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Comparative Study on Saikosaponin Contents in Root of Unsexual Propagation and Sexual Reproduction in *Bupleurum falcatum*

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Production of major constituents in medicinal plants mainly depends on their origin; hence it is possible to produce more of such constituents through selection of potential cultivars. Differences in saikosaponin content and agronomic characteristics between the roots of regenerated plantlets through anther culture and plants cultivated by seed in *Bupleurum falcatum* were examined. There were significant differences between the means of regenerated plantlet and plant cultivated in fresh weight of aerial part, dry weight of aerial part and dry root length. The saikosaponin contents differed between the different origins. The content of Saikosaponin a in plants with chromosome number 2n=20, originated from Korea, was higher than that of 2n=26, from Japan, in both regenerated plantlets through anther culture and plants cultivated by seed propagation. But, total saikosaponin contents determined by HPLC were similar between the roots of regenerated plantlets and cultivated plants. Unsexual propagated plants through anther culture have proven to be more homogeneous than those of sexually reproductive plants by seed. To obtain uniform characteristics in *B. falcatum*, the unsexual propagation through anther culture is one of the effective methods for stable and uniform production in *B. falcatum*.

INTRODUCTION

Recently, demand on production of medicinal plants is increasing especially to supply for crude herbal drugs with elevation of economic development. The demand of *Bupleurum falcatum* is also extending year by year.

B. falcatum shows variation in histological and chemical characteristics in roots of cultivated plants (Shimokawa *et al.*, 1980; Tani *et al.*, 1987; Shon *et al.*, 1997a). Efforts for quality improvement, especially increasing of saikosaponin content in roots, was well studied through cultivation methods, soil ventilation (Hosoda and Noguchi, 1990), cultivation years (Shimokawa and Ohashi, 1980; Park *et al.*, 1992; Sohn *et al.*, 1998) and variety (Shimokawa *et al.*, 1980; Mizukami *et al.*, 1991). The evaluation of plants propagated through somatic embryogenesis of callus culture and cultivated in the field was performed in *B. falcatum* (Hiraoka *et al.*, 1986), fennel (Miura *et al.*, 1988) and *Angelica acutiloba* (Nakagawa *et al.*, 1982). But there is no report on the production of saiko-

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saponin and its agronomical characteristics in plantlets produced through anther culture and plants propagated by seeds, and as well as varieties of *B. falcatum*. Recently, the anther culture has become widely used as a method for obtaining haploid plants and for shortening the breeding intervals for obtaining high yield and quality of roots in *B. falcatum*. A successful system is thus required for obtaining stable production of major constituents and dry matter.

In this report, to clarify further the production of saikosaponin and its agronomical characteristics, we examined the differences by propagation method using plantlets regenerated through the anther culture and plants propagated by seed to obtain a genetically homogeneous population in two varieties of *B. falcatum*.

MATERIALS AND METHODS

Plant materials

B. falcatum, originated from Japan (2n=26) and Korea (2n=20), were grown in pots at an experimental field for two years. The cultivation was carried out as described in the following a cultivation method of *B. falcatum* (Shon *et al.*, 1998). Seeds were distributed from National Crop Experimental Station Suwon, Korea and Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, Japan. Nine months old plants flowers grown after seeding under natural state were used for anther culture. Production of regenerated plantlets through the anther culture was carried out as described in the method of Shon *et al.* (1997). Regenerated plantlets and nine months old plants after the transplanting into soil were used for saikosaponin analysis. To investigate the agronomic characteristics such as plant height, stem node, fresh weight, dry weight, root length and xylem ratio, the plants were sampled late November and were dried at natural condition for one week. After drying, it was preserved in tight container for saikosaponin analysis using HPLC.

