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STUDIES ON PRODUCTION PHYSIOLOGY AND HAPLOID INDUCTION IN *Bupleurum falcatum* L.

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STUDIES ON PRODUCTION PHYSIOLOGY AND HAPLOID INDUCTION IN *Bupleurum falcatum* L.

BY

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6. Shon, T.K.,Totok Agung Dwi Haryanto and T. Yoshida. Variation of chromosome numbers for plants and regenerated plantlets in *Bupleurum falcatum* L.（in preparation）.

CONTENTS

Bupleurum falcatum L. （Mishimasaiko in Japanese; Fig. 1） is a perennial herb of Umbelliferae species and is indigenous to China. Its roots have been used as one of the most important Chinese crude drugs prescribed together with other herbs as a current drug therapy and widely used for pain-killers, anti-allergy, anti-inflammatory, anti-pyretic medications and so on. Effective ingredients are saikosaponin a, c, d（Shibata et al., 1973; Fig. 2）.

Cultivation of medicinal plants is being done to supply the increasing demand for crude herbal drugs. *Bupleurum falcatum* is one of the important medical plants and its demand is increasing year by year. The root yield depends on both of environmental and genetical conditions, which the author studied in this thesis.

This thesis consists of two chapters; I. Production Physiology, and II.

Haploid Induction in *B. falcatum.*

The former chapter describes about production physiology for biomass, saikosaponin, efficiency for solar energy utilization and growth characteristics of *B. falcatum*. The latter chapter shows about the optimum condition for callus induction and haploid induction through anther culture of *B. falcatum*.

Fig. 1. The root and aerial part of *Bupleurum falcatum*.

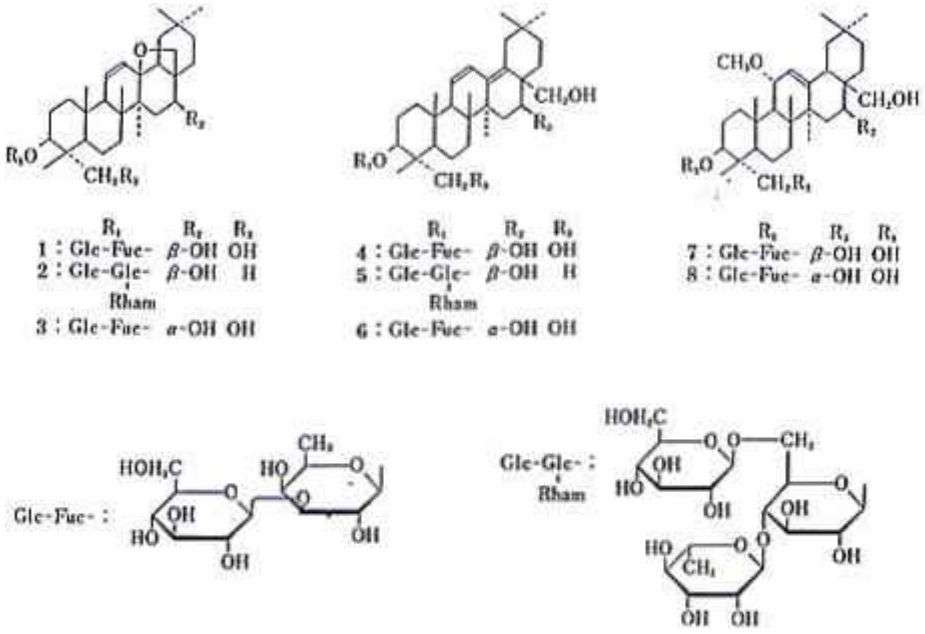


Fig. 2. Structure of saikosaponins.

1:saikosaponin a; 2:saikosaponin c; 3:saikosaponin d; 4:saikosaponin b_1; 5:saponin; 6:saikosaponin b_2; 7,8:secondary products.

I. PRODUCTION PHYSIOLOGY

I. Introduction

B. falcatum is usually produced under extensive culture, and also has many variation of geographical origins or plant characters（Kim et al., 1995: Minami and Sugino, l995; Shimokawa et al., l980; Tani et a1., l987）. The fundamental research work on the production of *B. falcatum* is scarce, especially about the difference among cultivars for growth characteristics, saikosaponin production, and efficiency for solar energy utilization, which are needed for more and further studies.

