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Cytogenetic Analysis of *Bupleurum falcatum* L. Cultivated in Korea

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한국 재배종 시호의 세포유전학적 분석

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ABSTRACT : Karyotype analysis was carried out in four lines of *Bupleurum falcatum* L. cultivated in Korea and SDS-PAGE was applied to determine the seed protein profiles among the lines. Chromosomes were classified into two groups, large and small ones. Two kinds of karyotype, 2n=20 and 2n=26, were identified. Chromosome 1 of 2n=20 were all submedian, while that of 2n=26 were median. Chromosomes 2, 3 and 5 of 2n=20 showed polymorphism in size and arm-ratio. Chromosome 2 was submedian, while others were median in the line of 2n=26. Karyotypes of cultivars native of Korea were similiar each other, while those introduced from Japan showed different patterns. In SDS PAGE gels, qualitative differences in high molecular weight proteins, more than 45KD, were detected among the lines. The numbers of specific band were three in lines of 2n=20 and two in 2n=26.

Key words: *Bupleurum falcatum* L., karytype, submedian, median SDS-PAGE,

Bupleurum falcatum L., widely distributed in Europe, Central Asia, Korea, China, and Japan, is perennial herbaceous species of the family Umbelliferae. The root of the plant contains about 0.5% saikosaponin which is one of the important crude drug for antipyretic in East Asia(Tomita and Umori, 1976). Many intraspecific variations in external morphology were found in this species. Thus, classification and identification of cultivars and clones of the plants have been needed to develope the breeding program.

The chrompsome number of *B. falcatum* was firstly reported to be 2n=28 and the basic chromosome number was x=7 and 8 (Suzuka, 1950). The variation in chromosome numbers has been also reported. Plants with variable chromosome numbers such as 2n=19, 21, 22, 23, 25, 27, 30, 31, 33, 34, 37, and 40 have been identified in Japanese population and three basic cytogenetic types, 2n=20, 26, and 32, have been established (Arano and Saito, 1977; Amano et al., 1989; Kohda, 1990; Ohta et al., 1986; 1986; Ohta, 1991). Chromosome number of the plant in Korean natural population was firstly reported to be 2n=22(Lee, 1967) and those of

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Table 1. The plant materials of *Bupleurum falcatum* L.

Lines (Local name)	Chromosome number	No.of plants investigated	Origin of plants[a]	Abbreviation for lines
B.falcatum (Shiho)	2n=20	5	SCES	Bf
B.faicatum (Chamshiho)	2n=20	26	CRDA	BfC
B.faicatum (Taejon-samdoshiho)	2n=20	11	CRDA	BfJ
B.falcatum (Suwon-samdoshiho)	2n=26	13	SCES	BfS
Total		55		

[a] SCES : Suwon Crop Experiment Station, CRDA : Chungnam branch of Rural Development Administration

cultivars were reported to be 2n=19, 20, 21, 22(20+2B), 23(20+2B) and 26(20+6B) (Ohta, 1991). But Ohta's data from cultivars have no genetical back ground, because the seeds applied in his expriment were obtained from market not from natural population. Moreover, plants called Samdoshiho (Table 1) have been imported from Japan and cultivated with Korean cultivars together. Identification of Korean native species among the lines has been needed to establish the genetic marker for breeding program and conservation of the natural resource.

Seed proteins are products of many genes and they can provide useful informations about evolutionary relationships among plant species. Comparison of seed poteins using SDS-PAGE(sodiumdodecyl sulfate - polycrylamide gel electrophoreis) gel can provide a clue to identify the variability among species(Irwin and Boucaud, 1974; Cole et al., 1981). Thus, SDS-PAGE analysis between cultivars may support the cytogenetic results in studying the genetic background.

In the present study, karyotype and SDS PAGE analyses were carried out to establish the genetic system of *B. falcatum* L. cultivated in Korea.

MATERIALS AND METHODS

Plant materials

The seeds and the plants were obtained from Suwon Crop Experimant Station and Chungnam branch of Rural Development Administration (Table 1).

Karyotype analysis

Cytological analysis of the chromosomes was carried out in root-tip cells. Root-tips were pretreated in the saturated 1-bromonaphalene solution for 5 hours at room temperature. They were fixed in 1 : 3 solution (acetic acid glacial : ethanol) for 24h at 4℃, macerated in 1N HCl for 5 min at 60 ℃, double stained with aceto-carmine(1%) and Feulgen solution, and squash preparation was applied. Analysis of karyotypes followed Lavan *et al.* (1964)

SDS-PAGE

Protein extraction and SDS-PAGE followed Laemmli's mathods (1970). To avoid the error in analysis, 50mg of material (about 20 seeds) was ground in liquid nitrogen using mortar and pestle. Protein samples were denatured in sample buffer, and separated on 10% acryamide gel. Molecular standards were as follows : serum album (66KD), ovalbumin (45KD), glycerald- ehyde-3-phosphate dehydrogenase (36KD), carbonic anhydrase (29KD), trypsinogen (24KD) and trypsin inhibitor (20KD).

