開花結実が始まった。

2) カンランを台木とした場合：

カンラン：活着率は3%と低いものの、活着した個体の生育は順調である。

3) センダンを台木とした場合：

インドセンダン：活着率は 100%で、生育は良好である。

4) イスノキを台木とした場合：

シナマンサク：活着率は良好で、生育は順調である。

アメリカマンサク：活着率は良好で、生育は順調である。

5) ヤマザクラを台木とした場合：

カンヒザクラ：活着率は 90%以上で、成育は順調で、日陰にあるにもかかわらずよく開花している。

カンザクラ：活着率は 100%近くで、成育旺盛な個体は地際部が直径 30cm 以上に成長し、よく開花・結実している。

サトザクラ：活着率は90%以上で、生育は良好である。

カラミザクラ：活着率は100%近くで、成育旺盛で 2004 年に開花する個体もあった。

6) ウメを台木とした場合：

スモモ：活着率は約 70%で、生育は良好である。

7) モモを台木とした場合：

モモ(ハナモモ)：花芽がある部分では、施術後に開花も見られた。活着率は 90%以上で、生育は旺盛である。

8) イヌザンショウを台木とした場合：

ブドウザンショウ：活着率は10%程度で、生育は緩慢である。

9) タチバナを台木とした場合：

タチバナ：生育は良好で、2003 年から開花・結実が始まり、2004 年には 50 個前後の果実を着ける株もある。

シキキツ：生育は非常に良好で、2003 年から開花・結実が始まり、名前のとおり年数回開花し、結実している。

斑入りダイダイ：2004 年には開花・結実が始まった。

D．考察

1) ハマセンダンを台木とした場合：

キハダ、タイワンキハダ：現時点では、恒久的台木として使用可能かどうかは不明で、生育の経過を観察する必要がある。暫定措置としての活用には問題ないことが確認された。

ゴシュユ：現時点では、生育の経過を観察する必要がある。

ホンゴシュユ：春の接ぎ木では当年に開花結実し、その後の生育も良好で、今後暖地での栽培法として十分期待できる。ゴシュユの株分け繁殖の場合のような地下部のランナーによる小苗の叢生がないため、栽培地や個体の管理に好適だと考えられる。

2) カンランを台木とした場合：

カンラン：低い活着率の原因が冷蔵したことによるものか不明であり、熱帯・亜熱帯植物に対しての穂木の移動方法として冷蔵輸送は検討する必要がある。

3) センダンを台木とした場合：

インドセンダン：自根の個体と生育上の違いは判らない状況である。今後、成分についての検討が必要である。

4) イスノキを台木とした場合：

シナマンサク：園芸界でもイスノキを台木として利用しており、その面では問

題はない。

アメリカマンサク：生薬となった場合の成分についての検討が必要である。

5) ヤマザクラを台木とした場合：

カンヒザクラ、カンザクラ：両種とも生育も良好で、特に問題はないように思われる。

サトザクラ：現時点では、生育の経過を観察する必要がある。

カラミザクラ：現在までは良好な生育をしているが、開花結実期には至っておらず、今後の生育の経過を観察する必要がある。

6) ウメを台木とした場合：

スモモ：系統によっては台負けの傾向もあるようなので、今後の経過を観察する必要がある。

7) モモを台木とした場合：

モモ（ハナモモ）：花・葉を採取する場合には問題ないように思われる。桃仁生産の場合の系統選択にあたっては検討の必要性がある。

8) イヌザンショウを台木とした場合：

ブドウザンショウ：和歌山県では、フユザンショウまたはサンショウに接ぎ木しているが、緊急の場合の暫定措置としてイヌザンショウでも不可能ではない。もう少し、活着した穂木の今後の生育状況を観察する必要がある。

9) タチバナを台木とした場合：

タチバナ、シキキツ、斑入りダイダイの３種については、他の台木（カラタチ、ダイダイなど）との比較も必要であるものの、暖地でのタチバナの活用が可能であることが確認できた。

薬用植物の場合、利用部位の成分が、共台の場合と相違ないのか、検討の余地がある。

E．結論

接ぎ木による繁殖法は、従来は種々の制約（季節、管理方法など）があったものの、パラフィルムの使用により大幅に緩和され、技術の差も少なくなり活着率の向上にもなった。また、落葉樹の接ぎ木の多くは早春に行うことが通例となっていたが、夏期においても実施可能であることが確認できた。

台木と穂木の親和性については未知・不明なことが多く、実施して確認する必要がある。

活着率が高いということは、相互の組織の親和性が高いということも言えるわけで、今後の遺伝子組み換え体の創造に関するひとつのヒントを提示しているようにも思われる。

F．研究発表

1.論文発表

なし

2.学会発表

なし

G．知的所有権の取得状況

なし

H．参考文献

1)香月茂樹「熱帯動植物友の会会報」第93号(1997年4月):pp.11-13

2)中平幸助・染郷正孝：造園木の手引き つぎ木・とり木の実際、地球社、1973

3)大野正夫：図解 果樹の接木・挿木と

高接更新、博友社、1973
4)農耕と園芸・ガーデンライフ編：図解 植木のふやし方、誠文堂新光社、1972
5)飯島亮・安蒜俊比古：庭木と緑化樹 1 針葉樹・常緑高木、誠文堂新光社、1974
6)飯島亮・安蒜俊比古：庭木と緑化樹 2 落葉高木・低木類、誠文堂新光社、1974
7)安田勲：植木園芸ハンドブック、養賢堂、1973
9) 香月茂樹他「日本植物園協会誌」第37号(2003年):pp.103-111

厚生労働科学研究費補助金（ヒトゲノム・再生医療等研究事業）
分担研究報告書

遺伝子組み替え植物の栄養器官の系統的保存と野生遺伝子の導入の法の研究

分担研究者　佐竹　元吉　お茶の水女子大学生活環境研究センター　教授

昨年度、遺伝子組替え植物が環境に適合し、自然生態系を乱すことなく増植させるためには、植物の生殖様式と深く関わっていることを報告した。特に、維管束植物（シダ、裸子植物、種子植物）の生殖様式からこの問題点の解明をおこなった。今回は、栄養器官の増殖方法と野生遺伝子の導入方法に関して報告する。栄養器官の繁殖方法には、栽培した植物体の増殖方法と試験管内での増殖方法の二つがある。また、野生遺伝子の導入方法には、自然環境中での交配によう方法があり、この方法に関して雌の遺伝資源受容植物を野生植物花粉供給植物の周辺に植えて、開花させる試験を試みたので報告する。

A．研究目的：

栄養器官の繁殖方法は、広く知られている草本類で行われる根茎の株分け方法と樹木植物で行われる挿し木、接ぎ木、芽接ぎ方法などがある。草本植物の一部では葉挿しや不定芽による増殖方法もある。ミャンマーの山間部で行った増殖方法とその成績を報告する。導入した植物は、薬用植物と果樹植物を約１００種である。これらの増殖方法に関して３年間の実験結果を報告する。

