Studies on the Cultivation of *Bupleurum falcatum* L. I. Effect of Cultivation Conditions on the Root Growth and Saponin Contents[1)]

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Cultivation of *Bupleurum falcatum* L. and determination of saikosaponins by high-performance liquid chromatography (HPLC) were studied. The yield of dry roots decreased, while the weight of each root increased, as the cultivation period became longer in one-year-old plants. The yield and the weight of each root were shown to be higher in two-year-old plants than in one-year-old plants. The number of plants that survived, however, was greatly decreased by two-year cultivation. By clipping the aerial part of the plant at the height of 50 or 70 cm, the weight of each root was increased, while the yield of the root was decreased. Mulching of the field with transparent or black vinyl sheet increased the weight of root while silver sheet was ineffective. The content of saikosaponin a or d (SA or SD) was higher in one-year- than in two-year-old plant. The correlation between the root weight and the content of SA or SD was poor. However, a close correlation was found between the contents of SA and SD in the same root.

Keywords *Bupleurum falcatum*; saikosaponin a; saikosaponin d; clipping; high-performance liquid chromatography; mulching; root weight; cultivation

The root of *Bupleurum falcatum* L. (mishima-saiko in Japanese) is one of the most important crude drugs in kampo, the traditional medicine in Japan. Recently, the pharmacological activities of saikosaponins have been widely recognized[2)] and the content of saponins has been used for the quality evaluation of *B. falcatum*.

In this paper, we deal with the cultivation method of *B. falcatum* to increase both the root yield and saponin content by way of mulching of the field with vinyl sheets and clipping off the flowers to prevent their fruiting.

Experimental

Location of Cultivation The study was conducted in 1987 and 1988 on a clay loam soil field at Wakayama Experimental Station of Medicinal Plants, Institute of Hygienic Sciences Japan at Kawabe, Hidaka, Wakayama.

Cultivation Method The field was given 20, 70 and 4 kg/a of poultry manure, barnyard compost and a 3-2-3 (N-P-K) fertilizer, respectively. Soil conditioner Kips-PX-S (PX, Eisai Co., 5 kg/a) was also applied in some parts of the field. The seeds of *B. falcatum* were sowed in two line plots with 30 cm spacing on a ridge sowing bed of 90 cm width, 20 cm height and 25 m length at October, November, December or April. The seedlings were thinned in a zigzag pattern so that they were located within 20 cm distance of each other. In some plots of 2-year-old plants, the aerial parts were clipped at the height of 50 or 70 cm. The roots of each plot were harvested in June or November, washed, dried in hot air (Phoenix C-11DX, Taisho Noukou)[2)] and then weighed.

Cultivation Method with Mulching Three kinds of seeds of *B. falcatum* cultivated in different districts (Tsukuba, Totsukawa or Miyazaki in Japan) were planted in April, in the same manner as mentioned above. Three kinds of mulching (transparent, black or silver) were applied on the field when the plant had reached about 30 cm in height. The roots were harvested in November and weighed after being washed and dried in hot air as mentioned before.

Quantitative Analysis of Saikosaponins Powdered *Bupleurum* roots (500 mg) were extracted with 20 ml of ethyl ether in an ultrasonic apparatus. The solvent was removed and the powder was then extracted 3 times with 30 ml each of MeOH containing 0.2% KOH. The MeOH solution was evaporated *in vacuo* and the residue was dissolved in MeOH (10 ml). After filtration, the filtrate was subjected to high-performance liquid chromatography (HPLC). Analyses were performed under following conditions: LC-6A apparatus (Shimadzu) equipped with an Inertsil ODS stainless steel column (4.6 mm i.d. × 150 mm, Gasukuro Kogyo), an ultraviolet detector (SPD-6A, Shimadzu) and a computing integrator (CR-4A, Shimadzu). We used 34.5% MeCN as the mobile phase with a flow rate of 2.5 ml/min. The wavelength was set at 203 nm with a sensitivity of 0.04 a.u.f.s. Linear calibration plots were obtained from the peak areas of 40—500 μg/ml of saikosaponin a or d (SA or SD) in MeOH ($r=0.9999$). Authentic saikosaponins were purchased from Wako Pure Chemical Industries, Ltd.

Method of Regeneration of HPLC Column The used column was connected reversely, outlet to pump and inlet to detector, on the HPLC apparatus, and 80% EtOH was passed from the HPLC apparatus to the column at 1.0 ml/min for 20 min. The column was then reconnected in the normal way, inlet to pump and outlet to detector, and 15% MeCN was passed to the column at 1.0 ml/min for 20 min and then 34.5% MeCN at the same flow rate for more than 30 min.

