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https://doi.org/10.5109/24185

出版情報：九州大学大学院農学研究院紀要. 42 (1/2), pp.17-22, 1997-12. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **42** (1·2), 17-22 (1997)

Variation and Distribution and Saikosaponin in *Bupleurum falcatum* L.

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(*Received July 24, 1997 and accepted August 25, 1997*)

Variation and distribution of saikosaponin were studied for *Bupleurum falcatum*, originated from seven different regions. There were differences in saikosaponin content among cultivars and cultivation year. The saikosaponin content in roots of one-year-old plants for the cultivar from Ibaraki (Tsukuba) was higher than for the cultivars from Shizuoka, Ibaraki (Yasato), Fukuoka, Jeongseon and Kumamoto. Two-year-old plants had more xylem area than one-year-old plants as 26.1% and 23.2%. Saikosaponins were predominantly produced in phloem tissues regardless cultivation year. Total saikosaponin content was higher in the upper part of a root than lower part of a root. It was higher in lateral roots than main roots.

INTRODUCTION

Distribution of saikosaponin is attributed to the morphological characters of a root. Variation in morphology (Tani *et al.*, 1987), and individual plants (Kim *et al.*, 1995), effects of mineral fertilizers (Minami and Sugino, 1995), distribution in a root (Tani *et al.*, 1986), difference between asexually and sexually propagated plants (Hiraoka *et al.*, 1986), and saikosaponin contents in different geographical origins (Mizukami *et al.*, 1991; Tanaka *et al.*, 1988) have been reported. However, regarding the variation among cultivars in saikosaponin content, there is no report comparing between cultivars from Japan and Korea.

In order to obtain further information on distribution among root parts and geographical variations for saikosaponin content in roots of *B. falcatum*, the cultivars from different origins were cultivated under the same conditions to be compared here.

MATERIALS AND METHODS

(1) Difference of Saikosaponins Content among Cultivars

Seven cultivars of *B. falcatum*, originated from Korea (Jeongseon, Suwon) and Japan (Fukuoka, Kumamoto, Shizuoka, Ibaraki (Tsukuba and Yasato)) were grown. Cultivation, sampling methods and Saikosaponin analysis (Fig. 1) were described in materials and methods of the previous reports (Shon *et al.* 1997). From the outer layer, the roots of the cultivar from Jeongseon were sliced using a microtome to obtain the phloem and xylem tissues separately. Xylem tissues included wood fiber, vessel, pith, etc. Phloem tissues included pericycle, phloem, parenchyma, phloem, secretory, cannal, etc. Main and lateral roots were separated. Saikosaponin contents were analyzed separately for these components. The maximum diameter of main roots was measured using a caliper.

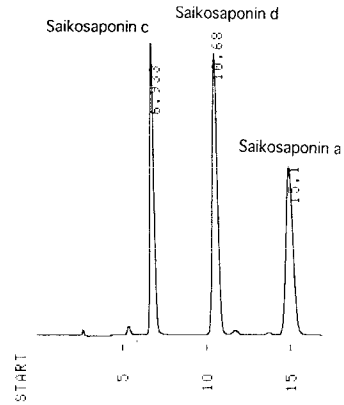

Fig. 1. Chromatogram of standard saikosaponin by HPLC. Absorbance 251nm.

(2) Distribution of Saikosaponin in a Root

The thickest part was cross-sectioned using a razor blade with twelve roots randomly sampled. Thin cross-sections obtained were examined using a light microscope to determine the xylem and phloem ratio for several plants of the cultivar from Jeongseon. Xylem and phloem ratios were calculated as;

Xylem ratio (%) = (xylem tissues area /total area) $\times 100$

Phloem ratio (%) = (phloem tissues area /total area) $\times 100$

The cross-sections of a root for tissue observation were obtained using a microtome technique. Total saikosaponins of each 50 plants of the cultivar from Jeongseon were measured to find the variation among roots. After roots were separated into main and lateral ones, both of which were cut to obtain upper, middle and lower part, and these parts were analyzed for saikosaponins content for the cultivars from Jeongseon.

RESULTS

(1) Difference of Saikosaponin Content among Cultivars

Table 1 represents root dry weight, root diameter and saikosaponin content in *B.*

Table 1. Dry weight and saikosaponin content of *B. falcatum*.

