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Analysis of *Populus* cpDNA by Restriction Fragment Length Polymorphism(RFLP) Technique[1]

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RFLP技法을 利用한 포플러 葉綠體 DNA의 分析[1]

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ABSTRACT

In woody species with a long life span, the studies on inheritance of any trait may be very time consuming and laborious. Chloroplast DNA(cpDNA) has been a valuable tool in such studies since it has several unique features such as limited genome size and cytoplasmic inheritance.

In the present study, cpDNAs from five different species of *Populus*(*P. alba*, *P. glandulosa*, *P. alba*×*P. glandulosa*, *P. davidiana*, and *P. nigra*), and *Nicotiana tabacum* were compared with regard to restriction fragment length polymophism. The results showed that cpDNAs among the species were very conserved, although some polymorphisms were observed when the DNAs were digested with restriction enzyme *Eco*RI or *Kpn*I. The other enzymes(*Bgl* II, and *Pst*I) tested produced identical restriction fragmentation pattern among the species. However, cpDNAs from all the five *Populus* species showed different restriction fragmentation pattern from that of tobacco with the four restriction enzymes tested. Southern hybridization with tobacco *rbc*L gene fragment as a probe also produced identical pattern among *Populus* species. The results indicate that cpDNAs in the genus are very well conserved during evolution.

要 約

生長期間이 긴 林木의 경우 어느 形質의 遺傳樣式을 究明한다는 것은 상당한 時間과 努力이 필요하다. 葉綠體의 DNA는 그 크기가 비교적 작고 細胞質 遺傳現象을 보이기 때문에 林木을 對象으로 하는 硏究에 適切할 것으로 생각된다. 본 硏究에서는 포플러 5개 樹種의 葉綠體 DNA를 4가지의 制限酵素로 處理하여 切斷된 DNA의 크기를 樹種間 및 比較 植物인 담배와 比較하였다. 그 결과 포플러의 葉綠體 DNA는 서로 아주 類似하여 사용된 2가지의 制限酵素(*Bgl* II 및 *Pst*I)에서는 種間에 차이를 전혀 볼 수 없었으며 다른 두 酵素(*Eco*RI 및 *Kpn*I)에서도 비슷하나 약간의 差異가 觀察되었을 뿐이었다. 그러나 比較 植物로 사용한 담배와는 전혀 다른 切斷 pattern을 보이고 있었다. 담배 葉綠體의 *rbc*L 遺傳子를 probe으로 한 DNA-DNA 交雜 결과 역시 같은 傾向을 보이고 있었다. 따라서 포플러의 葉綠體 DNA는 進化 과정중 비교적 安定이 되어 있는 것으로 나타났다.

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Introduction

Populus species are widely distributed throughout the northern hemisphere. The Genus *Populus* consists of 5 subgenera : Leuce, Eupopulus, Turanga, Tacamahaca, and Aigeiros(Bogdanov 1965). It is known that most of the species can be crossed with each other. In addition to natural hybridization, there has been so much artificial hybridization between species and the hybrids have been distributed so widely that it is difficult to find a tree type of a pure species throughout the world(Wright 1976). For the identification of each species or hybrid, several techniques could be applied. Techniques such as isozyme analysis, restriction fragment length polymorphism(RFLP), and random amplified polymorphic DNA(RAPD) might be the examples.

Chloroplasts are cytoplasmic organells which contain their own DNAs. Chloroplasts DNA(cpDNA) has been studied intensively with various plant species. The cpDNA in most land plants is known to be circular and 140-170 kb in size. A typical cpDNA in land plant contains about 120 genes(Sugiura 1987). The complete nucleotide sequence of cpDNA has recently been published in tobacco(Shinozaki *et al*. 1986), rice(Hiratsuka *et al*. 1989), and liverwort(Ohyama 1986). Comparison of those works revealed that nucleotide sequence of cpDNA among land plants are very well conserved during evolution. The mode of its inheritance is also noteworthy. While cpDNA is known to be strictly maternally inherited in most plant taxa, in some other species it is either paternally or bisexually inherited(Corriveau and Coleman 1988). Most coniferous species examined so far have exhibited paternal inheritance (Neale et al. 1986, Wagner et al. 1989). The two unique features(i.e. sequence stability and cytoplasmic inheritance) make the cpDNA very attractive in phylogenetic studies as well as pedigree researches.

The purpose of the present study was to explore the possibility of identifying *Populus* species by cpDNA analysis.

Materials and methods

Plant materials :

Following species and hybrids of *Populus* and a *Nicotiana tabacum* were used in the experiment : *Populus davidiana* Dode, *P. glandulosa* Uyeki, *P. alba*×*P. glandulosa*, *P. alba* L., *P. nigra* var. *italica* Koehne, *P. maximowiczii* Henry, *P. nigra*× *P. maximowiczii*, and *Nicotiana tabacum* L.