Results and Discussion

Relationship between Cultivation Period and the Yield of *Bupleurum* Roots To observe the effect of cultivation period on the growth of *Bupleurum* roots, *Bupleurum* seeds were sown in October, November, December and April and harvested in November. The dry weight of each root increased while the root yield (kg/a) decreased as the cultivation period became longer in one-year-old plants (exposed to one warm season). It is assumed from this result that the number of plants decreased as the cultivation period was increased (Table I). In two-year-old plants, both the root weight and the yield increased compared with one-year-old plants, while the number of plants that survived

TABLE I. Relationship between Cultivation Period and the Yield of Dry Root of *Bupleurum falcatum*

Period		Clipping	Dry weight (g)	Yield (kg/a)	No. plant
Seed.	Harvest				
One-year-old[a)]					
Apr.	Nov.	No	1.30±0.22	2.5	1923
Dec.	Nov.	No	1.73±0.36	2.7	1561
Nov.	Nov.	No	1.93±0.56	1.6	829
Oct.	Nov.	No	2.09±0.58	1.5	718
Two-year-old					
Apr.	Jun.	No	4.80±1.62	—	—
Apr.	Nov.	50 cm	4.98±1.15	3.1	646
Apr.	Nov.	70 cm	6.70±2.10	3.4	507
Apr.	Nov.	No[b)]	5.22±1.59	4.2	805

a) Plants which experienced one warm season (spring, summer and autumn). *b*) The plant height was 145 cm.

was greatly decreased. The root weight was the highest when the aerial parts of the plant were clipped at 70 cm height, while the number of plants and the yield of roots were decreased compared with the untreated control. This result agrees with that of Ohashi *et al.*,[3] who reported that the yield of *Bupleurum* root was increased by pinching off the plant at 40 cm plant height, but was unchanged at 20 or 30 cm. At the first stage of the experiment, there were 4500 seedlings on the sowing bed, so more than half of the plants disappeared in one-year and 4/5ths of the plants in two-year cultivation.

Effect of Mulching on the Cultivation of *B. falcatum* As reported before, mulching (covering the field with vinyl sheets) increases the yield of *Platycodon* roots owing to the increase of the soil temperature and soil moisture.[4] So, three genotypes of *Bupleurum* obtained from different areas in Japan (Tsukuba, Totsukawa or Miyazaki) were planted on the field treated with three types of mulching (transparent, black or silver). As shown in Table II, the yield of roots was increased by both transparent and black mulch but not by silver mulch. Ohashi *et al.*[5] have already reported that the yield of roots of *B. falcatum* increased at lower temperature (15 °C/10 °C, day/night), but our result is different, as the soil temperature was increased by mulching with transparent or black vinyl sheet[4] but decreased with silver sheet. Further investigation will be necessary to clarify the effect of mulching on the growth of *B. falcatum*.

TABLE II. Effect of Mulching on the Fresh Root Weight (g) of *Bupleurum falcatum*

Mulching	Tsukuba	Totsukawa	Miyazaki
Control	—	10.0±2.4	7.2±2.5
Transparent	11.1±3.0	11.5±3.8	11.6±3.3
Black	12.2±4.6	12.9±5.4	9.4±3.8
Silver	12.4±5.8	9.1±4.5	7.2±3.6

The seed was sown in November, mulching was applied at April the following year. Harvesting was done in November.

Determination of Saikosaponins by HPLC MeOH containing ammonia[6] (A) or MeOH containing 0.2% KOH[7] (B) has been widely used for the HPLC analysis of saikosaponins in *Bupleurum* root. But more complicated patterns were observed on the chromatogram with solvent A (Fig. 1a, b). So, we used solvent B for the analysis. The peak shape of saikosaponins became broader and contaminants appeared on the chromatogram upon repeated HPLC analysis. When the column was cleaned by the method mentioned in Experimental, more than 100 samples could be analyzed without difficulty.

The recovery of SA and SD added (0.5 and 0.6 mg) to the sample was 94.9 and 100% with the coefficient of variation of 1.8 and 2.5%, respectively.

Relationship between Cultivation Period and Content of Saikosaponins in *B. falcatum* Table III shows the data on the dry weight of the root, the content of SA or SD at different cultivation periods and the correlation between

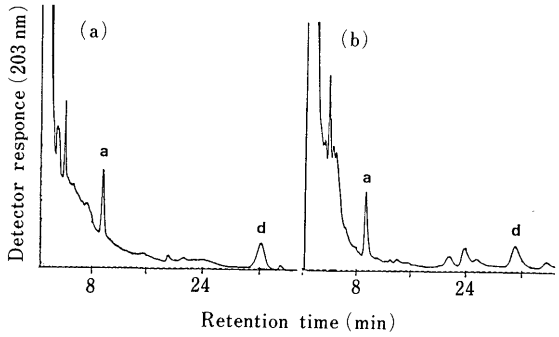

Fig. 1. High-Perfomance Liquid Chromatograms of the Root Extract of *Bupleurum falcatum* L. with Various Solvents

a, saikosaponin a; d, saikosaponin d. (a) 0.2% KOH in methanol, (b) 5% ammonia in methanol.