Generally, plant growth depends on photosynthesis, respiration and distribution of assimilates into various plant parts in relation to light, temperature and age of plants. There are several reports about the growth, dry matter （DM） production and saikosaponin production in *B. falcatum*（Hiraoka et al., 1986; Horikoshi et al.; Katano et al.; Kimata et a1., l979; Shimokawa et al., l980）. Shimokawa and Ohasi （1980） reported that the root growth of the cultivars introduced from Shizuoka and Kagawa was more vigorous than that from Yamaguchi. Comparing two-year-old plants of *B. falcatum* collected from six different habitats revealed that the cultivars from Hokkaido were inferior in the growth of top, but superior in the yield of root（Horikoshi et al., l976）. But, the research of the growth analysis and the efficiency for solar energy utilization （Eu） of *B. falcatum* have not found yet.

Recently much information about the DM production and Eu for many crops in different localities and growth conditions has been obtained （Aboagye et al., 1995; Kokubun and Watanabe, 1981; Shibles and Weber, l966; Sinclair et al., 1992; Yoshida, l979）. On the basis of these data, statistical analysis has been made on the relationship between climatic factors and the yield or productivity of plants. The estimation of the Eu and DM production can be used in improving the production of many plants. Murata （198l） made an analysis of the relationship between crop growth rate （CGR）, leaf photosynthetic activity and Eu among various crop species, and concluded that Eu corresponding to the max CGR was shown to be higher in the C4 species than in the C3 species. Further, a very close correlation was found between photosynthetic rate and Eu. Ensuing experiments reported about Eu within crop species, important differences among species were also found. Yamamoto et al.（1996） summarized a number of field experiments conducted by other researchers as well as data of their own, and reported Eu of 1.09 % for barely, 1.15 ～ l.94 % for rice and 0.92 % for soybean. From a theoretical derivation of Eu, Sinclair and Horie （l989） showed that difference in radiation-utilization efficiency （RUE） among species should be expected. In addition, they showed RUE would vary within a species, depending on the leaf photosynthesis. Shibles and Weber （l965） reported that DM production in equidistantly-spaced soybeans was a linear function of solar radiation interception by leaves.

Sequentially, the rate of DM production increased with leaf area development. The efficiency of utilization of intercepted energy differed between years（Shibles and Weber, 1966）. Thus, the relevance of leaf area and occupancy of inter plant spaces resulted in increasing solar radiation interception, which resulted in an increasing rate of DM production.

Eu is one of the important determinants for DM production and crop productivity, and depends on plant type and a spatial distribution of leaves. The spatial distribution of the leaves consists of a horizontal or a vertical distribution of the leaves and leaf orientation. Kawashima（1969）pointed out the significance of the leaf-adjusting movement for DM production in soybean. These factors markedly affect the photosynthetic rate of the crop community and Eu（Hirota and Takeda, 1982; Isoda et al., 1994; Lang et al., 1985）. DM production and Eu have been stressed by several authors and considered as a major factor in determining yield（Aboagye et al., 1994, 1995; Kokubun and Watanabe, 1981; Yoshida, 1979）. Thus, for obtaining higher yield of *B. falcatum* is very crucial to study about the growth characteristics and Eu, and the difference among cultivars and the growth habits of the crop.

Major ingredient of *B. 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., l979; Shibata et al., 1973）. Variation in saikosaponin content has been attributed to the environmental and genetical conditions, but there is almost no information 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.

Objectives in this chapter are;

-to compare differences of biomass and saikosaponin production and efficiency for solar energy utilization using one-year and two-year-old plants for cultivars originated from Japan and Korea.

-to compare variation and distribution of saikosaponins in the root of one-year-old and two-year-old plants.

2. Dry Matter and Saikosaponin Production, and Efficiency for Solar Energy Utilization

There are several reports about the growth, dry matter（DM） production and saikosaponin production in *B. falcatum*（Hiraoka et a1. J986; Horikoshi et al., l976; Katano et al., 1989; Kimata et al., l979; Shimokawa et al., 1980）. But, as far as I have been informed, there is no report about the growth analysis and the efficiency for solar energy utilization（Eu） of *B. falcatum*. Eu is one of the important determinants for DM production and crop productivity.

In this chapter, Eu as well as DM production and their relationships to growth characteristics of one-year-old and two-year-old plants were studied. In addition, saikosaponin a, c, d contained in the roots were chemically analyzed and the differences between plant age（one-year-old and two-year-old） and cultivars were studied.