B．試験方法と材料：

試験地：ゴールデントライアングルと呼ばれるケシの不法栽培地にケシの代替に薬用植物を植え込み，ケシ栽培をしている山岳民族・少数民族の人々のために営農出来るように指導しているばしょで行った．場所はミャンマー北部のカチン州，海抜 1,200m から 1,800mの地域である．ミャンマー政府との交換文書で，本研究のミャンマーでの研究展開が認められている．

試験材料：日本から導入した植物は表１に示す。

試験方法：

（１）森林局の職員とミャンマー北部のカチン州，海抜 1,200m から 1,800mの地域でケシ不法栽培地に薬用植物を導入するために、モデル研修薬草園を作り、そこでの研修に必要な薬用植物を導入した。

（２）野生薬用植物のカチン州での分布調査を行い、実験に利用可能な植物を発見したのでこれらの実験を開始した。

（3）野生モモの発見とモモの木の周辺に日本の優良3系統（オドロキ、カワナカジマ、紅錦香）を植え込んだ。

C．研究結果

ミャンマー連邦山間地域での導入植物の生育状況は表1、表2に示した。3年間の成育状況は移植苗で

（1）成長のよいものはナンテン、ユズ、ミカン、ブドウ、ミカン、チャノキクチナシ（100本　成育中)、イチョウ（大形の果実の出来るトウクロウは接ぎ穂として育成中)、ウメ（成育が良いが北部で大量生産地に植え込む)、カキ、イチジク（新緑　15株)、マルメロ、ホウノキ、ヒノキ、ミシマサイコ、ダイウイキョウ、モッコウ、チクセツニンジン（9本)、オウレンとセンキュウ及びキクは成育がやや良いが、更なる生産量を期待して北部へ移植する。ヤマノイモ、キキョウ、ツバキ、オオバク

（2）あまり良くないものにはオオツズラフジがある。この植物は更に北部の地域へ移植する。クリも成育が悪い。シャクヤクも北部へ移植する。

（3）ミャンマーの薬用植物

① ボケ　株分け、地元のもの、

② カギクルマバナルコユリ100株は根茎を切断して増殖中である。成育良好である。黄精（根茎）として利用可能である。

③ ミャンマーニンジンは学名が明確ではないが、ブータンのニンジンと類似している。根茎はチクセツニンジン状であるが、数節毎に肥大する特徴がある。

（4）台木の育成と現地で見つけだされた台木植物

① ミカン（早川早生）の苗木2500本を育成中であり、これらの接ぎ木用台木としてカラタチ苗も育成中である。

② ナシ類の台木となる植物が地元に成育している。播種試験中。

③ バラの増殖用に野生種を見つけだしたので、接ぎ木の台木用に育成中である。

④ 現地の古くからあるミカン類を発見したので、この種を育成して台木に栽培中である。

⑤ リンゴの野生種を見つけだしたので、台木としての育苗中である。

⑥ カシグリは栗の台木になるか検討中であるが、属が異なるので困難かもしれない。

（5）特別の栽培条件　チャノキはヤブキタ種及びトウチャ種を導入したが、増殖のため挿し木を黒い布の覆いのある場所で行っている。成育は良い。

今回の導入植物の中で栄養繁殖法を行っているものを表1に示す。ミャンマーの導入種の中で株分け又は根茎で増殖するものは①　ボケ（地元のもの花は深紅ト白色)、②はカギクルマバナルコユリは野生品で、100株を根茎を切断して増殖中である。成育良好である。黄精（根茎）として利用可能である。③　ミャン

マーニンジンも落葉樹林帯に野生しているもので、根茎を増殖中である。(写真１、２)

自生植物の花粉と日本の優良品種との交配：強い品種を作るために自生のモモの樹下に日本から持ち込んだ品種を植え付けて、虫媒にのる花粉の受け渡しさせる試みをおこなった。昨年、植え込んだ植物の生長のようす。(写真3-１０)

D．考察

遺伝子組み換え植物体を保存し、自然界との交配を防ぐためには、隔離も重要であるが、栄養繁殖方法の確立も重要な技術である。また、人工交配で新しい遺伝子を入れ込んだ植物体を作り出し、組み換え研究の材料に供給することも新しい試みと思われる。ミャンマーの奥地で多くの薬用植物を栽培し、適地を見つけだすことに成功したので、これらの情報を基に遺伝子組み換え分野活用されることを期待する。

表1 導入植物の中で栄養繁殖法を行っているもの

	生薬名	植物名	増殖方法	成育状況
1	オウレン	セリバオウレン	根茎の株分け、種子	良い
2	ガジュツ	ガジュツ	根茎の株分け	良い
3	カンゾウ	カンゾウ	株分け、種子	やや良
4	キクカ	キク	株分け、挿し穂	良い
5	ケイヒ	シナニッケイ	取り木、種子	良い
6	サンキライ	サンキライ	株分け	良い
7	サンシシ	クチナシ	種子、挿し木	良い 開花
8	サンショウ	アサクラザンショウ	接ぎ木、種子	良い 開花
9	シャクヤク	シャクヤク	株分け	良い 開花
10	シュクシャ	シュクシャ	株分け	良い
11	タイソウ	ナツメ	株分け	良い
12	チクセツニンジン	トチバニンジン	株分け	良い
13	ナンテンジツ	ナンテン	株分け	良い
14	ハンゲ	カラスビシャク	株分け	良い
15	ビャクジュツ	オケラ	株分け	良い
16	ボタンピ	ボタン	株分け	栽培中
17	ヤクチ	ヤクチ	株分け	良い
18	リョウキョウ	リョウキョウ	株分け	良い
19	センキュウ	センキュウ	株分け	良い

資料 ミャンマーの山岳地帯での種子繁殖の薬用植物の成育状況

(05年3月20日現在)

	生 薬 名	植 物 名	増殖方法	成育状況
1	ウイキョウ	ウイキョウ	種子	良い 結実
2	オウゴン	コガネバナ	種子	良い
3	オウバク	キハダ	種子	良い
4	カミツレ	カミツレ	種子	良い
5	キキョウ	キキョウ	種子	良い
6	ケイヒ	シナニッケイ	取り木、種子	良い
7	コウカ	ベニバナ	種子	良い 開花、結実
8	シコン	ムラサキ	種子	やや困難
9	シャゼンソウ	オオバコ	種子	良い
10	ソヨウ	シソ	種子	良い
11	ダイオウ	ダイオウ	種子	困難
12	トウキ	トウキ	種子	良い 芽繰り苗の植え付け
13	ヨクイニン	ハトムギ	種子	良い 結実

写真 1 野生植物のカギクルマバナルコユリ

（写真　1）野生植物のカギクルマバナルコユリ

（写真　2)野生植物のミャンマーのニンジン

(写真 3)モモの野生種の廻りにおしべの退化した3系統(オドロキ、カワナカジマ、紅錦香)を植え込み、原産地の遺伝子を入れ込む試み行っている。ミャンマー北部のセイロン地区、海抜1800m。2005. 2. 27.