HPLC analysis

After alkaline treatment of extract in *B. falcatum*, saikosaponin a content was determined by high performance liquid chromatography (HPLC) following the modified method of Kimata *et al.* (1979). Roots sampled from the field were dried at room temperature, and were randomly selected and pulverized using a grinder. Extraction of 500 mg of the powdered *B. falcatum* roots was treated with 10 ml MeOH containing 0.2% KOH at 60 °C and extracted by ultrasonic apparatus for 3 min. After extraction, the solution was centrifuged with 3000 rpm for 1 min. at 5 °C. After separation of the supernatant, it was extracted twice by same method. This solution was evaporated to dryness at 60 °C. The extracted solution was filled up to 10 ml with MeOH and filterated by disposable syringe filter of 0.5 mm. 10 ml of solution was subjected to HPLC. The saikosaponin content in the extract was measured by HPLC (LC–10AD, Shimadzu) with a stainless column of TSK–GEL ODS–120A (4.6×250 mm, TOSOH). The mobile phase was compared to acetonitrile with 50 mM KH_2PO_4. The flow rate was 0.5 ml per min and the detection was done at 203 nm.

RESULTS AND DISCUSSION

Table 1 shows the means and S.D. values in agronomical and morphological characteristics of *B. falcatum* between plantlets regenerated through the anther culture and plants cultivated by seeding using mean value of two cultivars, originated from Korea (2n=20) and Japan (2n=26). There were significant differences between the means of regenerated plantlets and plants cultivated in some factors. The fresh weight of aerial part in sexual reproduction plants was higher than in unsexual propagated plants. Dry weight of aerial part was significant at 5% level. The unsexual propagated plants showed smaller coefficient variance value than sexual reproduced plants with 1000-seed weight and root length. Therefore, unsexual propagated plants through the anther culture proved to be more homogeneous than those of sexually reproductive plants by seed. The morphological and agronomical characteristics of the regenerated plantlet were found to be uniform than that of plants cultivated by seed. The general characteristics trend compared among the variables showed higher values in sexual reproduction than unsexual propagation. Hiraoka *et al.* (1986) reported that the morphological characteristics and secondary metabolite contents of *in vitro* propagated plants were remarkably uniform as compared with those plants propagated by seeds.

Fig. 1 shows chromatograms of standard saikosaponin by high performance liquid chromatograms at absorbance 251 nm. Retention time was detected as 6.93, 10.68, 15.10 min. for saikosaponin c, d, a. The saikosaponin composition were as follow: saikosaponin d >saikosaponin a >saikosaponin c in both of cultivars originated from Korea and Japan

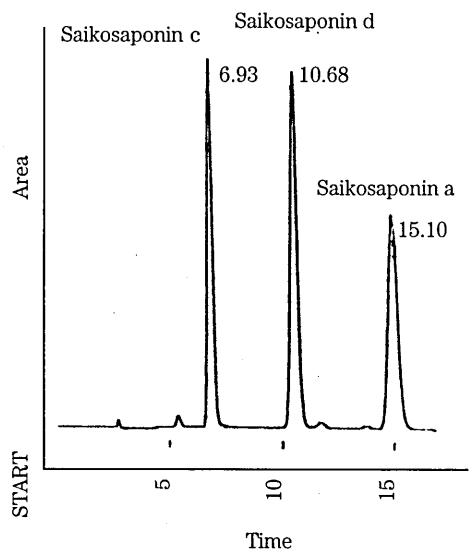

Fig. 1. Chromatograms of standard saikosaponin by HPLC. Detection was performed at 203 nm.

Table 1. Agronomical and morphological characteristics of *B. falcatum* plants regenerated through anther culture and seed reproduction.

Variables	Unsexual propagation			Sexual reproduction	
	Mean±S.D.	C.V (%)		Mean±S.D.	C.V (%)
No, of stem nodes	20.6±2.72	17	NS	22.9±2.88	27
Plant height (cm)	71.2±5.49	11	NS	69.5±4.87	19
Fresh wt. of aerial part (g)	97.0±28.3	32	*	112.0±38.6	45
Dry wt. of aerial part (g)	34.0±11.2	21	*	47.0±12.0	26
Dry root length (cm)	9.1±1.32	29	*	10.4±1.76	22
Dry wt. of root (g)	1.4±0.31	26	NS	1.5±0.42	39
Main root diameter (mm)	8.2±0.65	29	NS	8.9±0.77	37
Xylem ratio (%)	62.3±3.78	37	NS	65.7±4.43	42
1000-seed weight (g)	1.64±0.43	25	NS	1.64±0.34	18

* Represent significance of the differences between unisexual propagation and sexual reproduction according to Duncan's Multiple Range Test at 5% level, respectively. NS: no significance. Values represent the mean±S.D. from measurement of samples. The xylem tissues which include the inner tissues from cambium, wood fiber, vessel, pith etc.