（1） Dry Matter Production and Efficiency for Solar Energy Utilization

Materials and Methods

Two cultivars of *B. falcatum*, originated from Jeongseon, Korea and Kumamoto, Japan, were grown at the Experimental Farm, Faculty of Agriculture, Kyushu University from 1995 to 1996.

Fertilizer application was 18 g m^{-2} of N, P_2O_5, and K_2O as a basal dressing. Seeds were row-seeded by hand on April 5, 1995, 30 cm between rows. After germination, plants were thinned to maintain the inter-plant space of 20 cm on May 20. Plants were sampled from the 2 sites of 0.3 m^2 at 2 week intervals from 90 days after sowing. Sampled plants were divided into three parts; leaf, stem and root. Each part was dried at 70 ℃ for a day and weighed. On the basis of DM weight and leaf area obtained, growth parameters such as leaf area index （LAI）, DM production, crop growth rate （CGR）, relative growth rate （RGR） and net assimilation rate （NAR） were obtained by following the classical growth analysis method. CGR, RGR, and NAR were calculated from the equations shown below.

$$CGR = \frac{dw}{dt} = \frac{W2 - W1}{t2 - t1}$$

$$RGR = \frac{1}{W}\frac{dw}{dt} = \frac{\log W2 - \log WI}{t2 - t1}$$

$$NAR = \frac{1}{A}\frac{dw}{dt} = \frac{\log A2 - \log A1}{A2 - A1}\cdot\frac{W2 - WI}{t2 - t1}$$

where W is DM weight per unit area, i is sampling day and A is leaf area per unit area.

Eu was expressed by the ratio of the total combustion energy contained in DM, produced on a unit field area during a definite period (△ w), to the total shortwave solar radiation incident on the unit area during the same period (△ S), as shown by the following equation:

$$Eu = \frac{\triangle w\ (g\ m^{-2})\ \times\ 16.8\ (kJ\ g^{-1})}{\triangle S\ (kJ\ m^{-2})} \times 100$$

where 16.8 kJ g^{-1} (= 4 kcal g^{-1}) stands for heat combustion per gram of DM

After the harvesting of one-year-old plants in autumn, all of the aerial parts were cut and roots were left underground. March of 1996, shoots began growing again from previous year's roots. Plant sampling and data collection methods are the same as the case for one-year-old plants. Data on temperature, precipitation and △ S were obtained from Fukuoka District Meteorological Observatory.

Results

The temperature in l995 was slightly higher than average, but precipitation was almost similar to average, though it fluctuated monthly in 1995 and 1996 (Fig. 3). Solar radiation was higher than average with the exception of June 1996 (Fig. 4)

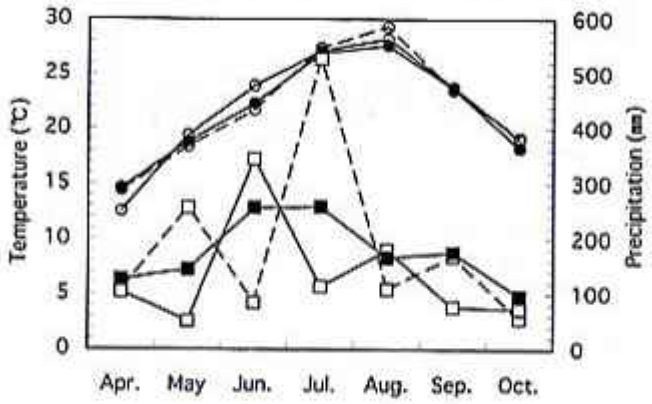


Fig. 3. Changes in mean air temperature and mean precipitation in a month from April to October in Fukuoka, Japan.
Temperature ; --○--:1995, ○ :1996, ●:Average.
Precipitation ; --□--:1995, □ :1996, ■:Average.