(写真 4) 2003年の植え込み作業現場

（写真5） 自生のモモの木に日本の品種を植え付ける 2003年

（写真6） 桃の類でおしべが無く花粉が出来ない種類の苗 2003年

（写真7）接ぎ木苗

（写真8） 接ぎ木の方法

（写真9） メイミョウ第一試験地で薬用果樹モモの植え付け

（写真 10） 自生のモモの木に日本の品種を植え付ける。

桃の類でおしべが無く花粉が出来ない種類の苗

III. 研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
C. Long, N. Kakiuchi,A. Takahashi, K. Komatsu, S. Cai, M. Mikage	Phylogenetic analysis of the DNA sequence of the non-coding region of nuclear ribosomal DNA and chloroplast of *Ephedra* plants in China	*Planta Medica*	**70** (11)	1080-1084	2004
C. Long, N. Kakiuchi,G. Zhong, M. Mikage	Survey on resources of *Ephedra* plants in Xinjiang	*Biol. Pharm. Bull*	**28** (2)	285-288	2005
姉帯正樹，熊谷健夫，柴田敏郎	白止の調製法と化学的品質評価（第5報）保存中におけるフロクマリンの減少	*Natural Medicines*	**58**	209-213	2004
Y. Kaminaga, F. P. Sahin, H. Mizukami	Molecular cloning and characterization of a glucosyltransferase catalyzing glucosylation of curcumin in cultured *Catharanthus roseus* cells	*FEBS Letters*	**567**	197-202	2004
K. Yoshimatsu, H. Sudo, H. Kamada, F. Kiuchi, Y. Kikuchi, J. Sawada, K. Shimomura	Tropane alkaloid production and shoot regeneration in hairy and adventitious root cultures of *Duboisia myoporoides – D. leichhardtii* hybrid	*Biol. Pharm. Bull.*	**27** (8)	1261-1265	2004
S. Oguro, T. Akashi, S. Ayabe, H. Noguchi, I. Abe	Probing Biosynthesis of Plant Polyketides with Synthetic *N*-Acetyl- cysteamine Thioesters	*Biochem. Biophys. Res. Commun.*	325 (2)	561-567	2004
I. Abe, Y. Utsumi, S. Oguro, H. Morita, Y. Sano, H. Noguchi	A Plant Type III Polyketide Synthase that Produces Pentaketide Chromone	*J. Am. Chem. Soc.*	**127** (5)	1362-1363	2005

書籍（出版予定原稿）

「薬用植物　栽培と品質評価」　Part 11

ウイキョウ、オミナエシ、オオツヅラフジ、カラスビシャク、ヨロイグサ

IV. 研究成果の刊行物・別刷

Phylogenetic Analysis of the DNA Sequence of the Non-Coding Region of Nuclear Ribosomal DNA and Chloroplast of *Ephedra* Plants in China

Changfeng Long[1]
Nobuko Kakiuchi[1]
Akira Takahashi[2]
Katsuko Komatsu[3]
Shaoqing Cai[4]
Masayuki Mikage[1]

Original Paper

Abstract

Twenty-four *Ephedra* plants belonging to 8 species grown in the northern and western parts of China were phylogenetically analyzed for their non-coding DNA sequences, internal transcribed spacers (ITSs) of nuclear ribosomal DNA as well as *trnL* intron and intergenic spacers between *trnL* and *trnF* (*trnL/trnF*) of the chloroplast. Based on the ITS sequences, the 8 species could be divided into 3 groups: Group 1 (*Ephedra intermedia, E. sinica, E. przewalskii*), Group 2 (*E. equisetina, E. monosperma, E. gerardiana*), and Group 3 (*E. likiangensis, E. minuta*). The species classified into Group 1 grow mainly in the north, Group 3 in the south and Group 2 in the center, suggesting their genetic and geographic relationships. A specific primer set was designed to classify the 3 groups by routine PCR. Combined analysis of ITS and *trnL/trnF* differentiated the 8 *Ephedra* species.

Key words

Ephedra · Ephedraceae · nuclear ribosomal DNA · internal transcribed spacer (ITS) · chloroplast DNA · intron of *trnL* and intergenic spacer between *trnL* and *trnF* (*trnL/trnF*)

Abbreviations

ITS1:	internal transcribed spacer 1
ITS2:	internal transcribed spacer 2
trnL/trnF:	intron of *trnL* and intergenic spacer between *trnL* and *trnF*
EI:	*Ephedra intermedia*
ES:	*Ephedra sinica*
EP:	*Ephedra przewalskii*
EE:	*Ephedra equisetina*
EM:	*Ephedra monosperma*
EG:	*Ephedra gerardiana*
EL:	*Ephedra likiangensis*
EMu:	*Ephedra minuta*

Introduction

Mahuang is a crude drug that has been utilized for perspiratory, antitussive, antipyretic and anti-inflammatory purposes in traditional Chinese medicine for centuries. The herbal origin of Mahuang is confined to the aerial part of *Ephedra sinica, E. intermedia* and *E. equisetina* in the Pharmacopoeia of China [1]. The habitat of these 3 species is mainly the northern and northwestern parts of China, such as Inner Mongolia, Gansu and Qinghai Provinces. Although other *Ephedra* species such as *E. gerardiana, E. likiangensis, E. przewalskii, E. minuta*, are also used as Mahuang [2], their reputation is not as good as the *Ephedra* species listed in the Pharmacopoeia of China, and their ephedrine content is reportedly lower. It is therefore inappropriate to use these species because their therapy effect has not been confirmed scientifically. However, it is difficult to exclude these spe-

Affiliation
[1] Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan
[2] Museum of Nature and Human Activities, Hyogo, Japan
[3] Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan
[4] Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Beijing, P.R. China

Correspondence
Dr. N. Kakiuchi · Faculty of Pharmaceutical Sciences · Kanazawa University · 13-1 Takaramachi · Kanazawa 920-0934 · Japan · Fax: +81-76-234-4490 · E-mail: kakiuchi@p.kanazawa-u.ac.jp

Received February 6, 2004 · **Accepted** March 14, 2004

Bibliography
Planta Med 2004; 70: 1080–1084 · © Georg Thieme Verlag KG Stuttgart · New York
DOI 10.1055/s-2004-832651
ISSN 0032-0943

cies from clinical use because of the morphological resemblance among *Ephedra* species when they do not bear flowers or seeds. Anatomic research has been conducted to differentiate them [3], [4], [5], but its accuracy was discounted due to geographic variation. DNA analysis is a powerful tool for the identification of *Ephedra* plants: the RAPD method was used by Takeuchi et al., but the results were unclear because the plants in the study were not differentiated [6]. In the course of research into medicinal plants in 2001 and 2002, we surveyed *Ephedra* plants in northern and western China during flowering and collected 8 *Ephedra* species [7]. Since non-coding regions tend to evolve faster than the coding region, nuclear ribosomal internal transcribed spacers (ITSs) were often used to identify the botanical origin of the herbal drugs as well as to resolve the phylogenetic relationship [8], [9], [10], [11]. The ITS of *Ephedra* plants, a member of the gymnosperm family whose ITS are longer than in angiosperm plants, can provide further information for identification [12], [13]. The *trnL/trnF* of chloroplast DNA is another molecular marker effective in differentiating the herbal origin [14]. The plastids are inherited maternally in *Ephedra*, and nuclear DNA is inherited biparentally [15]. The combined data derived from rDNA and chloroplast DNA provide a more precise resolution in phylogeny [16].