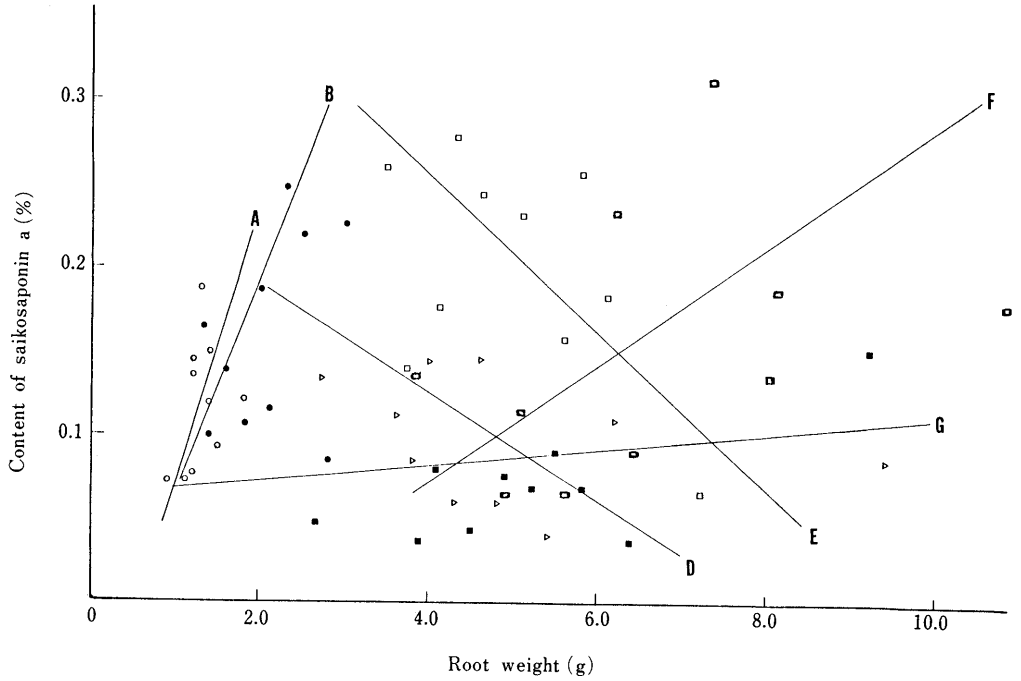

Fig. 2. Relationship between the Root Weight of *B. falcatum* L. and the Content of Saikosaponin a

A—G: see Table III. ○, A; ●, B; △, D; □, E; □, F; ■, G.

TABLE III. Relationship between Cultivation Condtions and Content of Saikosaponins in Dry Root of *Bupleurum falcatum*

	Period		Clipping	Cont. of saponins (%)		Correlation		
	Seed.	Harvest		SA	SD	SA/root	SD/root	SA/SD
One-year-old								
A	Dec.	Nov.	No	0.116±0.036	0.147±0.037	0.283	0.457	0.820
B	Oct.	Nov.	No	0.158±0.055	0.201±0.114	0.361	0.261	0.763
C	Dec.	Nov.	No	0.155±0.077	0.183±0.097	0.034	0.043	0.977
Two-year-old								
D	Apr.	Jun.	No	0.097±0.036	0.095±0.038	−0.297	−0.486	0.936
E	Apr.	Nov.	50 cm	0.199±0.063	0.193±0.062	−0.509	−0.704	0.844
F	Apr.	Nov.	70 cm	0.153±0.072	0.147±0.063	0.384	0.249	0.943
G	Apr.	Nov.	No	0.070±0.033	0.087±0.035	0.730	0.798	0.922

them. The contents of both SA and SD increased as the cultivation period becomes longer in one-year-old plants. They showed higher values in one-year- than in two-year-old plants, as described by Tanaka *et al.*[8] By clipping the aerial part of two-year-old plants at 70 or 50 cm, the contents of SA and SD in the root was increased compared with the untreated control (145 cm height). This result agrees with that of Ohashi *et al.*,[5] who showed that pinching of the plants increased the content of SA or SD in *B. falcatum*. A poor correlation was found between the root weight and the content of SA or SD in all cases except in two-year-old plant (G). Tani *et al.*[9] have shown that small roots contained higher concentrations of saikosaponins than big roots did. However, our result shows that the bigger roots contains more SA or SD in one-year-old plants (Table III). Overall data on the correlation between the weight of each root and SA content in it are shown in Fig. 2.

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