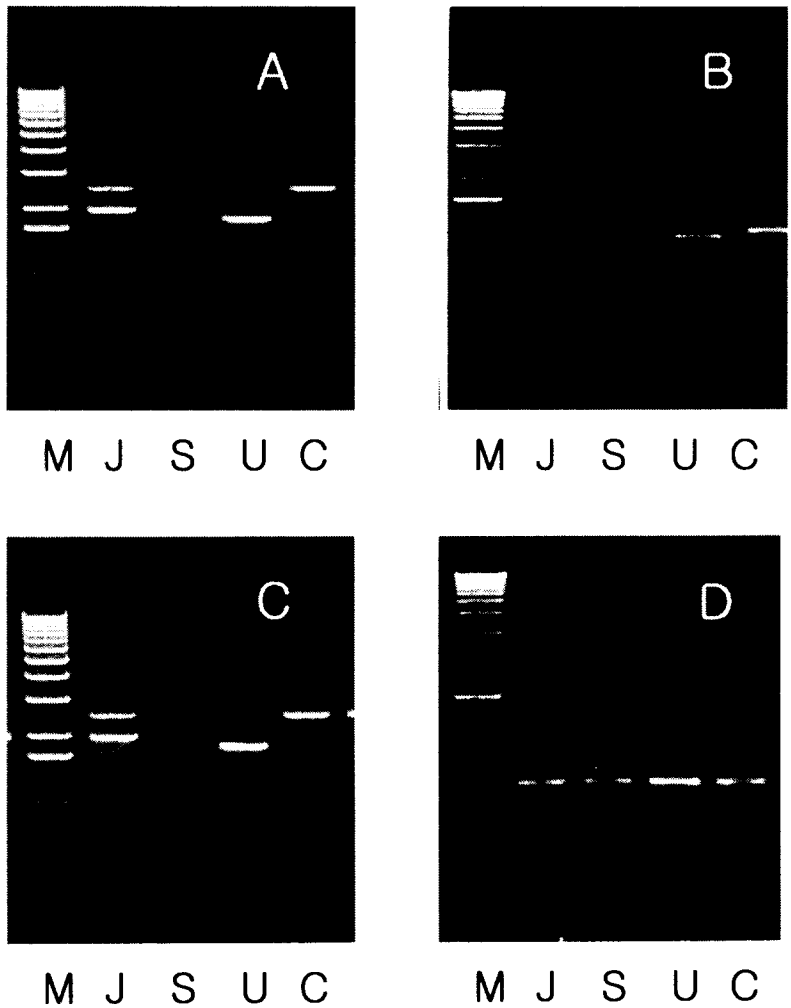

Figure 1. Amplication of Korean native yews. Genomic DNA samples were amplified from four location using 4 primers of arbitrary nucleotide sequence (Materials and Methods). Amplication products were resolved by electrophoresis in a 1.4% agarose gel which was stained with ethidium bromide and photographed. Lane M to C represented M(molecular weight markers of lamda DNA), J(Mt. Jiri), S(Mt. Sobaek), U(Ullung Island) and C(Cheju Island yew) DNA, respectively. A, B, C and D are B11, B13, B14 and B15, amplified primer, respectively.

could be used quantify the distances among them(Figure 2). These calculators were based on the presence of specific RAPD markers among locations. The Korean native yews among locations were readily separated into location groups. The average distance between Mt. Jiri and Cheju Island was 0.48, and between those of two location and Mt. Sobaek yew was 0.56, and above three locations and Ullung Island was 0.62. That is, a short distance was observed between Mt. Jiri and Cheju Island, and the greatest distance found between three location yews(Mt. Jiri, Cheju Island and Mt. Sobaek) and Ullung Islands.

Table 2 shows needle morphology of Korean native yews. Morphology among Korean native yews was varied in length, width, and dry weight of needle. Variation of needle morphology also observed within location, between individual trees. The small differences of needle morphology was also found in other domestic location(Mt. Sobaek and Mt. Jiri). In particular, the width as well as dry weight of Ullung Island needle was bigger and broader than those of other locations. Therefore, differences between needle morphology and RAPD markers have a correlation. The results presented is reliable and will be repetitive.

Differences of polymorphism among 4 locations may be due to geographic isolation and consequently, differences in environmental factors. Also, Ullung Island yew (*T. cuspidata* var. *latifolia*) which showed biggest difference from other locations is one variety of native Korean yew(var. *latifolia* means broad morphology of yew needle). The explanations for these appearance may be depletion of opportunities of out crossing by pollen, and migration of parent lines.

It could be also assumed that polymorphisms of Korean native yews and variations for taxane content caused by genetic parameter. The Pacific yew genetic study, which has been supported by the National Cancer Institute, was also reported at all levels of genetic variation on taxol contents.

The large range of variation among individuals indicates an opportunity for improvement based on selection and cloning. The extensive polymorphism present in the *Taxus* and speed of the techniques makes it feasible to use the RAPD techniques as an efficient tool for germplasm analysis and characterization of yews. *Taxus* spp. in Korea peninsula inflowed to Ullung Island and Cheju Island yew. Intensive nersery cultures provides the most rapid scale up of

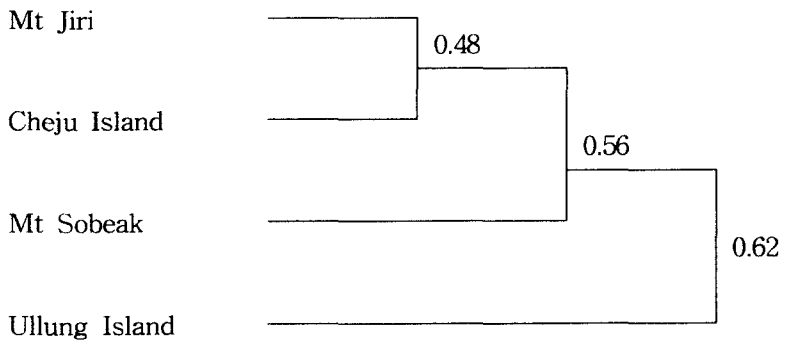

Figure 2. Dendrogram of cluster analysis of Ullung Islands yew and other native Korean yews.

Table 2. Needle morphology of native Korean yews

Species/ Location	Lengtha of needle (cm)	Width of needle (cm)	Dry wt per needle (mg)
***T. cuspidata* Sieb et Zucc**			
Mt. Jiri	1.82±0.17b	0.28±0.03	6.40
Mt. Sobaek	2.00±0.11	0.29±0.02	7.00
Cheju Island	1.92±0.13	0.27±0.10	7.09
T. cuspidata* var. *latifolia			
Ullung Island	2.63±0.22	0.31±0.02	11.30

[a] All samples measured needle number in 5 cm shoot of 5 trees per locations.

[b] The values represents mean±standard deviation.

production of acceptable biomass in the shortest time possible. Application of RAPD markers could be helful on breeding as well as conservation of Ullung Island and Cheju Island yews which contain high amount of taxol.

IV. References

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