In this paper, we report the results of the combined analysis of rDNA ITS and *trnL/trnF* to differentiate 8 *Ephedra* species grown in China.

Materials and Methods

Plant materials

All 24 *Ephedra* plant specimens belonging to 8 species (1 – 5 plant specimens per species) were collected in the Inner Mongolia Autonomous Region and the Sichuan, Hebei, Shanxi, Gansu and Qinghai Provinces, as shown in Table 1. *Gnetum leptostachyum* was a generous gift from Dr. M. Tamura, identified by M. Tamura and J. F. Maxwell. The plants were deposited in the Herbarium of the Medicinal Plants of the Faculty of Pharmaceutical Sciences in Kanazawa University.

DNA extraction and PCR amplification

The plant stem was cut into 2-mm pieces, frozen in liquid nitrogen and ground into powder. Using the DNeasy Plant Mini Kit (QIAGEN, Germany), the DNA was extracted according to the manufacturer's protocol. Total DNA was used as a template for amplifying the ITS1 and ITS2 by PCR. The primers were designed based on 18S, 5.8S and 26S nuclear ribosomal DNA sequences from the Genbank. The primers, Eph-F (GAC GTC GCG AGA AGT TCA TT) and 5.8S-R (CGG GAT TCT GCA ATT CAC AC), were used to amplify ITS1 and primers 5.8-F (GAA CGT AGC GAA ATG CGA TA) and Eph-R (GTA AGT TTC TCT TCC TCC GC) were used for ITS2. The primers C (CGA AAR CGG TAG ACG CTA CG) and F (ATT TGA ACT GGT GAC ACG AG) were used to amplify the region of *trnL/trnF* [17]. PCR was performed in 50 μL of reaction mixture

Table 1 Plant materials

Species	*Voucher No.*	*Locality of voucher*	*Date of collection*	*GenBank Accession No.*		
				ITS1	*ITS2*	*trnL/trnF*
E. intermedia (EI)	KANP02309	Guinan, Qinghai Prov., China	2002.7.31			
	KANP02341	Akese, Gansu Prov., China	2002.8.7			
	KANP02363	Yuzhong, Gansu Prov., China	2002.8.13	AY394070	AY394062	AY423430
	KANP02364	Qingshui Xiang, Yuzhong, Gansu Prov., China	2002.8.13			
	KANP02369	Longxi, Gansu Prov., China	2002.8.14			
E. sinica (ES)	KANP02109	E-er-duo-si, Neimenggu, China	2002.6.5			
	KANP02133	Datong, Shanxi Prov., China	2002.6.8			
	KANP02139	Chengde, Hebei Prov,, China	2002.6.10	AY394071	AY394063	AY423431
	KANP02143	Chifeng, Neimenggu, China	2002.6.11			
	KANP02145	Tongliao, Neimenggu, China	2002.6.11			
E. przewalskii (EP)	KANP02321	Dulan, Qinghai Prov., China	2002.8.4			
	KANP02326	Ge-er-mu, Qinghai Prov., China	2002.8.4	AY394072	AY394064	AY423432
	KANP02340	Xi-meng-gu-zu-zi-zhi-zhou,, Qinghai Prov., China	2002.8.7			
	KANP02351	Jiuquan, Gansu Prov., China	2002.8.10			
E. equisetina (EE)	KANP02303	Xunhua, Qinghai Prov., China	2002.7.30			
	KANP02314	Xining, Qinghai Prov., China	2002.8.1	AY394073	AY394065	AY423433
	KANP02356	Shandan, Gansu Prov., China	2002.8.11			
	KANP02136	Zhangjiakou, Hebei Prov., China	2002.6.8			AY423434
E. monosperma (EM)	KANP02378	Qilianshan, Qinghai Prov., China	2002.8.1	AY394077	AY394066	AY423435
E. gerardiana (EG)	KANP0101117	Shiqu Xian, Sichuan Prov., China	2001.8.6	AY394074	AY394067	AY423436
E. likiangensis (EL)	KANP0101001	Kangding Xian,, Sichuan Prov., China	2001.7.27	AY394075	AY394068	AY423437
	KANP0101126	Xinlong Xian, Sichuan Prov., China	2001.8.8			
E. minuta (EMu)	KANP0101136	Litang Xian, Sichuan Prov., China	2001.8.11	AY394076	AY394069	AY423438
	KANP0101039	Kangding Xian, Sichuan Prov., China	2001.7.28			AY423439
G. leptostachyum		Doi Suthep, Thailand	2002.3	AY445622	AY445623	AY445621

containing 5 μL of 10× PCR buffer for KOD-Plus, 0.2 mM each of dNTP, 1 mM $MgSO_4$, 0.4 μM of each primer, 100 ng of the template and 1 unit of KOD-Plus DNA polymerase (TOYOBO). PCR was carried out as follows: hot start at 94 °C for 2 min, 30 cycles of denaturation at 94 °C for 15 sec, annealing at 55 °C for 30 sec and elongation at 68 °C for 45 sec, and final elongation at 68 °C for 5 min. Five μL PCR product was checked with 1.5% agarose gel electrophoresis and the remaining product was purified using the QIA quick PCR Purification Kit (QIAGEN, Germany).

Sequencing and phylogenetic analysis

The purified PCR product was subjected to direct sequencing using a Bigdye Terminator Cycle Sequencing Kit (Applied Biosystem) with ABI PRISM 310 (Applied Biosystem). The primers Eph-F, 5.8S-R and ITS-1A (GCG GGG ACG TGG ACG GTC TT) were used for sequencing ITS1 and primer 5.8S-F was used for ITS2. Primers C and F were used to sequence the region of *trnL/trnF*. The DNA sequences were aligned by 'DNASIS' version 3.0 (Hitachi).

Based on ITS1, ITS2 and *trnL/trnF* sequences, the phylogenetic trees were constructed using the program PAUP* (Version4.0b10, Sinauer Assoc. Inc., U.S.A.). Parsimony analysis was performed using the heuristic search method with TBR branch swapping with *Gnetum leptostachym* as an outgroup. Confidence in the tree was estimated by bootstrap analysis.

Results

The sequence analysis of multiple specimens of EI, ES, EP and EL showed their uniformity in the sequences analyzed in this study, ITS 1 and 2 (Fig. **1**), and *trnL/trnF*, within the same species. This is true for EE and EMu in the ITS1 and 2. The length of ITS2 found to be conserved was 246 bp, whereas that of ITS1 ranged from 1120 to 1139, as shown in Table **2**. EI and ES, EL and EMu had identical ITS1 and ITS2 lengths and sequences. Sequence divergence of ITS1 in 8 *Ephedra* species ranged from 0 to 3.49% while that of ITS2 ranged from 0 to 3.69%. At the end of ITS1, 76 bp were repeated 3 times in EI, ES and EP, whereas each of the sub-repeat units of EE, EM, EG, EL and EMu has a 4 bp deletion (Table **3**). According to the sequences of ITS1 and ITS2, 8 *Ephedra* species can be divided into 3 groups: Group 1 (EI, ES and EP), Group 2 (EE, EM and EG), and Group 3 (EL and EMu).