Table 2. Saikosaponin content in different genetic origins of the root of *B. falcatum*.

Genetical origin	2n=20		2n= 26	
Saikosaponin contents	Mean±S.D	CV (%)	Mean±S.D	CV (%)
Sexual reproduction				
Saikosaponin a	0.632±0.112	20	0.472±0.093	18
Saikosaponin c	0.083±0.028	29	0.195±0.046	23
Saikosaponin d	0.689±0.102	14	0.734±0.187	32
Total saikosaponin	1.334±0.389	23	1.401±0.338	21
Unsexual propagation				
Saikosaponin a	0.671±0.134	17	0.513±0.122	22
Saikosaponin c	0.123±0.027	28	0.181±0.031	23
Saikosaponin d	0.779±0.134	23	0.722±0.149	18
Total saikosaponin	1.573±0.301	20	1.416±0.269	19

Value represents the mean±S.D from measurement of nine samples.

(Table 2). This result showed the same as reports of Shon *et al.* (1997, 1998). Hosoda and Noguchi (1990) also reported that content of saikosaponin in roots was affected by soil ventilation, which was increased by stream or mountainous sand.

Further more, *B. falcatum* has wide variation in growth, yield, and quality among others. Therefore, genetically uniform cultivars with high yield and quality are urgently needed. From this study, it is revealed that saikosaponin content in the roots is caused by various cultivars. The cultivars originated from Japan (2n=26) showed higher mean values of saikosaponin production parameters measured than that from Korea (2n=20) regardless of plant line. This is interpreted to suggest the difference between genotypes for potential crop productivity in *B. falcatum*. The saikosaponin a content was varied

between genotypes were 1.334% for 2n=20 and 1.401% for 2n=26 at harvesting stage, which were similar with our previous report (Shon *et al.*, 1998). The cultivars from 2n=20 showed higher saikosaponin a content than that from 2n=26. This shows that saikosaponin productions are related to genotypes. Therefore, it is proposed that for higher root yield and high saikosaponin production per unit in *B. falcatum*, high root yield of the 2n=20 and high saikosaponin content 2n=26 must be combined by crossbreeding between the two cultivars. Different genetic origins had not same saikosaponin contents. In case of the saikosaponin content, comparison made in 2n=20 showed an increased value than that of 2n=26 in sexual reproduction, similar tendency were observed in that of unsexual propagation. Total saikosaponin content showed no differences between the root of unsexual and sexual propagation in the same cultivars at 5% level of Duncan's Multiple Range Test. Our results showed similar in both of sexual reproduction and unisexual propagation as described by Hiraoka *et al.* (1986). Also, the results are in agreement with that of Mizukami *et al.* (1991) and Shimokawa (1980) who showed that saikosaponin contents in the roots plants were observed with different results by their geographical origins.

Saikosaponin contents in *B. falcatum* might be affected by some genetic factors. There were significant differences between the means of regenerated plantlets and plants reproduced by seed in total saikosaponin content. *B. falcatum* showed variation in morphological, histological and chemical characteristics and growth period. The xylem ratio and saikosaponin content showed differences between bolting and non-bolting roots cultivated in *B. falcatum*. The non-bolting roots were higher than bolting roots in saikosaponin content (Tani *et al.*, 1987). Kim *et al.* (1995) reported that saikosaponin contents in adventitious roots formed from callus of *B. falcatum* was differed by growth period, the contents of saikosaponin a and d were the highest at 150 days of culture, whereas the content of saikosaponin c was the highest at 60 days of culture. Park *et al.* (1992) determined the differences in cultivation by years, and cultivars in saikosaponin content. The saikosaponin contents of one-year-old roots were higher than those of two year old.