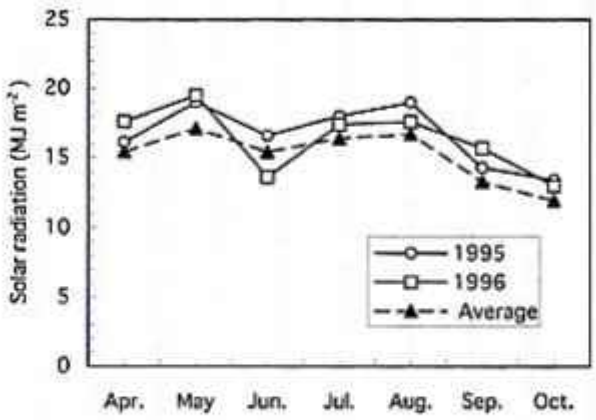


Fig. 4. Changes in mean solar radiation in a day from April to October in Fukuoka, Japan.

Fig. 5 represents changes in percent of dry weight of organs in *B. falcatum*. Percentage of dry weight of organs was not different between the cultivars from Kumamoto and Jeongseon in one-year-old as well as two-yew-old plants. One-year-old plants had higher percent of leaf dry weight than two-year-old at early growth period. The percent of root dry weight exhibited a similar tendency regardless of cultivars and plant age, showing values of 6 ~ 8%.

DM accumulation of aerial parts and roots of *B. falcatum* is shown in Fig. 6. Total DM of one-year-old and two-year-old plants was higher in the cultivar from Kumamoto than in the cultivar from Jeongseon. For the yield of roots, the cultivar from Kumamoto out yielded than from Jeongseon either in the one-year-old or in the two-year-old plants. Basically, the tendency of DM accumulation showed a similar pattern for the cultivar from Jeongseon and Kumamoto in one-year-old as well as two-year-old plants. One-year-old plants showed a very rapid growth in aerial parts and roots. Two-year-old plants showed a gentle growth in aerial parts compared to one-year-old plants. The increase of total dry weight in two-year-old plants was not due to an increase in roots weight but mainly due to aerial parts. DM accumulation of roots in two-year-old plants was not found during the sampling time as 54 to 60 g m^{-2} for the cultivar from Jeongseon and 55 to 66 g m^{-2} for the cultivar from Kumamoto. DM between sampling times, plotted as a function of mean LAI, is shown in Fig. 7. These data were characterized by a similar response to LAI for DM produced regardless of plant age. They showed positive correlations, though the correlation in one-year-old plants of the cultivars from Jeongseon was not significant.

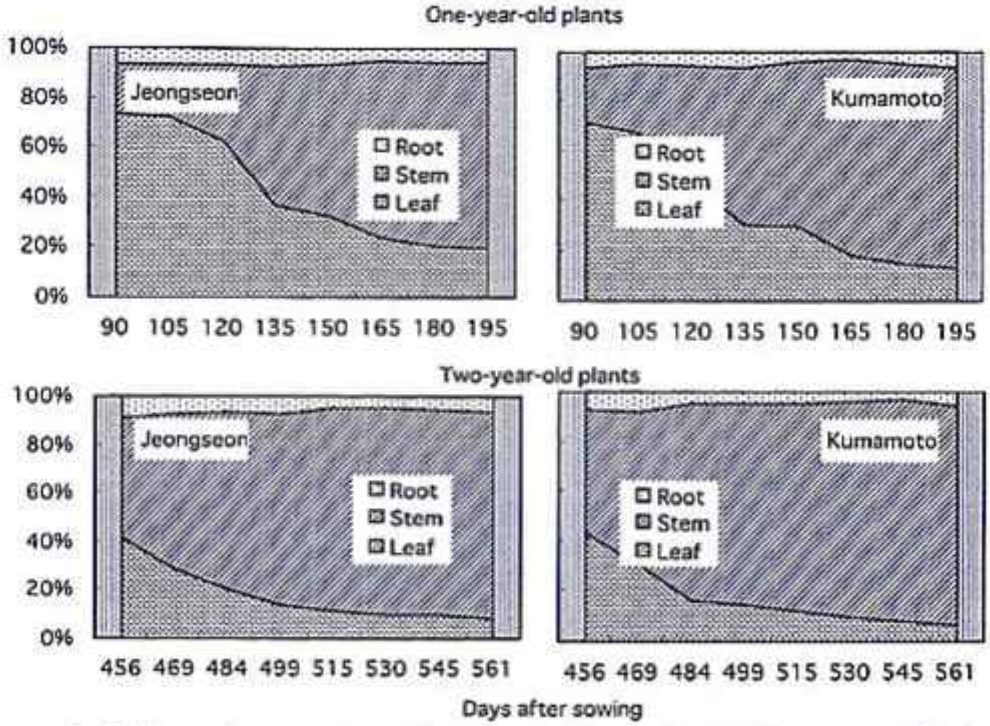


Fig. 5. Changes in percent dry weight of organs to the total dry weight in one-year-old and two-year-old *Bupleurum falcatum* for the cultivars from Jeongseon and Kumamoto.