Based on the difference in the sub-repeat unit of ITS1 (Table **3**), a specific primer to Group 1, ITS-B-R (GTG AGC GGC AAG TAA GAT CC), was designed. Differentiation of the 3 groups of *Ephedra* species was conducted using a primer set of ITS-B-R and ITS-1A (357–376). When the annealing temperature was set at 55 °C, PCR products with about 450 bp were detected in Group 1 and Group 3, but no amplified fragment was seen in Group 2 (Fig. **2A**). In addition to 450 bp, faint amplified bands of about 530 and 610 bp were also detected in Group 1. When the annealing temperature rose to 65 °C, PCR fragments were detected only in Group 1 analysis (Fig. **2B**).

The sequences of *trnL/trnF* were found at 465 bp in EI, EP, EE, EM, EG, EMu, EL, and 463 bp in ES (Fig. **3**). One specimen of EE, EE02136 was found to be different at the 398th position from the other 3. Similarly, 2 EMu were found to differ from each other at the 271st position. EI and EP have identical sequences. EE has an identical sequence to EM, EG, EL, but not EE02136.

The phylogenetic tree based on the combined sequences of ITS1, ITS2 and *trnl/trnF* is shown in Fig. **4**. The results suggest that 8 *Ephedra* species were divided into two clusters, i.e., 1 (EI, ES and EP) and 2 (EE, EM, EG, EL and EMu) with high bootstrap probabilities. Cluster 2 can be divided into two sub-clusters, i.e., EE, EM and EG, and EL and EMu.

Discussion

Ephedra and other Gnetalean genera are thought to share evolutional positions between the angiosperm and gymnosperm. Out of 50–60 *Ephedra* species distributed worldwide, 12 species and 4 varieties are reported to grow in China [18], [19]. The length of ITS1 varies greatly within the same genus in many gymnosperm plants. On the other hand, variations in the ITS1 length of the 8 *Ephedra* species were limited in range from 1120 bp to 1139 bp. The region of *trnL/trnF* was about 460bp in the *Ephedra* plants, much shorter than that in the angiosperm. Nuclear ribosomal DNA of plants is often reported to be heterogeneous due to DNA recombination. However, we observed no ambiguous sequences in the ITS2 of *Ephedra* plants determined by direct sequencing, except for *E. equisetina* whose 16th and 17th positions of ITS2 were C or T, suggesting that sequence heterogeneity is limited. As for ITS1, although *E. intermedia*, *E. sinica* and *E. przewalskii* were determined without ambiguity, *E. likiangensis* and *E. minuta* that were classified into Group 3, were not clear at the 497th, 595th and 731st positions, supposedly due to heterogeneity in the nucleotide sequence of these points. Furthermore, *Ephedra*

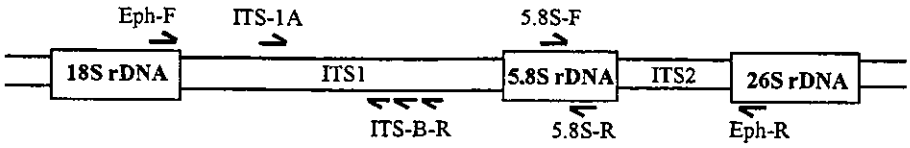

Fig. 1 Organization of plant ITS1 and ITS2. Arrows indicate orientation and approximate position of the primers.

Table 2 The length of ITS1 and ITS2 of *Ephedra* species (bp)

	EI	*ES*	*EP*	*EE*	*EM*	*EG*	*EL*	*EMu*
ITS1	1139	1139	1139	1120	1120	1120	1124	1124
ITS2	246	246	246	246	246	246	246	246

Table 3 The place where primer ITS-B-R (GTG AGC GGC AAG TAA GAT CC) possibly attaches. The numbers above sequences are the aligned nucleotide positions. Hyphens represent gaps.

	791–810	*866–885*	*945–964*
EI, ES	GGATCTTACTTGCCGCTCAC	GGATCTTACTTGCCGCTCAA	GGATCTTACTTGCCGCTCAC
EP	GGATCTTACTTGCCGCTCAC	GGATCTTACTTGCCGCTCAC	GGATCTTACTTGCCGCTCAC
EE, EM	GGATCTCAC- - - -CGCTCAA	GGATCTCAC- - - -CGCTCAA	GGATCTCAC- - - -CGCTCAC
EG	GGATCTCAC- - - -CGCTCAA	GGATCTCAC- - - -CGCTCAA	GGATTTCAC- - - -CGCTCAC
EL, EMu	GGATCTTAC- - - -CGCTCAC	GGATCTCAC- - - -CGTTCAA	GGATATCAC- - - -CGCTCAC

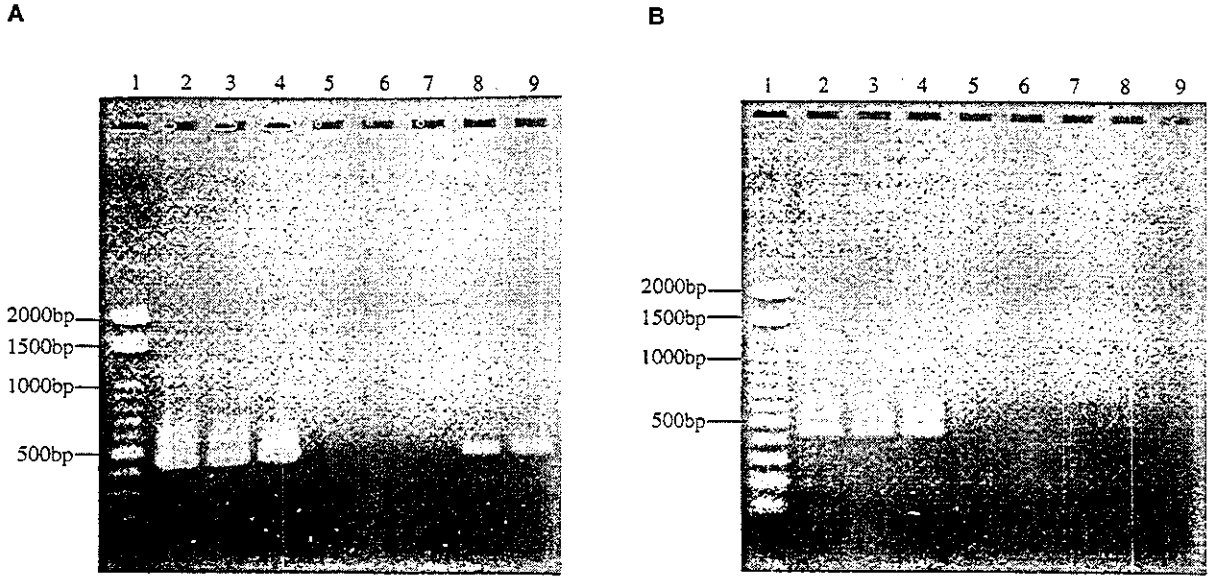

Fig. 2 Specific amplification using primers ITS-1A and ITS-B-R. **A:** Annealing temperature at 55 °C. **B:** Annealing temperature at 65 °C. Lane 1: 100-bp marker. Lane 2: *E. intermedia*. Lane 3: *E. sinica*. Lane 4: *E. przewalskii*. Lane 5: *E. equisetina*. Lane 6: *E. monosperma*. Lane 7: *E. gerardiana*. Lane 8: *E. likiangensis*. Lane 9: *E. minuta*.