RESULTS AND DISCUSSION

Karyotypes

A total of 55 plants were examined cytologi-

Table 2. Chromosome analysis in four lines of *B. falcatum* L.

Lines / Chr. No.	Bf	BfC	BfJ	BfS
1	SM	SM	SM	M
2	SM	M	M	SM
3	M-SM	SM	M	M
4	M	M	M	M
5	SM	M-SM	M	M
6	M	M	M	M
7	M	M	M	M
8	M	M	M	M
9	M	M	M	M
10	M	M	M	M
11			-	M
12			-	M
13	-	-		M

M : median, SM : submedian, M-SM : median-submedian

cally in four lines of *B. falcatum* L., Bf., BfC, BfJ and BfS (Table 1). Two kinds of chromosome complements, 2n=20 (Bf, BfC and BfJ) and 2n=26 (BfS), were identified (Fig. 1 and 2). Two pairs of chrom osomes, No. 1 and No. 2, was large in all the lines and others were relatively small in size. The lar gest chromosome was submedian in the lines of 2n =20, whereas median in 2n=26. Two lines of plants in troduced from Japan carried different chromosome numbers, 2n=20 in BfJ and 2n=26 in BfS.

The sizes of small chromosomes in the line 2n =26 were significantly different from those in the lines 2n=20.

Lines 2n=20 : The total length of the large chromosome pairs ranged from 3.4 μm to 3.7 μm. The remaining eight pairs showed a gradual de crease in length from 2.9 μm to 1.7 μm. The largest chromosome was submedian in all the lines and chromosomes 2, 3 and 5 showed polymorphisms in arm-ratio (Table 2).

The highly varied chromosome numbers caused by polyploidy, aneulpoiy, and B chromosome, such as 2n=19, 21, 22, 23,25, 27, 30, 30, 31, 34, 37, and 40, were reported in the Japanese populations (Amano et al., 1989; Kohda et al., 1990; Ohta et al., 1986). Plants with 2n=19, 20, 21, 22(20+2B), 23 (20+3B) and 26(20+6B) were found in Korean cul

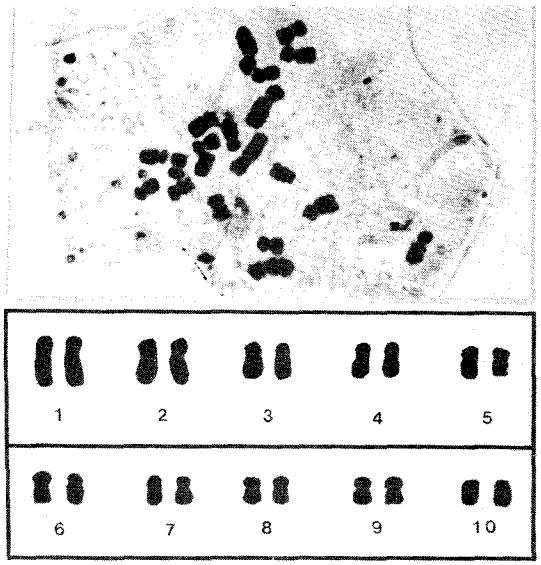

Fig. 1. Chromosome complement and karyotype of *Bupleurum falcatum* L., 2n=20

tivars (Ohta, 1991). However, any aneuploid plant and B chromosome were not found in the present study, though two lines (BfJ and BfS) have been introduced from Japan. That reason was estimated that chromosome complement in cultivated plant became stable.

Karyotype of Bf and BfC native of Korea were similiar each other, while BfJ introduced from Japan showed different pattern(Table 2). Studies on wild plants from Korea, China and Japan is needed for detail anaysis of the genetic lines of 2n=20.

Line 2n=26 : Two pairs of large chromosomes were quite distinct from the rest eleven pairs and the lengths ranged from 3.6 μm to 3.4 μm. Small chromosomes showed a gradual decrease in length from 2.8 μm to 1.2 μm. A pair of chromosome (No. 2) was submedian, and the remaining twelve pairs were all median(Table 2). In plants of 2n=26 reported in Korean cultivars, six of twen-ty six chromosomes were not somatic chromosomes but B chromosomes(Ohta, 1991). In the presen study, however, no B chromosome and no aneuploid were

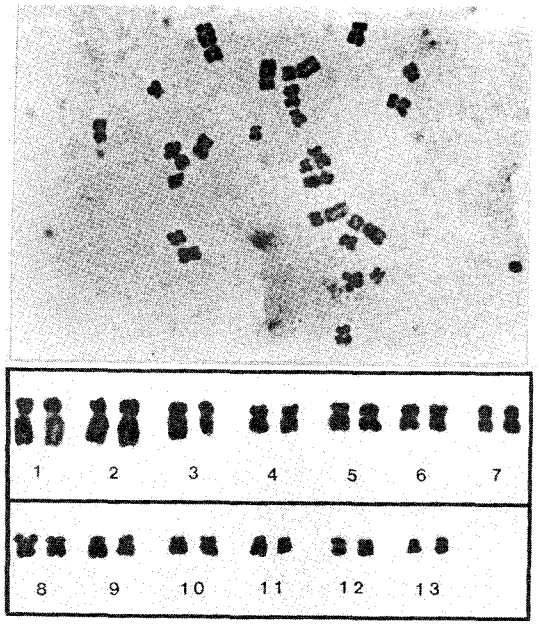

Fig. 2. Chromosome complement and karyotype of *B. falcalum* L., 2n=26

found.