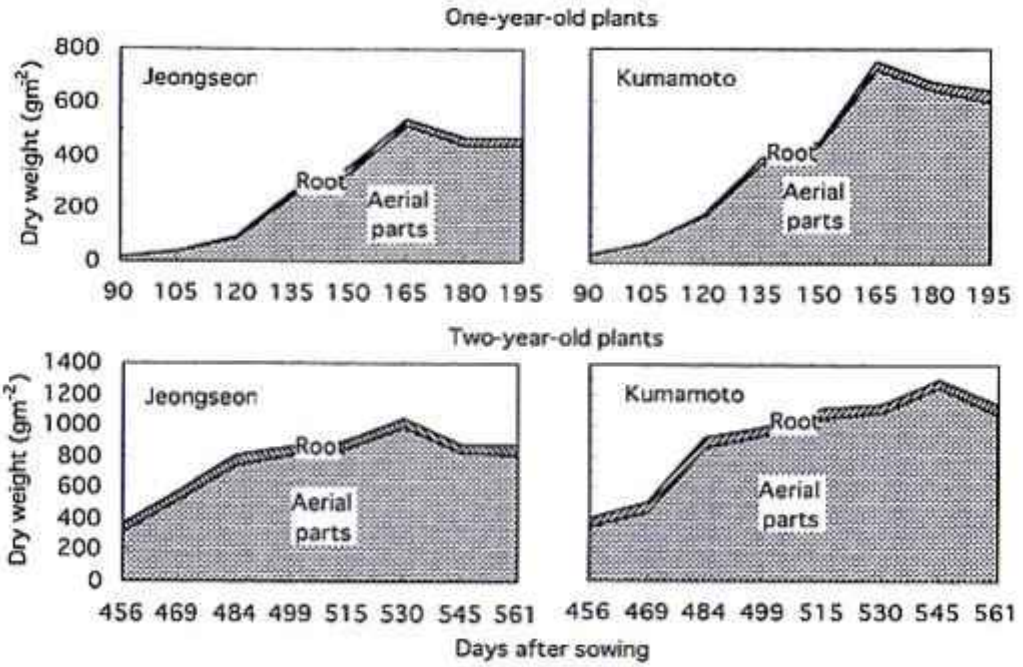


Fig. 6. Dry matter accumulation of aerial parts and roots in one-year-old and two-year -old *Bupleurum falcatum* for the cultivars from Jeongseon and Kumamoto.

The seasonal change in Eu during the growth period of one-year-old and two-year-old plants is shown in Fig. 8. The highest Eu value was observed at l80 （one-year-old plants） and 469 （two-year-old plants） days after sowing. Both cultivars attained high Eu around flowering stage regardless of plant age. Generally Eu was higher in the cultivar from Kumamoto compared to the cultivar from Jeongseon.

Significant correlation between the total DM and Eu was obtained in the one-year-old plants （r = 0.721 for the cultivar from Kumamoto, r = 0.761 for the cultivar from Jeongseon）. Though not significant, it showed a tendency of negative relation in two-year-old plants （Fig. 9）.

In both of one-year-old and two-year-old plants, Eu was associated with CGR and NAR with highly significant positive correlations （Table 1）.

Generally, correlation coefficients among the growth parameters of one-year-old plants differed from those of two-yew-old plants except some parameters as root length-root diameter. In one-year-old plants, total dry weight was closely related with another parameter. But negative or very low correlations were observed in two-year-old plants.