	96	219	271	325	398	459	460
E. intermedia (**EI**)	C	T	T	G	A	G	T
E. sinica (**ES**)	*	*	*	*	*	-	-
E. przewalskii (**EP**)	*	*	*	*	*	*	*
E. equisetina (**EE**)	A	*	*	*	*	*	*
E. equisetina (**EE**)02136	A	*	*	*	C	*	*
E. monosperma (**EM**)	A	*	*	*	*	*	*
E. gerardiana (**EG**)	A	*	*	*	*	*	*
E. likiangensis (**EL**)	A	*	*	*	*	*	*
E. minuta (**EMu**)	*	G	*	A	*	*	*
E. minuta (**EMu**)0101039	*	G	C	A	*	*	*

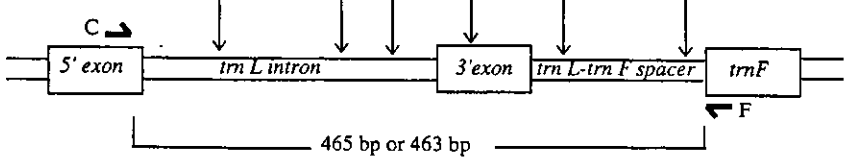

Fig. 3 Comparison of the region of *trnL* intron and *trnL-trnF* intergenic spacer sequence. Arrows indicate orientation and approximate position of the primers. The numbers above sequences are the aligned nucleotide positions. Asterisks indicate the identical nucleotides with *E. intermedia* in the first line, and hyphens represent gaps.

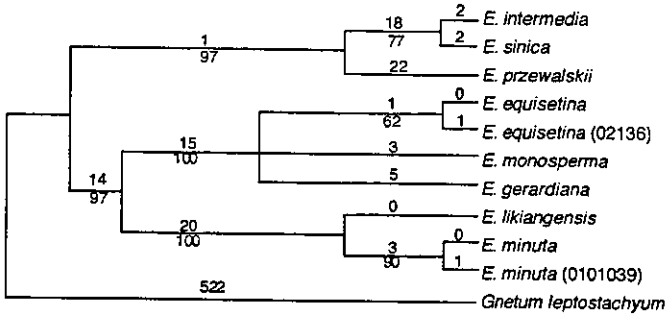

Fig. 4 Phylogenetic relationship of 8 *Ephedra* species based on DNA sequences of ITSs and *trnL/trnF*. The 50% majority rule consensus tree built on the basis of maximum parsimonious analysis of combined ITS1, ITS2 and *trnL/trnF*. Tree length = 609, CI = 0.9803, RI = 0.9226, RC = 0.9044. Number above line is branch length, and number below line is the bootstrap value with 1000 replicates.

species classified into Group 2 showed heterogeneity, C or T, at the 33rd position. Those nucleotide sequences were excluded from the sequence analysis. As reported previously on *E. fragilis*, ITS1 of the 8 *Ephedra* species had three tandem repeats of about 70 bp, the sequences of which were unique to each group and can be used to discriminate the groups. Although *E. intermedia* and *E. sinica* as well as *E. likiangensis* and *E. minuta* are identical in ITS1 and ITS2, they differ in the region of *trnL/trnF*. The information deduced from chloroplast DNA sequence analysis is different to that from ribosomal DNA ITS. Furthermore, one *E. equisetina* that was collected in Hebei Province, northeast China, had a different *trnL/trnF* sequence from the other 3 samples collected in the northwest. Two *E. minuta* that were collected in Sichuan Province, also showed differences in the *trnL/trnF* region. Chloroplast non-coding DNA mutates faster than nuclear DNA.

In the traditional classification of *Ephedra* species, according to the morphological difference in seed cones, *Ephedra* species in China were divided into 2 sections: Sect. Alatae and Sect. Ephedra. The seed cones in Sect. Alatae are membranous at maturity, whereas the seed cones in Sect. Ephedra are fleshy at maturity. *E. przewalskii* belongs to Sect. Alatae, and the other 7 *Ephedra* species belong to Sect. Ephedra [19]. However, analysis of ITS and the region of *trnL/trnF* shows that *E. przewalskii* has a genetically close relationship with *E. intermedia* and *E. sinica*. In addition, *E. likiangensis* and *E. minuta* that inhabit southern China differ greatly in size of plant, but are genetically close according to the ITS variation. The results from ITS and *trnL/trnF* did not correlate well with the morphology.

Karyomorphological studies showed that *Ephedra* species are diploid with 2n = 14 (*E. przewalskii* , *E. equisetina* and *E. minuta*), or tetraploid with 2n = 28 (*E. sinica, E. likiangensis*), as well as a mixture of those two 2n = 14, 28 (*E. intermedia, E. gerardiana*) [20]. No polyploid relationship of either species distribution or DNA classification was observed. On the other hand, in our survey, the species classified into Group 1 grew mainly in northern China; Inner Mongolia, Hebei, Shanxi, Gansu, and Qinghai. Group 3 grew in the south, Sichuan, and Group 2 grew in the center, suggesting genetic and geographic relationships. The species of Group 1 are thought to better tolerate harsh climates such as water-deficiency and low temperatures. The narrow DNA divergence within the species may result from selection under such climate stress. Considering their adaptive character, they may be suitable for cultivation in the dry land of northern China where progressive desert conditions have accelerated in the last 10 years.

Acknowledgements

This work was supported by Grant-in-Aids from the Ministry of Education, Science, Sports, and Culture, Japan.

References

[1] Chinese Pharmacopoeia Editorial Committee. Chinese Pharmacopoeia.; 2000 edition, Volume 1 Beijing: Chemical Technology Press, 2000: p 262

[2] Zhonghua Bencao (Chinese Herb) Editorial Committee. Zhonghua Bencao (Chinese Herb).; Volume 2 Shanghai: Shanghai Science & Technology Press, 1999: pp 349–357

[3] Zhang JS, Li SH, Lou ZC. Morphological and histological studies of Chinese *Ephedra* mahuang I. Seven species produced in north China. Acta Pharmaceutica Sinica 1989; 24: 937–48

[4] Zhang JS, Li SH, Lou ZC. Morphological and histological studies of Chinese *Ephedra* mahuang II. Species produced in southwestern China and other species. Acta Pharmaceutica Sinica 1990; 25: 54–65

[5] Mikage M, Kondo N. Medico-botanical studies of *Ephedra* plants from the Himalayan region, part I. Anatomical studies of the herbal stems and botanical origin of Tibetan crude drugs "TSHE" and "BALU". J Jpn Bot 1996; 71: 323–32