Cultivars from	Dry weight of a root (g)	Root diameter (mm)	Saikosaponin content (%)			
			a	c	d	Total
Shizuoka	2.31	9.68	0.64±0.013	0.39±0.035	0.77±0.111	1.795±0.159
Ibaraki (Y)*	3.27	12.19	0.53±0.128	0.41±0.135	0.63±0.205	1.565±0.468
Ibaraki (T)**	3.06	10.69	0.62±0.091	0.58±0.094	0.90±0.136	2.095±0.323
Kumamoto	3.02	10.90	0.37±0.290	0.39±0.093	0.68±0.018	1.445±0.141
Jeongseon	2.81	8.76	0.62±0.045	0.38±0.008	0.86±0.083	1.855±0.135
Fukuoka	2.89	9.70	0.46±0.023	0.27±0.063	0.55±0.079	1.275±0.165
Suwon	3.10	11.10	0.33±0.021	0.18±0.018	0.32±0.052	0.820±0.090

*: Yasato, **: Tsukuba.

falcatum originated from seven different regions. There was a difference among cultivars in total saikosaponin content for one-year-old plants and the values were in the order of Ibaraki (Tsukuba) > Jeongseon > Mishima > Ibaraki (Yasato) > Kumamoto > Fukuoka > Suwon. The order of saikosaponin composition was saikosaponin d > saiko-saponin a > saikosaponin c for all cultivars.

(2) Distribution of Saikosaponin in a Root

Fig. 2 represents a profile of one-year-old and two-year-old *B. falcatum* roots. Two-year-old plants had more xylem area as 26.1% (Table 2).

Fig. 3 represents the frequency distributions of saikosaponin content in one-year-old plant roots. The large variation of saikosaponin content was observed as 0.3% ~ 2.1%. The highest frequency of saikosaponin contents was 1.2% ~ 1.5%.

Correlation between saikosaponin content and xylem ratio is shown in Fig. 4. The correlation was negative (r = −0.837) at 1% level of significance.

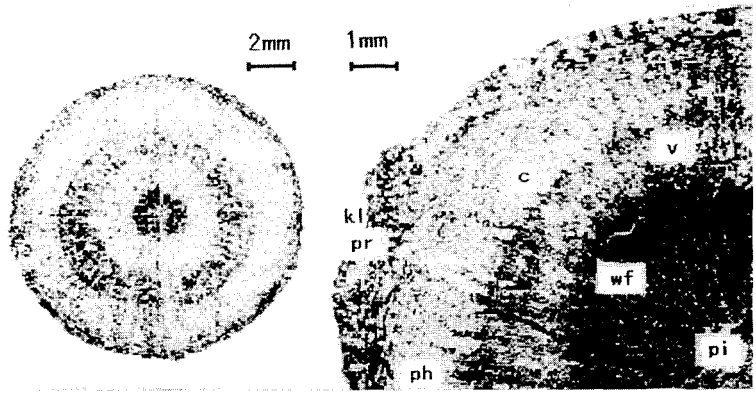

Fig. 2. A cross-section of one-year-old (left) and two-year-old (right) *B. falcatum* root.
kl, cork layer; **pr**, pericycle; **c**, cambium; **v**, vessel; **wf**, wood fiber; **pi**, pith; **ph**, phloem.

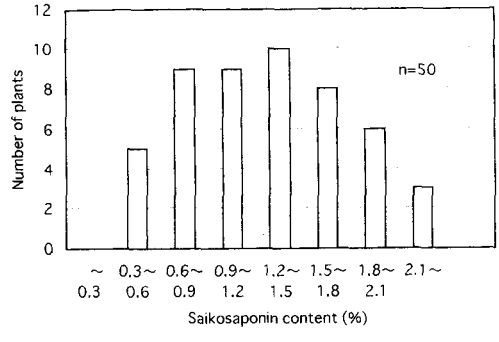

Fig. 3. Frequancy distribution of total saikosaponin content of one-year-old plants.

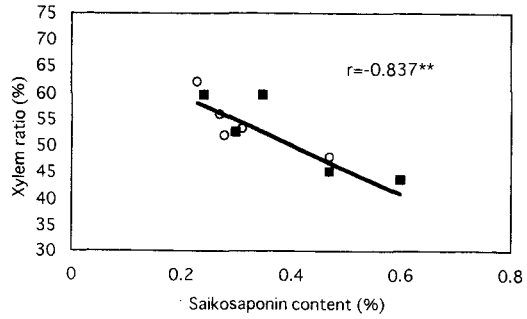

Fig. 4. Relationship between saikosaponin content and xylem ratio in *B. falcatum*. ○: one-year-old plants; ■: two-year-old plants; **: 1% level of significance.

Distribution of saikosaponin in different tissues of the root of *B. falcatum* is presented in Table 2. The total saikosaponin content of phloem tissues as 1.80 and 1.66 % was higher than those of xylem tissues as 0.16 and 0.25 % in one-year-old and two-year-old plants. Almost all total saikosaponin located in phloem tissues as 91.8 and 86.9 % for one-year-old and two-year-old plants, respectively. The similarity of differences between phloem tissues and xylem tissues was observed for total saikosaponin content, distribution rate, and area ratio in one-year-old and two-year-old plants. Consistently, in phloem tissues, saikosaponin a, c, d and their total content, and distribution rate were considerably higher than the values of xylem for both of one-year-old plants and two-year-old plants.