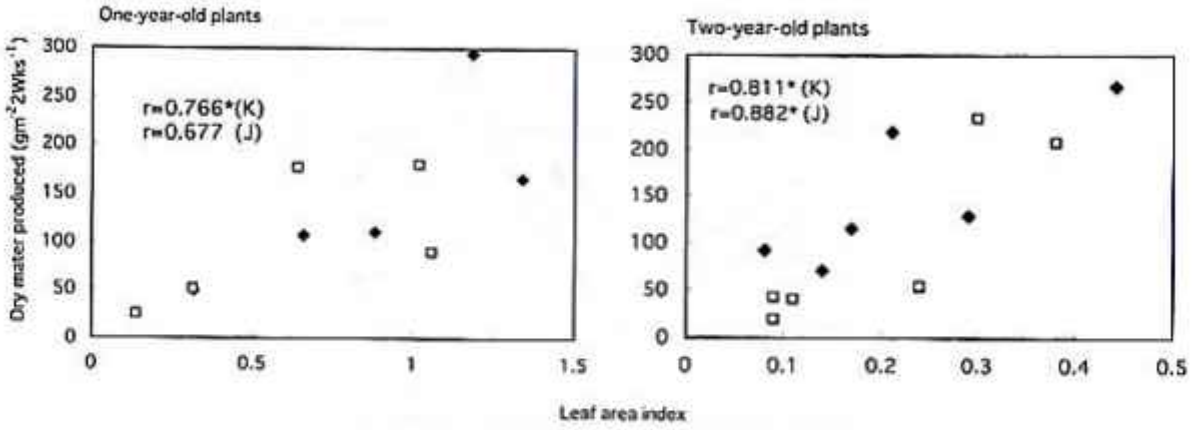


Fig. 7. Dry matter increase between sampling times as a function of LAI. *:5% level of significance. ◆:the cultivar from Kumamoto (K), □ :the cultivar from Jeongseon (J).

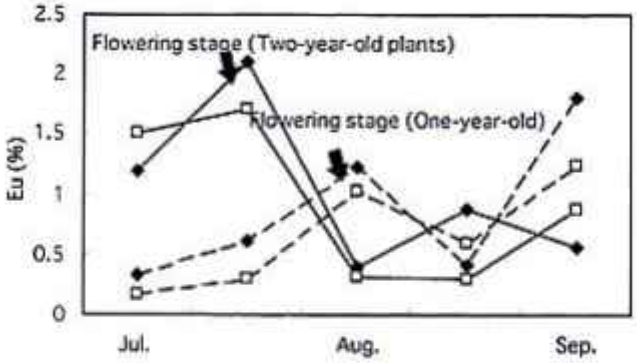


Fig. 8. The seasonal change in solar energy utilization (Eu) of one-year-old and two-year-old *Bupleurum falcatum*. □;the cultivar from Jeongseon and ◆;the cultivar from Kumamoto. ---;One-year-old plants, — ;Two-year-old plants.

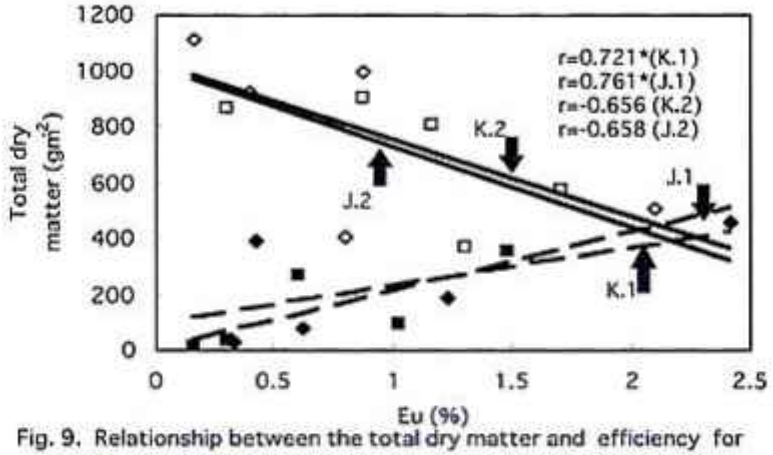


Fig. 9. Relationship between the total dry matter and efficiency for solar energy utilization (Eu) in one-year-old and two-year-old *Bupleurum falcatum*. *:5% level of significance.

K.1:One-year-old, the cultivar from Kumamoto (◇); J.1:One-year-old, the cultivar from Jeongseon (□); K.2:Two-year-old, the cultivar from Kumamoto (◆); J.2:Two-year-old, the cultivar from Jeongseon (■).

Table l. Correlation coefficients among growth parameters and plant characters.