[6] Takeuchi M, Nakashima A, Mizukami H, Hiraoka N, Kohda H. RAPD analysis of *Ephedra* plants. Natural Medicines 2003; 57: 50–4

[7] Mikage M, Takahashi A, Chen HB, Li QS. Studies of *Ephedra* plants in Asia. Part 1. On the resources of *Ephedra* plants in China. Natural Medicines 2003; 57: 202–8

[8] Lau DTW, Shaw PC, Wang J, But PPH. Authentication of medicinal *Dendrobium* species by the internal transcribed spacer of ribosomal DNA. Planta Medica 2001; 67: 456–60

[9] Ding XY, Xu LS, Xu H, Wang ZT, Zhou KY. Morphological and DNA molecular evidence for authentication of *Dendrobium flexicaule* from ITS allied species of *Dendrobium*. Acta Pharmaceutica Sinica 2001; 36: 868–73

[10] Chen YQ, Wang N, Zhou H, Qu LH. Differentiation of medicinal *Cordyceps* species by rDNA ITS sequence analysis. Planta Medica 2002; 68: 635–9

[11] Wen J, Zimmer EA. Phylogeny and biogeography of *Panax* L. (the Ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. Mol Phylogenet Evol 1996; 6: 167–77

[12] Liston A, Robinson WA, Oliphant JM. Length variation in the nuclear ribosomal DNA internal transcribed spacer region of non-flowering seed plants. Syst Botany 1996; 21: 109–20

[13] Maggini F, Marrocco R, Gelati MT, Dominicis RID. Lengths and nucleotide sequences of the internal spacers of nuclear ribosomal DNA in gymnosperms and pteridophytes. Pl Syst Evol 1998; 213: 199–205

[14] Yang MH, Zhang DM, Liu JQ, Zheng JH. A molecular marker that is specific to medicinal rhubarb based on chloroplast *trnL/trnF* sequences. Planta Medica 2001; 67: 784–6

[15] Mogensen HL. The hows and whys of cytoplasmic inheritance in seed plants. Am J Bot 1996; 83: 383–404

[16] Zhu S, Fushimi H, Cai SQ, Komatsu K. Phylogenetic relationship in the Genus *Panax*: inferred from choroplast trnK gene and nuclear 18S rRNA gene sequences. Planta Medica 2003; 69: 647–653

[17] Taberlet P, Gielly L, Pautou G, Bouvet J. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 1991; 17: 1105–9

[18] Price RA. Systematics of the Gnetales: a review of morphological and molecular evidence. Int J Plant Sci 1996; 157: 40–9

[19] Agenda academiae sinicae edita. Flora Reipublicae Popularis Sinicae.; Volume 7 Beijing: Science Press, 1978: pp 468–89

[20] Chouhdry AS. Karyomorphological and cytogenetical studies in *Ephedra*. J Sci Hiroshima Univ Ser B, Div 2 1984; 19: 57–109

Survey on Resources of *Ephedra* Plants in Xinjiang

Changfeng LONG,[a] Nobuko KAKIUCHI,*[,a] Guoyue ZHONG,[b] and Masayuki MIKAGE[a]

[a] *Kanazawa University Graduate School of Natural Science and Technology; Kakuma, Kanazawa 920–1192, Japan: and*
[b] *Chongqing Academy of Chinese Materia Medica; Chongqing, 400065 China.*
Received August 25, 2004; accepted November 1, 2004

The resources of wild *Ephedra* plants in the Xinjiang Uygur Autonomous Region were surveyed. *Ephedra* plants mainly grow on the fringes of the Taklimakan Desert and Gureban-tonggute Desert. We found six genotypes of *Ephedra przewalskii* growing widely in Xinjiang. Three genotypes of *Ephedra intermedia* were limited to the northern and eastern parts, and *Ephedra regeliana* scattered in the northern part of Xinjiang. These *Ephedra* specimens were analyzed for DNA sequences of nuclear ribosomal DNA, internal transcribed spacers 1 and 2, chloroplastic DNA, *trn*L intron and *trn*L-*trn*F intergenic spacer. Intraspecific variation of the nucleotide sequence in *E. przewalskii* was found in different habitats. Norephedrine, ephedrine, pseudoephedrine, and methylephedrine contents of the specimens were determined. Although *Ephedra intermedia* of all three genotypes contained ephedrine alkaloids, ephedrine alkaloids were not detected in *E. regeliana* and *E. przewalskii*.

Key words *Ephedra*; internal transcribed spacer; *trn*L intron; *trn*L-*trn*F intergenic spacer; ephedrine alkaloid

The herbal drug Mahuang is commonly used in traditional Chinese and Japanese medicines to cure colds. Botanical origin of the drug is confined to *Ephedra intermedia* SCHRENK *et* C. A. MEYER, *Ephedra sinica* STAPF and *Ephedra equisetina* BUNGE in the pharmacopoeia of both China and Japan. Ephedrine alkaloids, ephedrine, pseudoephedrine, norephedrine, and methylephedrine that are pharmaceutically active the anodyne and antifebrile principles, are abundant in those *Ephedra* plants. In Mongolia, North America, and some other parts of the world, however, *Ephedra* species that do not contain ephedrine alkaloids are used traditionally for various diseases. *Ephedra przewalskii* STAPF, for example, which is reported not to contain any ephedrine alkaloids, is used for stomatitis, nephritis, and inflammation of organs in Mongolian traditional medicine.[1] Thus the *Ephedra* plants that are excluded from the Chinese and Japanese pharmacopoeia are still important for their medicinal usefulness.

To protect *Ephedra* plant resources and prevent the encroachment of the desert, the Chinese government has banned exports of Mahuang since 1999. Most Mahuang used in Japanese traditional medicine (kampo) is imported from China. Aiming at easing the shortage of Mahuang, we surveyed the resources of *Ephedra* plants in China. In a previous paper, we reported the results of the survey in the Inner Mongolia Autonomous Region, and Sichuan, Hebei, Shanxi, Gansu, and Qinghai provinces that are known habitats of *Ephedra* plants.[2] We collected specimens of eight *Ephedra* species,[2] and their phylogenetic relationships were studied in DNA sequence analysis of internal transcribed spacers 1 and 2 (ITS1 and -2) and the *trn*L (UAA) intron and the intergenic spacer between the *trn*L (UAA) 3′ exon and the *trn*F (GAA) gene (*trn*L/F) and found that those *Ephedra* species could be classified into three groups. We developed a simple PCR method for the classification based on the DNA sequence variation between the species in ITS1.[3] We found no variation within a species in ITS and *trn*L/F in *E. przewalskii*, *E. sinica*, and *E. intermedia*.

This time, we surveyed the resources of *Ephedra* plants in Xinjiang, located on the west of the previous research sites. With a large area including huge deserts and mountains, Xinjiang has varied vegetation from the neighboring provinces on the east, and it continues with variation to the west. We examined whether there is genetic variation in *Ephedra* plants according to different habitats and tried determine the relationship among morphology, nucleotide sequence, and content of ephedrine alkaloids.