Molecular technique :

Chloroplast DNAs were isolated by the method of Charbonnier *et al*. (1987). The extracted DNAs were quantified by Flourometer according to the protocol supplied by manufacturer(Hoefer Scientific Co. USA). Three hundred ng of DNA were digested with 20 units of restriction enzymes(*EcoR*I, *Kpn*I, *Pst*I, or *Bgl* II) overnight and electrophoresed on 0.8% agarose gel in TBE buffer(0.089M Tris, 0.089M boric acid, 0.02mM EDTA, pH 8.0). All these processes were done according to Ausubel *et al*. (1987). The gel was stained in ethidium bromide solution(final conc. 5 ng/ml) for 5 min and washed for 20 min in running tap water.

Southern hybridization : cpDNAs digested with restriction enzyme(s) were run on agarose gel and transferred to Nylon membrane by semi-capillary transfer method(Nakano *et al*. 1990). The probe for Southern hybridization was a fragment of *rbc*L gene from tobacco cloned into pTZR19 vector. It was a gift from Dr. K. Scott of Indiana University, USA. The probe was prepared using commercial DIG-labelling kit in which DIG-dUTP was added to dNTP mixture(Boehringer Manheim Co.). Prehybridization and hybridization were done according to the manual supplied by the manufacturer. Immunological detection using DIG-specific antibody was also done according to the manual.

Results and discussion

Restriction digestion and Southern hybridization

Restriction fragmentation patterns of cpDNA in both *Populus* spp. and *Nicotiana tabacum* are shown in Fig. 1-4. The fragmentation patterns appear to be

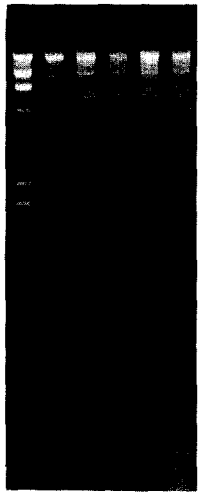

Fig. 1. Restriction digestion pattern of cpDNA from *Nicotiana tabacum* and *Populus* spp. Three hundred ng of cpDNA were digested with restriction enzyme *Pst*I.
Lanes 1 to 6 : DNA molecular marker (*Hin*d III cut lambda phage DNA), *N. tabacum*, *P. nigra*, *P. davidiana*, *P. alba*×*P. glandulosa*, and *P. alba*.

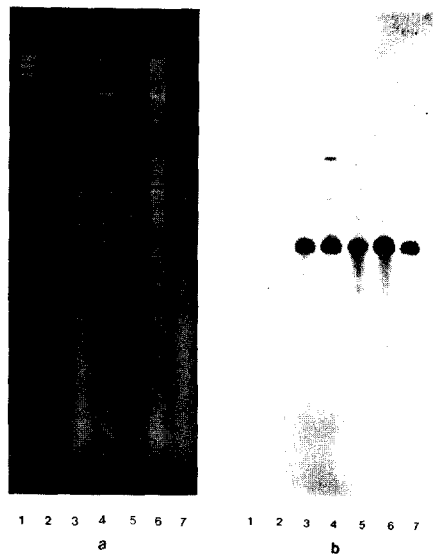

Fig. 2. Restriction digestion and Southern hybridization of cpDNA from *Populus* spp. and *N. tabacum* digested with *Eco*RI.
(a) Restriction digestion pattern of cpDNA from *Nicotiana tabacum* and *Populus* spp. Three hundred ng of cpDNA were digested with restriction enzyme *Eco*RI.
Arrow indicates the band showing polymorphism among species.
(b) Southern hybridization with tobacco *rbc*L gene probe. Lanes 1 to 7 : *Hin*dIII cut lambda phage DNA, *N. tabacum*, *P. nigra*, *P. davidiana*, *P. glandulosa*, *P. alba*×*P. glandulosa*, and *P. alba*.

similar among *Populus* species. The restriction enzyme *Pst*I digests produced identical fragmentation pattern among *Populus* species (Fig. 1). However, it is quite different from that of tobacco. The enzyme *Pst*I has been known to produce simple restriction fragmentation pattern (i.e. produces fewer bands than does any other enzymes) when used to cut cpDNA (Salts *et al.* 1984). In the case of *Populus*, the pattern also looks simpler than that produced by any other enzymes tested.

The restriction enzyme *Eco*RI digest reveals almost identical pattern among *Populus* species (Fig. 2-a). One exception was *P. nigra* that showed a different band (in size of around 2.4 Kb). However, Southern hybridization with tobacco *rbc*L gene as a probe again produced identical pattern among *Populus* species suggesting conserved gene order among *Populus* species (Fig. 2-b). As in the case of *Pst*I digestion, the difference between *Nicotiana* and *Populus* was far greater than that between *Populus* species.