Based on karyotype analysis of somatic chromosome in *B. falcatum*, we propose that two cytogenetically different lines are growing in Korea population.

SDS-PAGE

More than thirty bands were identified on the SDS-PAGE gel and specific bands were deteced in lines of 2n=20 and 2n=26, respectively. It was interesting that qualitative differences were detected among the lines in high molecular weight proteins, more than 45KD region. The numbers of specific bands were three in the lines of 2n=20 and two in the line of 2n=26 (Fig. 3).

Bands on 85, 70 and 53 KD region were specific for 2n=20, while those on 80 and 75 KD were specific for 2n=26. Three lines with 2n=20 appeared to be more similar to one another than to the line of 2n=26, except 50 KD region which showed slight differences among BfC and others in the lines with 2n=20.

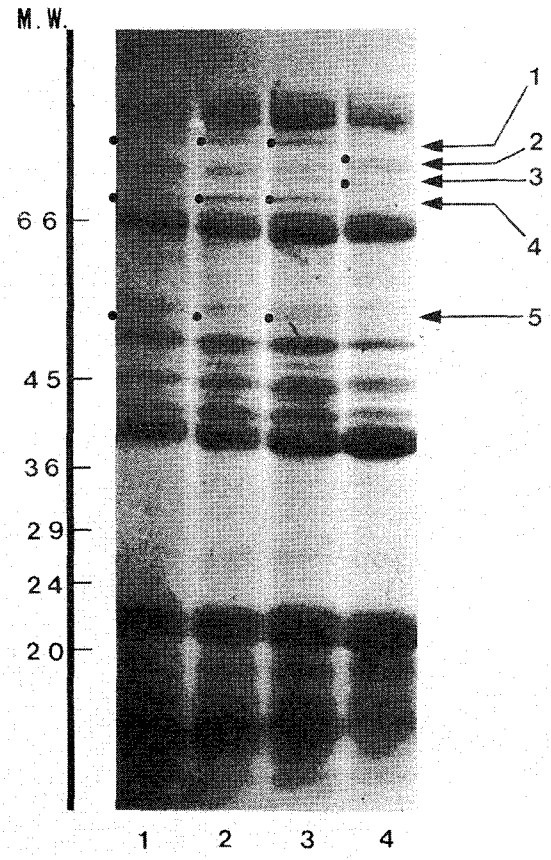

Fig. 3. SDS-PAGE patterns of seed proteins form *B. falcatum*. Lane 1 : Bf, Lane 2 : BfC, Lane 3 : BfJ, Lane 4 : BfS. The migration pattern of molecular weight markersare shown ; serum albumin (66KD), ovalbumin (45KD), glyceraldehyde-3-phosphate dehydrogenase (36KD), carbonic anhydrase (29KD), trypsinogen (24KD), trypsin inhibitor (20KD), Arrows indicate specific bands.

Soluble protein of each lines with different chromocome number of plants had qualitative and quantitative characteristics which are usually distinctive for each line (Cole *et al.*, 1981). This result showed that different homoeologous chromosomes in different lines may control endosperm pretein which differs in molecular weight (Brown, 1981). SDS-PAGE analysis was proved to be a useful technique in describing the close intraspecific

relationships among four lines of *B. falcatum* L.

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摘 要

한국에서 재배되고 있는 시호(*Bupleurum falcatum* L.)의 유전적 계통의 확립을 위해 시호(Bf), 참시호(BfC), 대전 삼도시호(BfJ) 및 수원 삼도시호(BfS) 등 4계통을 대상으로 핵형분석을 통해 염색체의 다형현상을 비교하고, SDS PAGE를 이용하여 저장단백질의 양상을 분석하였다. 체세포 염색체 수는 3계통(Bf, BfC, BfJ)에서 2n=20, 1 계통(BfS)에서 2n=26으로 구분되었다. 염색체 1번은 2n=20 계통의 경우 차중부 염색체였으나, 계통 2n=26에서는 중부 염색체로 관찰되어 차이를 보였다. 계통 2n=20에서 2번, 3번 및 5번염색체가 다형현상을 보였으며, 한국에서 재배되고 있는 계통의 핵형은 일본에서 도입되어 재배되고 있는 대전 삼도시호 및 수원 삼도시호와 차이를 보였다. 이러한 결과는 세포유전학적으로 다른 2가지 이상의 계통이 한국에서 재배되고 있음을 보여주는 것이다. 종자에서 추출한 저장 단백질은 45KD 이상의 단백질에서 수적 차이를 보였으며, 2n=20의 계통에서 3개, 2n=26 계통에서 2개의 특이한 밴드가 관찰되었다.

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