MATERIALS AND METHODS

Plant Materials Eighteen specimens of three *Ephedra* species were collected from different habitats in the Xinjiang Uygur Autonomous Region from July 9 through July 23, 2003 (Table 1). All plant specimens were identified by Dr. M. Mikage.

PCR Amplification and Sequencing of Target DNA Regions Total DNA was extracted from the stem of plants with a DNeasy Plant Mini Kit (Qiagen, Germany). Using the total DNA as a template, ITS1, ITS2, and *trn*L/F were amplified by PCR as reported previously.[3] The amplified PCR product was purified with a QIA quick PCR Purification Kit (Qiagen, Germany). The purified PCR product was subjected to direct sequencing using a Bigdye Terminator Cycle Sequencing Kit (Applied Biosystems) with ABI PRISM 310 (Applied Biosystems).

Analysis of Ephedrine Alkaloids The alkaloid content was determined by the reported method with slight modification.[4] The HPLC system is composed of a Shimadzu LC-6A pump, Shimadzu SPD-6A detector, and C-R6A recorder. Five hundred milligrams of powdered *Ephedra* specimen was extracted with 25 ml of a mixed solvent of CH_3CN: H_2O: H_3PO_4 (400 : 600 : 0.4) with 0.4% SDS by ultrasonic extraction for 30 min. Extracts (5 μl) were analyzed with HPLC as followings: column, Devesil ODS-5 Nomura Kagaku (Nagoya, Japan); mobile phase, CH_3CN: H_2O: H_3PO_4 (400 : 600 : 0.4) with 0.4% SDS; flow rate, 0.8 ml/min; column temperature, 40 °C. Contents of ephedrine, norephedrine, pseudoephedrine, and methylephedrine were calculated from peak areas of UV absorption at 210 nm by comparing with the standard curve of each compound.

* To whom correspondence should be addressed. e-mail: kakiuchi@p.kanazawa-u.ac.jp

RESULTS

Survey of *Ephedra* Plants in Xinjiang Our survey of *Ephedra* plants in Xinjiang started in Urumqi on July 9, 2003 and ended in Chongqing on July 30, 2003. The survey route traveled along the northern fringes of the Guerban-tonggute Desert in the Junggar Basin and the southern edge of the Taklimakan Desert in the Tarim Basin through the regions listed in Table 1. Specimens of three *Ephedra* species, *E. intermedia*, *E. regeliana* FLORIN, and *E. przewalskii*, were collected. We found one specimen, specimen 23-20, that was morphologically identical to *E. intermedia* collected in Gansu and Qinghai, with bush-like futures, three scaly leaves, and twisted integument tubes (Fig. 1B). Two specimens, 11-40 and 12-30 (Fig. 1C), showed different morphology from typical *E. intermedia*. They were smaller in height and had two scaly leaves. Specimens 15-10 and 17-10 had two scaly leaves, but were taller than specimens 11-40 and 12-30. The habitats of *E. intermedia* were limited to the northern and eastern parts. *E. regeliana* (Fig. 1D) was scattered in the northern part of Xinjiang. We found *E. przewalskii* (Fig. 1A) growing widely in Xinjiang. No remarkable morphologic change was detected within the specimens of *E. przewalskii* collected from different habitats.

Table 1. Plant Material Collected in Xinjiang

Species	Voucher no.	Locality of voucher	Date of collection (r.m.d.)	GenBank accession no. ITS1	ITS2	*trn*L/F
E. intermedia	11-40	Kushui, Qitai xian	2003.7.11	AY730603	AY394062	AY423430
	12-30	Aletai Shi	2003.7.12	AY730603	AY394062	AY423430
	15-10	Guhe Zhen, Jinghe Xian	2003.7.15	AY730604	AY394062	AY423430
	17-10	Kuergan, Kuche Xian	2003.7.17	AY730604	AY394062	AY423430
	23-20	Aerjin mountain, Ruoqiang Xian	2003.7.23	AY394070	AY394062	AY423430
E. regeliana	11-50	Saertuohai xiang, Fuyun xian	2003.7.11	AY730602	AY730607	AY730599
	12-20	Beitun Zhen, Aletai Shi	2003.7.12	AY730602	AY730607	AY730599
	13-10	Heishitou, Aletai Shi	2003.7.13	AY730602	AY730607	AY730599
E. przewalskii	303	Hami Shi	2003.7.9	AY394072	AY394064	AY423432
	306	Urumqi Shi	2003.7.9	AY394072	AY730606	AY423432
	11-10	Hongliugou, Qitai xian	2003.7.11	AY394072	AY730605	AY423432
	11-31	Hongliugou, Qitai xian	2003.7.11	AY730601	AY730606	AY423432
	13-40	Wuerhe Xiang, Kelamayi Shi	2003.7.13	AY730601	AY730606	AY730598
	15-20	Daheyanzi, Jinghe Xian	2003.7.15	AY730601	AY730606	AY730598
	19-10	Qilang Xiang, Keping Xian	2003.7.19	AY730601	AY730606	AY423432
	20-10	Mukuilai, Pishan Xian	2003.7.20	AY730601	AY730606	AY730600
	21-10	Aoyitogelake Xiang, Yutian Xian	2003.7.21	AY730601	AY730606	AY730600
	22-10	Longkou, Qiemo Xian	2003.7.22	AY730601	AY730606	AY730600

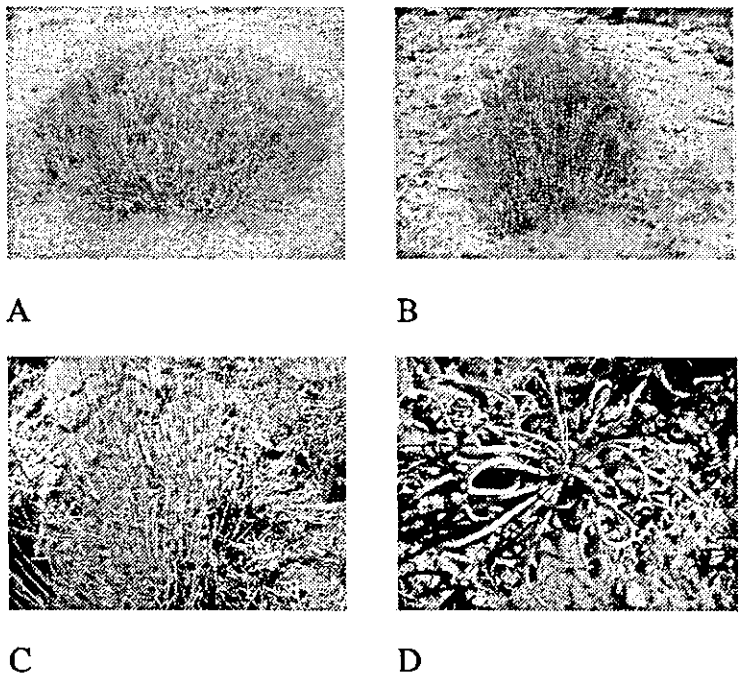

Fig. 1. Photos of *Ephedra* Plants Collected in Xinjiang

A, *E. przewalskii* (11-31); B, *E. intermedia* (23-20); C, *E. intermedia* (12-30); D, *E. regeliana* (11-50).