The restriction fragmentation pattern produced by the enzymes *Bgl*II and *Kpn*I and subsequent Southern hybridization with tobacco *rbc*L probe showed almost the same results as the other enzymes mentioned above (Fig. 3 and 4). Chloroplast DNA in higher plants has been known to be very stable in evolutionary process (Palmer 1987). Comparisons between species in the same genus show few differences often confined to one or two restriction site polymorphisms. This has been demonstrated in *Medicago* (Rose *et al.* 1988), *Nicotiana* (Salts *et al.* 1984), *Solanum* (Kawagoe and Kikuta 1991), and *Picea* (White *et al.* 1993). In the present study, *Populus* species also showed the similar tendency as the other plant taxa mentioned above. However, a

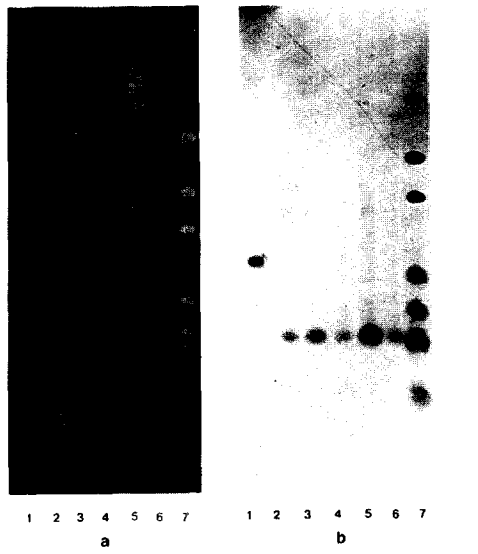

Fig. 3. Restriction digestion and Southern hybridization of cpDNA from *Populus* spp. and *N. tabacum* digested with *Bgl* II.
(a) Restriction digestion pattern of cpDNA from *Nicotiana tabacum* and *Populus* spp. Three hundred ng of cpDNA were digested with restriction enzyme *Bgl* II.
(b) Southern hybridization with tobacco *rbc*L gene probe. Lanes 1 to 7 : *N. tabacum*, *P. nigra*, *P. davidiana*, *P. glandulosa*, *P. alba*×*P. glandulosa*, *P. alba*, and pGEM DNA marker.

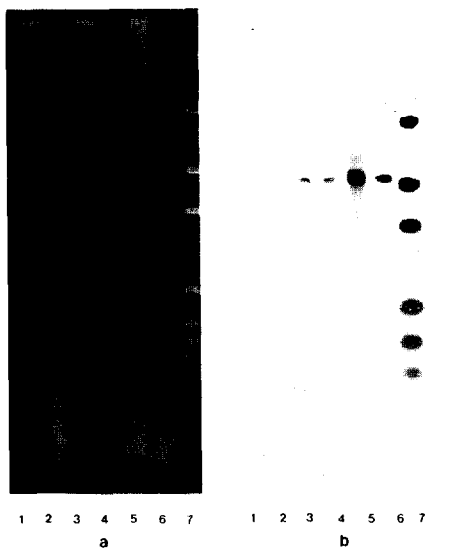

Fig. 4. Restriction digestion and Southern hybridization of cpDNA from *Populus* spp. and *N. tabacum* digested with *Kpn*I.
(a) Restriction digestion pattern of cpDNA from *Nicotiana tabacum* and *Populus* spp. Three hundred ng of cpDNA were digested with restriction enzyme *Kpn*I. Arrow indicates the DNA band showing polymorphism among species.
(b) Southern hybridization with tobacco *rbc*L gene probe. Lanes 1 to 7 : *N. tabacum*, *P. nigra*, *P. davidiana*, *P. glandulosa*, *P. alba*×*P. glandulosa*, *P. alba*, and pGEM DNA marker.

minor difference was observed between *P. alba* and other *Populus* species when the cpDNAs were digested with the enzyme *Kpn*I. It was a rather surprising result since *P. alba* and *P. alba*×*P. glandulosa* were supposed to have the same cpDNA. The difference may be due to the fact that the *P. alba* clone used for this study was not the parent of the hybrid (*P. alba*×*P. glandulosa*). It may be therefore possible to observe polymorphism in cpDNA in the species. Recently Rajora and Dancik (1992), studying with two *Populus* species (*P. deltoides* and *P. nigra*) and their F1 hybrid, observed that cpDNA of the species showed uniparental maternal inheritance. They used 13 different enzymes in combination with 4 different probes to detect polymorphisms between the two species. Therefore, the use of other gene probes such as *psb*A, *psb*B, and *atp*B may also be useful to illucidate the difference between the species in the genus.

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