Saikosaponin contents in different parts of the root in *B. falcatum* are shown in Table 3. There were differences in saikosaponin content between main and lateral roots and among upper, middle and lower parts in one-year-old and two-year-old plants. The lateral

Table 2. Distribution of saikosaponin in different tissues of the root of *B. falcatum*.

Tissues	Area ratio (%)	Saikosaponin content (%)				Distribution rate (%)*
		a	c	d	Total	
One-year-old plants						
Phloem tissues	76.8±4.43	0.58±0.059	0.47±0.088	0.75±0.08	1.80±0.226	91.8
Xylem tissues	23.2±5.62	0.06±0.013	0.04±0.004	0.07±0.009	0.16±0.026	8.2
Two-year-old plants						
Phloem tissues	73.9±8.66	0.60±0.059	0.53±0.177	0.54±0.120	1.66±0.357	86.9
Xylem tissues	26.1±4.32	0.07±0.004	0.09±0.022	0.09±0.021	0.25±0.047	13.1

The phloem tissues which include the outer tissues from cambium: pericycle, phloem, parenchyma, phloem, secretory, cannal, etc.

The xylem tissues which include the inner tissues from cambium: wood fiber, vessel, pith, etc.

*: (Total saikosaponins content in phloem tissues/total saikosaponins content in phloem and xylem tissues) × 100; (Total saikosaponins content in xylem tissues/total saikosaponins content in phloem and xylem tissues) × 100.

Table 3. Saikosaponin content in different parts of a root of *B. falcatum*.

Part of root	Saikosaponin content (%)			
	a	c	d	Total
One-year-old plants				
main root	0.32±0.053	0.21±0.009	0.38±0.061	0.91±0.122
lateral root	0.36±0.053	0.43±0.026	0.82±0.096	1.61±0.175
upper part of root	0.33±0.042	0.23±0.023	0.39±0.051	0.94±0.116
middle part of root	0.53±0.073	0.37±0.028	0.73±0.139	1.62±0.241
lower part of root	0.57±0.036	0.32±0.031	0.80±0.090	1.68±0.156
Two-year-old plants				
main root	0.28±0.032	0.26±0.051	0.35±0.054	0.89±0.137
lateral root	0.59±0.078	0.34±0.045	0.76±0.020	1.68±0.143
upper part of root	0.31±0.029	0.34±0.019	0.41±0.078	1.05±0.125
middle part of root	0.57±0.012	0.41±0.078	0.81±0.018	1.78±0.108
lower part of root	0.58±0.027	0.43±0.047	0.89±0.110	1.90±0.184

roots and lower part showed higher contents compared to main roots and upper part of a root.

DISCUSSION

A wild strain introduced from China was compared with Japanese *B. falcatum* by Tanaka *et al.* (1988). The Chinese strain of *B. falcatum* was characterized by having smaller roots, a slower growth rate and a higher saponin content than the Japanese one. Therefore, they recommended that the Chinese strain of B. falcatum was more useful for one of the medicinal resources. In the present study, cultivars from Tsukuba and Jeongseon were found to have a high saikosaponin content.

Tani *et al.* (1987) found that non-bolting type had a smaller xylem ratio, larger phloem tissue and contained higher concentrations of bioactive saikosaponins in roots than bolting type. They also found a negative correlation between total saikosaponin content and xylem ratio of non-bolting and bolting type of *B. falcatum*. Minami *et al.* (1995) reported that total saikosaponin content was higher in outer tissues of roots than in inner tissues. Similarly a high negative correlation was obtained here between xylem ratio and total saikosaponin content with 1% significance level. This means that saikosaponins were stored predominantly in phloem tissues of roots.

Tani *et al.* (1986) reported that the lateral roots were higher in saikosaponins content compare to the main roots. The present study showed similar results. Major ingredient of *Bupleurum falcatum*, saikosaponin a, c, d, and many other minor saponins were isolated (Akahori and Kagawa, 1974; Akahori and Shimaoka, 1975) from the roots, and their structures and pharmacological value, effect and worth have been elucidated (Ishii *et al.*, 1980; Kimata *et al.*, 1979; Shibata *et al.*,1973). As variation in saikosaponin content has been attributed to the environmental and genetical conditions, more informations are need about the production of saikosaponin in *B. falcatum*. High quality *B. falcatum* must have high saikosaponin content in the roots. Saikosaponin yield, differences in saikosaponin content among genotypes and the relationship between root yield and saikosaponin content are also important subjects to be studied.

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