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Variations in Chromosome Numbers and Saikosaponin Contents of *Bupleurum falcatum* L. from Different Geographical Regions

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ABSTRACT

Seven genetic lines of *Bupleurum falcatum* L. from different geographical regions were analysed for saikosaponin contents and chromosomal numbers. The somatic chromosome numbers of *B. falcatum* originated from Euisong, Iri, Milyang, Sangnam, Taejon, and Youngchon were 2n=20 while Mishimasaiko showed 2n=26. However, chromosome features were different in plants grown in different geographical regions. Generally, Korean lines had higher saikosaponin contents than Mishimasaiko which is Japanese and Sangnam lines had highest saikosaponin contents compared to other tested lines.

Key Words : *Bupleurum falcatum* L., chromosome, saikosaponin

INTRODUCTION

Bupleurum falcatum L. is one of the well-known and important crude drug in oriental medicine in Korea. Recently, the demand of this drug has increased in Korea as well as in Japan. Usually, the root of *B. falcatum* have been used as a main source of crude drug which contains three major oleanine saponins, i.e. saikosaponin *a, c* and *d*(Shibata *et al.*, 1973).

The pharmacological activities of saikosaponins have been widely recognized and the contents of saponins have been used for the quality evaluation of *B. falcatum*. However, according to Shimokawa and Ohashi(1980), plants grown at different areas showed different saikosaponin contents and have different chromosome numbers. They also reported variation of chromosome number within same lines.

The chromosome numbers of *B. falcatum* were different, for example, 2n=16 in wild plants from Ashinoyu of Hakone region and mountain Kirishima, Kyushu in Japan(Hiroe, 1952). Similarly, Rostovtseva(1976) and Gorovoy et al.(1980) reported that the saiko, which was grown in the southern Siberia, had 2n=8 and 2n=12. There were not many reports regarding chromosome from saiko which is cultivated Korea. However, Lee(1967) found that the plant also grown in Korea showed 2n=22. Chung *et al.*(1995) observed two kinds of karyotype, 2n=20 and 2n=26. Ohta(1991) reported that an aneuploidal variation of the

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chromosome number, 2n=19, 20, 21, 25, 26, 27, 32, and 33, in somatic chromosome of 96 wild plants from 9 localities of Japan. Saikosaponin contents has been reported varies with the geographical variation of plant cultivation. Shimokawa *et al.*(1980) reported that saikosaponin contents showed significantly different from three regions of Japan. Mizukami *et al.*(1991) reported that *B. falcatum* plants collected in Japan showed polymorphic variation in saikosaponin contents.

However, there is limited information on variation in saikosaponin contents of *B. falcatum* of different geographical regions in Korea. This study was conducted to investigate chromosome and saikosaponin contents of *B. falcatum* cultivated in various geographical regions of Korea.

MATERIALS AND METHODS

Chromosome analysis

Seeds were collected from seven areas, Euisong, Iri, Milyang, Sangnam, Taejon, and Youngchon of Korea lines and Mishimasaiko originated from Japan, were provided by Youngnam Agricultural Experimental Station. The seven lines were grown in pots at green house of Kyungpook National University, Taegu. For somatic chromosomes observation, root tips were cut in the morning and treated with 2mM of 8-hydroxyquinoline for 5 hours at 20℃, fixed in 45% glacial acetic acid for 5-10 minutes at about 5℃ and then, macerated with a mixture of 1N HCl and 45% glacial acetic acid(2:1, v/v) for 2-3 minutes at 80℃. After maceration, material was stained with 1% acetoorcein and smashed on slide glass. The specimen of chromosome were examined by using microscope.

Quantitative analysis of saikosaponin

Powdered *B. falcatum* roots(500㎎) were extracted with 10ml of MeOH containing 0.2% KOH in an ultrasonic apparatus for 3 minutes. After that the MeOH solution was centrifused at 3,000rpm in 5℃ for 1 minute. After centrifuge, the MeOH solution was evaporized in vacuum, and the residues was dissolved with 10ml MeOH after filtration, the filtrate was subjected to high performance liquid chromatography (HPLC). Analysis was performed with Shimazu LC-9A apparatus equipped with a system controller(Shimazu, SCL-6B), UV detector(Shimazu, SPD-6A), column oven(Shimazu, CTO-6A) and data processor. CH_3CN was used as mobile phase with flow rate of 3ml per minute. Wave length was set at 203nm and column temperature at 40℃.

