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https://doi.org/10.5109/14032

出版情報：九州大学大学院農学研究院紀要. 54 (1), pp.25-28, 2009-02-27. Faculty of Agriculture, Kyushu University

J. Fac. Agr., Kyushu Univ., **54** (1), 25–28 (2009)

Effects of Cultivation Temperature on the Growth and Saikosaponin Production of *Bupleurum falcatum* L. Cultivars

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(*Received November 13, 2008 and accepted December 5, 2008*)

The effects of cultivar and cultivation temperatures on root extract, ash content and saikosaponin content of *Bupleurum falcatum* L. were investigated using four Japanese and one Korean cultivars. Results showed that the Korean cultivar obtained higher root extract and saikosaponin contents but lower ash content than the Japanese cultivars at cultivation temperatures of 16.5–17.6 °C. Higher cultivation temperature (21.0°C) consistently yielded low values in all parameters for both cultivars. Results also showed inverse relationships between root extract and ash content, and between ash content and saikosaponin content. On the other hand, root extract showed direct relationship with saikosaponin content. This study indicated the usefulness of component analysis of plant materials for traditional medicinal preparation since each plant material may have different concentration of their active ingredients.

INTRODUCTION

Bupleurum falcatum L. (Ch'Ai Hu or Misima–Saiko) is a rich source of pharmacologically active saikosaponin (Park *et al.*, 1992) which is used as an active ingredient in many drug preparations. Quality of *B. falcatum* is evaluated by mass content analysis of ash, extract, essential oil, saikosaponin concentration, etc. Higher values in these parameters indicate higher quality of *B. falcatum.* Thus, to increase secretion of saikosaponin in the *B. falcatum* roots has been a major focus of many scientific investigations.

Medicinal plant materials vary in quantity and quality depending on cultivation sites due to the influence of soil, climate and other factors (WHO, 2003). In *B. falcatum*, saikosaponin production and growth characteristics were found to be dependent on environmental and genetic factors (Shon *et al.*, 1997a–c, 1998). Cultivation site was also shown to greatly influence production of pharmacologically active components in *Aconitum* roots (Hikino *et al.*, 1983; Kitagawa *et al.*, 1984). Besides, studies showed that cultivation temperature affects root growth and saikosaponin production of *B. falcatum* (Hosoda *et al.*, 1992; Shimokawa and Ohashi, 1980; Otsuka *et al.*, 1977; Shimokawa *et al.*, 1980). In other medicinal plants, cultivation temperature also significantly affected growth and tuber productions of *Aconitum carmichaelii* Debx. (Shoyama *et al.*, 1993; Shiping *et al.*, 1998) and *Arnebia benthamii* (Wall. ex G. Don) (Kandari *et al.*, 2008).

Although the effects of agronomic and environmental factors on *B. falcatum* have been well explored already, studies on the effects of cultivar and cultivation temperature are still not fully conducted. Thus, this study investigated the effects of cultivar and cultivation temperature on the growth and saikosaponin production of *B. falcatum.*

MATERIAL AND METHODS

Plant materials

The experiment was carried out to determine the effect of genetic characteristics and environment factors on extract, ash and total saikosaponin contents of the root of *B. falcatum.* A total of five cultivars used were taken from Japan and Korea. Cultivars from Japan, Yasato, Nara, Mishima and Kumamoto, contained 26 chromosomes while the Korean cultivar contained 20 of them. These cultivars were planted in four different regions (A–1, A–2, A–3, and A–4) varying in the amount of precipitation and temperatures (Fig. 1). The average temperatures for the regions A–1 to A–4 were 21.0, 17.6, 16.9 and 16.5 °C, respectively. Besides them, two culti-

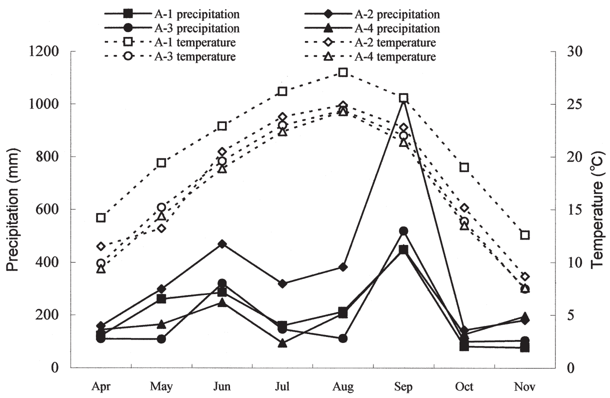

Fig. 1. Average amount of precipitation and temperature during the cultivation period of *Bupleurum falcatum.*

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vars of *B. falcatum*, originating from Korea and Japan, were grown at an experimental farm. Seeds were distributed from National Crop Experimental Station, Suwon, Korea and Tsukuba Medical Plant Research Station, National Institute of Health Sciences, Japan. Cultivation was carried out with the cultivation method of *B. falcatum* described by Shon *et al.* (1998). Sampling of plants was done in late November. Plant samples were air dried for one week and then stored in tight containers for analysis.