B. falcatum plants, unsexually propagated through the anther culture had uniform in agronomical and morphological characteristics compared to those from sexual reproduction. It could be concluded from this study that obtaining uniform characteristics in B. falcatum, unsexual propagation through anther culture is one of the effective methods for stable and uniform production in *B. falcatum*.

REFERENCES

Hiraoka, N., T. Kodama, M. Oyanagi, S. Nakano, Y. Tomita, O. Lida and M. Satake 1986 Characteristics of *Bupleurum falcatum* plants propagated through somatic embryogenesis of callus cultures. *Plant cell Reports*, **5**: 319-321

Hosoda, K and M. Noguchi 1990 Studies on the cultivation of *Bupleurum flcatum* L. II. Effect of soil ventilation on the root growth and saponin content. *Yakugaku Zasshi*, **110**: 823-833

Kimata, H., C. Hiyama, S. Yahara, O. Tanaka, O. Ishikawa and M. Aiura 1979 Application of high performance liquid chromatography to the analysis of crude drugs: separatory determination of saponins of *Bupleuri Radix*. *Chem. Pharm. Bull.*, **27**: 1836-1841

Kim, S. G., D. Y. Cho and W. Y. Soh 1995 Saikosponin content in adventitious root formed from callus of *Bupleurum falcatum* L.. *Korean J. Plant Tissue Culture*, **22**: 29-33

Miura, Y., H. Fukui and M. Tabata 1988 Reduced inhomogeneity of *Angelica acutiloba* plants propagated clonally through somatic embryoids. *Planta Medica*, **54**: 79–81

Mizukami, H., K. Matsunaga, H. Ohashi, A. Amano, T. Maekawa and K. Fujimoto 1991 Variation in saikosaponin content of *Bupleurum falcatum* L. of different geographical origins. Shoyakugaku Zasshi, **45**: 342–344

Nakagawa, K., Y. Miura, H. Fukui and M. Tabata 1982 *Plant tissue culture*. Marugen, Tokyo (Japan), pp. 701–702

Park, Y. J., H. S. Suh, J. W. Shim and S. K. Lee 1992 Comparative saikosaponin determination due to cultivars and root ages in *B. falcatum* L.. *Res. Rept. RDA*, **34**: 121–124

Shimokawa, Y and H. Ohashi 1980 Cultivation and breeding of *Bupleurum falcatum* L. (V) Relation among cultivation years, root growth and saikosaponin content. *Shoyakugaku Zasshi*, **34**: 235–238

Shimokawa, Y., I. Okuda, M. Kuwano and H. Ohashi 1980 Cultivation and breeding of *Bupleurum falcatum* L. (VI) geographical variation of *Bupleurum falcatum*. Shoyakugaku Zasshi, **34**: 239–244

Shon, T. K. and T. Yoshida 1997 Induction of haploid plantlets by anther culture of *Bupleurum falcatum* L.. *Jpn. J. Crop. Sci.*, **66**: 137–138

Shon, T. K., A. D. H. Totok and T. Yoshida 1997a Dry matter production and utilization of solar energy in one–year–old *Bupleurum falcatum*. *J. Fac. Agr. Kyushu Univ.*, **41**: 133–139

Shon, T. K., A. D. H. Totok and T. Yoshida 1997b Variation and distribution of saikosaponin in *Bupleurum falcatum* L.. *J. Fac. Agr. Kyushu Univ.*, **42**: 17–22

Shon, T. K., A. D. H. Totok and T. Yoshida 1998 Studies on dry matter production and efficiency for solar energy utilization in *Bupleurum falcatum* L. at different plant ages. *Plant Production Science*, **1**: 113–118

Tani, T., T. Katsuki, M. Kubo, Y. Okazaki and S. Arichi 1987 Histological and chemical characteristics of bolting and non–bolting roots of cultivated *Bupleurum falcatum* L.. *Chem. Pharm. Bull.*, **35**: 4530–4536