RESULTS AND DISCUSSION

Chromosome analysis

A total of 60 plants were examined cytologically in seven lines of *B. falcatum*. Two kinds of plants were found with chromosome numbers 2n=20 collected from Korea and 2n=26 originated from Japan. Morphological features of the chromosome were observed at resting stage and mitotic prophase. Euisong lines showed that the chromosome at metaphase 2n=20, were composed of 5 large chromosomes with 3.0~2.1㎛ in length. The median size chromosomes were 10 submedian and the small chromosome into 5 median chromosomes(Fig. 1A). The somatic chromosome at metaphase of Iri line with 2n=20 were 3.2~1.0㎛ in length. The chromosomes were composed of 4 large size in length and 10 medium size chromosomes 2.4~1.7㎛, 6 small size chromosomes about 1.2~1.0㎛, showing a biomodule variation in size(Fig. 1B). Somatic chromosomes were examined in 19 plants of Milyang line cultivated in greenhouse. Karyotype analysis was carried out on 10 of them. The chromosome numbers were 2n=20 in all plants. The total length of the large chromosomes was 2.1~2.4㎛, while length of other chromosomes was 1.5~1.0㎛. The large 6 chromosome group belonged to submedian, on the other hand, rest 14

chromosomes were median(Fig. 1C). Sangnam line have larger chromosomes than other 5 lines of Korean origin. The 9 large chromosomes were all submedian and 11 chromosome were median(Fig. 1D). Karyotype analysis was carried out on 9 plants of Taejon line that were grown from seed by the Youngnam Agricultural Experimental Station. The chromosome at mitotic metaphase, 2n=20, were composed of 12 large-size chromosome 3.3~1.8㎛ in length and 8 small-size chromosome. A secondary construction was observed

Table 1. Saikosaponin contents of *Bupleurum falcatum* roots from different geograpgical regions.

Geographical regions	Saikosaponin contents(% of dry wt.)			
	a	c	d	Total
Euisong	1.229	0.181	0.465	1.875
Iri	1.288	0.106	0.441	1.835
Milyang	1.215	0.088	0.361	1.664
Mishima	1.197	0.171	0.371	1.739
Sangnam	1.612	0.149	0.582	2.343
Taejon	1.251	0.079	0.312	1.642
Youngchon	1.336	0.088	0.307	1.731