Root extract analysis

Root extract analysis was conducted according to the modified method of the Korean Herbal Pharmacopoeia (KFDA, 2006). Two to three grams of the sample was accurately weighed, extracted with 70% ethanol in a suitable flask with intermittent shaking for 5 hours, and allowed to stand for 20 hours. The solution was filtered and the flask was washed with a small amount of dilute ethanol to make 100 mL of filtrate. Fifty ml aliquot of the filtrate was evaporated to dryness, dried at 105 °C for 4 hours, and cooled in a dessicator. The weight of the residue was measured and multiplied by 2 to determine the amount of dilute ethanol–soluble extract.

Total ash content analysis

Total ash analysis was done according to the modified method of the Korean Herbal Pharmacopoeia (KFDA, 2006). The platinum crucible was tared by placing in an oven with temperature between 500 °C for 1 hour. It was then cooled and weighed accurately. The sample (3 g) was put in the crucible, heated at low temperature first, and gradually exposed to high temperature upto 550 °C, for more than 4 hours to ignite the sample until no carbonized substance remained in the ash. After the crucible was cooled down, the ash was accurately weighed. The procedure was repeated until constant weight was obtained and then amount (%) of total ash was determined. The charred mass was added with hot water and the insoluble residue was collected on a filter paper which was incinerated until no carbonized substance remained in the ash. The filtrate was added and evaporated to dryness. After drying, the sample was incinerated as described above, cooled down and weighed accurately to determine the weight (%) of the total ash.

Saikosaponin content analysis

Saikosaponin content was analyzed according to the modified method of Kimata *et al.* (1979). Two to three grams of the sample was accurately weighed, extracted with 70% ethanol in a suitable flask with intermittent shaking for 5 hours, and allowed to stand for 20 hours. The solution was filtered, and the flask was washed with a small amount of dilute ethanol to make until 100 mL of filtrate. Fifty mL aliquot of the filtrate was evaporated to dryness, dried at 105 °C for 4 hours, and cooled in a desiccator. The weight of the residue was measured and multiplied by 2 to determine the amount of dilute ethanol–soluble extract.

RESULTS AND DISCUSSION

Effect on the root extract

The extraction rate of root extract did not follow the trend of temperature. The highest extraction rate of root extract (29.8%) was of the Korean cultivar planted at the lowest temperature (16.5 °C) while the lowest extraction rate was of Yasato cultivar (20.8%) at similar degree of low temperature (16.9 °C) (Table 1). The highest extraction rate of root extract among those planted in the highest temperature was obtained in Mishima (25.8%). The highest mean root extract across different cultivation temperatures was obtained in the Korea cultivar (25.6%). Generally, higher extraction rate of root extracts were obtained in the regions A–2, A–3 and A–4 with low temperatures (16.5–17.6 °C). This result is in contrast to the reports by Fujii *et al.* (1994) which showed that culture solution of *B. falcatum* obtained the maximum saponin content at 25/25 °C (photoperiod/dark period air temperature) and high efficiency in saponin production for 20 hour per day photoperiod.

Results also showed that *B. falcatum* harvested from the region A–3 (16.9 °C) obtained lower extraction rate of root extracts (20.8–4.2%) compared to those from the regions A–2 (17.6 °C) and A–4 (16.5 °C) which ranged from 23.7–6.9 and 24.6–29.8%, respectively. The extraction rate of root extract from the region A–3 was also lower than those from the region A–1 (22.4–25.8%). This could probably be due to very low amount of precipitation in A–3 (190.3 mm) compared to that in regions A–1 (206.3 mm), A–2 (371.5 mm) and A–4 (204.1 mm).

Table 1. Root extract of *Bupleurum falcatum* harvested from different cultivation temperatures

Cultivation temperature*	Root extract (%)				
	Yasato	Nara	Mishima	Kumamoto	Korea
A–1	23.55±0.764	23.17±0.749	25.81±0.810	23.18±0.264	22.41±0.393
A–2	25.08±0.635	23.67±0.688	24.57±0.498	26.93±0.067	25.89±0.111
A–3	20.84±0.109	23.00±0.273	21.42±0.533	21.04±0.590	24.19±0.444
A–4	25.33±0.749	24.61±0.167	25.76±0.208	25.59±0.248	29.83±0.448
Mean	23.70±1.786	23.613±0.626	24.39±1.785	24.185±2.259	25.58±2.745

* Average cultivation temperature (°C): A–1=21.0, A–2=17.6, A–3=16.9, A–4=16.5.