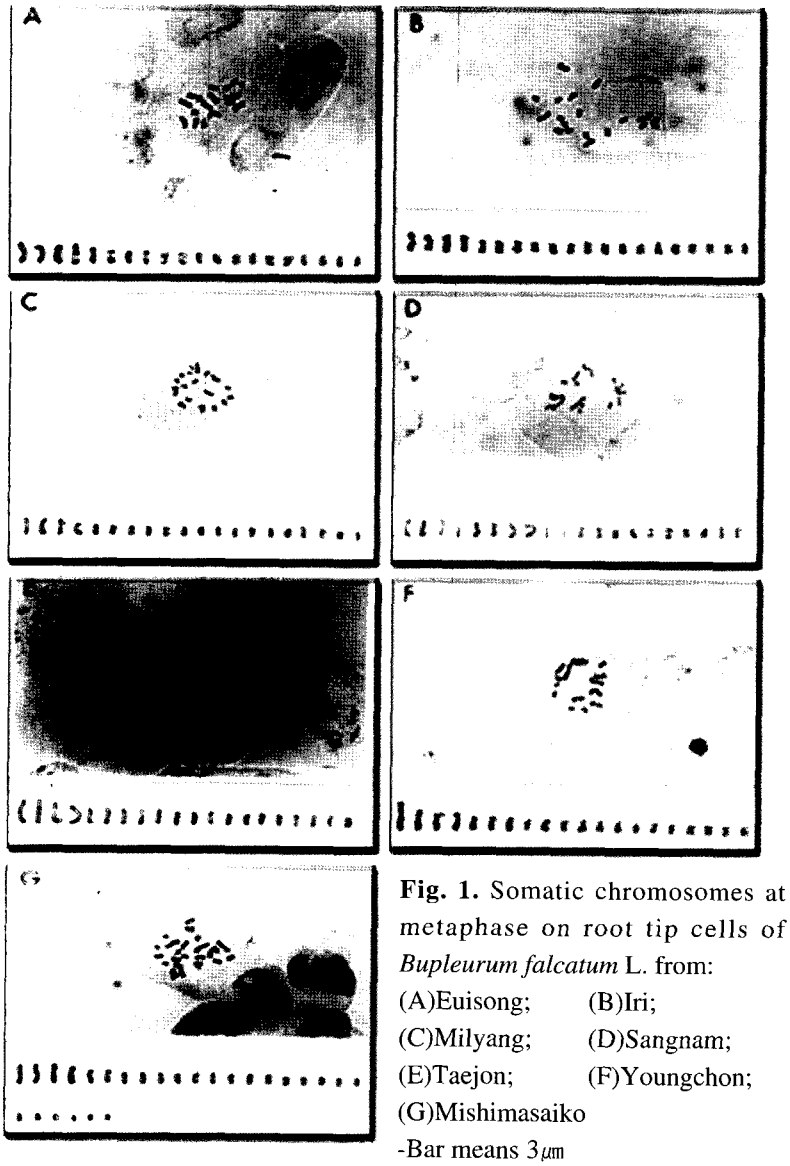

Fig. 1. Somatic chromosomes at metaphase on root tip cells of *Bupleurum falcatum* L. from:
(A)Euisong; (B)Iri;
(C)Milyang; (D)Sangnam;
(E)Taejon; (F)Youngchon;
(G)Mishimasaiko
-Bar means 3㎛

on the short arm of 4 of the large-sized chromosomes(Fig. 1E). Chromosome number was counted to be 2n=20 in 11 plant of Youngchon line. Chromosome at metaphase were 4 large and 10 submedian-sized and 6 small chromosomes. The large chromosomes belonged to the median(Fig. 1F). Chromosome number of Mishimasaiko was 2n= 26 in 12 plant. The morphological features of chromosome were observed at resting stage and mitotic prophase and metaphase. Chromosomes were composed of 4 large size, 12 submedian-sized and 8 small-sized and no secondary constriction was confirmed on the small chromosome(Fig. 1G). These results do not agree with earlier findings of Lee(1967). He found that the somatic chromosome numbers of *B. falcatum* were 2n=22. However, Chung(1993) suggested that the somatic chromosome numbers of *B. falcatum*(BF), *B. falcatum* var. Scorzomeraefolium(BFs), *B. falcatum* cv. Taejon-samdo(BFJ), and *B. falcatum* cv. Tsukuba-samdo(BFT) were 2n=20, and that of *B. falcatum* cv. Suwon-samdo(BFS) were 2n=26. *B. falcatum* has variation of chromosome number in different geographical regions as well as in the same line with 2n=20, 26, 32 of these basic types(Amano *et al.*, 1989; Chung *et al.*, 1995; Ohta *et al.*, 1986). Similarly, variation of chromosome number was observed in the regenerated plants from tissue culture and different origin(Amano *et al.*, 1989; Kohda *et al.*, 1990; Son *et al.*, 1997).

Therefore, the structural variation of chromosome are involved on the polymorphism of external morphology of *B. falcatum* and that differentiation has occurred in the species accompanied with the numerical and structural changes of chromosomes.

Variation in saikosaponin contents of *Bupleurum falcatum* L.

The saikosaponin contents were different among the different geographical lines grown under same condition at same places(Table 1). Sangnam had significantly higher saikosaponin a and d than those of the 5 other lines which were grown in Korea. In case of saikosaponin c, Euisong line showed highest content 0.181% compared with other 5 lines. Mishima line which was introduced from Japan, had lowest saikosaponin a content compared with Korean lines. However, saikosaponin c content of Mishima line was higher among tested lines except Euisong. The present study indicate that *B. falcatum* plants growing in different habitats in Korean areas genetically varied with respect to the saikosaponin contents of roots.

Among the tested lines, Taejon line had lowest saikosaponin contents. Euisong, Sangnam and Iri had some higher contents of saikosaponin d. Generally, Korean lines had higher saikosaponin contents than Mishimasaiko. It is interesting to note that somatic chromosome numbers of the plants belonging to the higher saikosaponin contents is mostly 2n=20, whereas that of lower saikosaponin content is 2n=26. Tanaka *et al.*(1988) reported that the Chinese race of *Bupleurum* is very similar to Japanese *B. falcatum*, morphologically, though the Chinese plant is smaller, and has a slower growth rate and a higher saponin content than the Japanese race.

These results were supported by Mizukami *et al.*(1998), they reported that the saikosaponin contents in the roots of plants from Itoda and Yufuin which had 2n=20 of chromosome number were higher than that of the plants from Hiraodai with 2n=26 chromosome number.

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