Table 2. Ash content of *Bupleurum falcatum* harvested from different cultivation temperatures

Cultivation temperature*	Ash content (%)				
	Yasato	Nara	Mishima	Kumamoto	Korea
A–1	0.66±0.021	0.58±0.057	0.51±0.057	0.68±0.014	0.50±0.026
A–2	0.87±0.120	0.86±0.085	0.73±0.025	0.68±0.080	0.52±0.055
A–3	0.50±0.078	0.59±0.033	0.56±0.052	0.53±0.084	0.57±0.045
A–4	0.96±0.082	0.72±0.066	0.79±0.036	0.66±0.045	0.42±0.056
Mean	0.75±0.180	0.69±0.114	0.65±0.116	0.64±0.063	0.50±0.054

* Average cultivation temperature (°C): A–1=21.0, A–2=17.6, A–3=16.9, A–4=16.5.

Table 3. Saikosaponin content of root of *Bupleurum falcatum* harvested from different cultivation temperatures

Cultivation temperature*	Saikosaponin content (mg/g)				
	Yasato	Nara	Mishima	Kumamoto	Korea
A–1	0.98±0.180	0.92±0.230	1.21±0.300	0.94±0.350	1.38±0.290
A–2	1.40±0.523	1.50±0.320	1.89±0.460	1.22±0.460	2.91±0.640
A–3	1.89±0.630	1.22±0.230	2.23±0.610	–	1.34±0.330
A–4	1.61±0.070	1.90±0.570	2.24±0.050	1.83±0.90	–
Mean	1.47±0.332	1.39±0.361	1.89±0.418	1.33±0.372	1.78±0.589

* Average cultivation temperature (°C): A–1=21.0, A–2=17.6, A-3=16.9, A–4=16.5.

Effect on the root ash content

The effect of cultivation temperature on ash content of *B. falcatum* root was investigated (Table 2). Ash content can be a measure of root growth. The Yasato cultivar obtained high production rate of ash contents in the regions A–2 (0.87%) and A–4 (0.96%). The Korean cultivar obtained lower ash content compared with that of the Japanese cultivars except in the region A–3. Also, ash content of cultivars harvested from the region A–3 obtained lower values compared with that harvested from the other regions. This trend was similar with the root extract.

Studies showed varying effects of temperature on root yield. Shimokawa *et al.* (1990) reported increasing root yield at lower temperature. On the contrary, Hosoda and Noguchi (1992) reported increasing root yield at higher temperature. Shimokawa *et al.* (1990) reported that root growth favored temperature (15/10 °C day/night) while decreased root growth was observed at higher temperature (35/30 °C day/night). Shoyama *et al.* (1993) reported increased number of leaves and flowers at higher temperature and promotion of tuber production at lower temperature at *in vitro* experiments.

Furthermore, the results did not show a consistent trend that observed in the root extract. The Korean cultivar obtained lower ash content than other four Japanese cultivars. It can be said that root extract is inversely related to ash content of *B. falcatum* root.

Effect on the saikosaponin content

Table 3 shows the effect of cultivation temperature on saikosaponin contents in *B. falcatum* root. The Korean cultivar planted in the region A–2 (17.6 °C) obtained the highest saikosaponin content of 2.61 mg/g. This was followed by Mishima cultivar planted at the lowest cultivation temperature, producing 2.24 mg/g. The other Japanese cultivars obtained relatively lower saikosaponin contents whether planted in lower or higher cultivation temperatures. Shimokawa *et al.* (1980) reported that only little difference was observed in the saikosaponin content of *B. falcatum* cultivated at different temperature conditions. The same was observed in this experiment for both Japanese and Korean cultivars.

CONCLUSION

Results showed that, at lower cultivation temperature (16.5–17.6 °C), the Korean cultivar obtained higher extraction rate of root extract and saikosaponin contents than the Japanese cultivars. As for cultivation temperature, the Japanese cultivars obtained lower extraction rate of root extract, higher production rate of ash content and lower saikosaponin content, except Mishima cultivar, which had seen increase saikosaponin contents. Higher cultivation temperature (21.0 °C) consistently yielded low values in all parameters for both cultivars. Results also showed inverse relationships between the root extract and ash content, and between ash content and saikosaponin content. On the other hand, root extract showed direct relationship with saikosaponin content. This study indicated the usefulness of component analysis of plant materials for traditional medicinal preparation since each plant material may have different concentration of their active ingredients.

ACKNOW LEDGMENT

We would like to than Ms. H. Ishioka and Ms. M. Kawaguchi for formatting and Ms. K. Inoue for proofreading.

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