	LAI	Height	Root length	Root diameter	Aerial part weight	Root weight	Total weight	CGR	RGR	NAR	
One-year-old plants											
LAI	-										
Height	0.639 *	-									
Root length	0.742 *	0.888 **	-								
Root diameter	0.661 *	0.966 **	0.874 **	-							
Aerial part weight	0.511	0.947 **	0.819 **	0.882 **	-						
Root weight	0.592	0.948 **	0.833 **	0.910 **	0.920 **	-					
Total weight	0.417	0.95l **	0.823**	0.886**	0.999**	0.927**	-				
CGR	0.553	0.717 *	0.66O *	0.68l *	0.619	0.417	0.609	-			
RGR	-0.870**	-0.770**	-0.759*	-0.740**	-0.860**	-0.947**	-0.863**	-0.180	-		
NAR	0.l96	0.442	0.400	0.379	0338	0.097	0.3Z5	0.912 **	0.155	-	
Eu	0.566	0.754 *	0,720 *	0.678 *	0.680 *	0.480	0.680 *	0.98l **	-0.250	0.906 **	
Two-year-old plants											
LAB	-										
Height	0.668 *	-									
Root length	-0.379	-0.266	-								
Root diameter	-0.l 59	-0.296	0.883 **	-							
Aerial part weight	-0.883 **	-0.484	0.217	0.058	-						
Root weight	0.l94	-0.l79	-0.435	-0,067	-0.091	-					
Total weight	-0.930 **	-0.488	0.210	0.057	1.000	-0.075					
CGR	0.559	0.078	0.030	0.211	-0.673 *	-0.028	-0.674 *				
RGR	0.716 *	0.255	-0.092	0.061	-0.808 **	-0.068	-0.810 **	0.95l **			
NAR	0.541	0.052	-0.l l8	0.137	-0.642 *	0.218	-0.640 *	0.883 **	0.795 **		
Eu	0.562	0.l87	0.025	0.196	-0.660 *	-0.079	-0.657 *	0.968 **	0.912 **	0.896 **	

Correlations are calculated among values of different growth stages.

*,**;5% andl % level of significance, respectively.

（2） Growth Characteristics and Saikosaponin Yield

Materials and Methods

Saikosaponin a, c, d contents were determined with a high performance liquid chromatography （HPLC; C-R6A, Shimadzu） following the method of Kimata et al. （l979）. After the roots sampled from field were dried at room temperature, eleven whole roots randomly selected per plot were pulverized using a grinder. For extraction, 2g of the powder was treated with 20 mL methanol at 60 ℃ for 2 hours. After filtration, the solution was adjusted to 25 mL with methanol. Five mL of this solution was evaporated to dryness. A solution of the residue in 0.5 mL of 2% HCl in 50% methanol was allowed to stand at 25 ℃ for 16 hours. The reaction mixture was neutralized with 2% NaOH. After the neutralized solution was made up to 10 mL with methanol, 10 μ L of solution was subjected to HPLC equipped with a UV-VIS detector （SPD-10A, Shimadzu）, a stainless column of 4.6 mm i.d. x 250 mm （Unisil Pack 5C18-250A, Gasukuro Kogyo） and a data processor （C-R6A, Hitachi）. Temperature was held at 30 ℃ and the mobile phase was composed of MeOH-H2O-AcOH-Et3N（80:20:0.2:0.2）. Flow rate was 1.0 mL min^{-1} and monitored at 251 nm （Fig. 10）.

Results

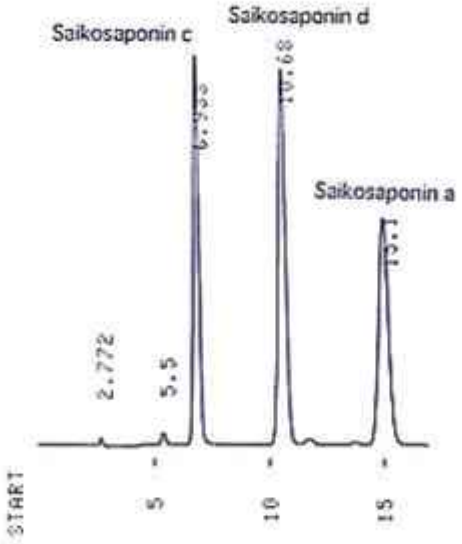


Fig. 10. Chromatogram of standard saikosaponin by HPLC. Absorbance 251nm.

There was a difference between plant age in the total saikosaponin content (Table 2). The roots of one-year-old plants had a higher concentration of saikosaponin than two-year-old plants. Saikosaponin a and d decreased with age, though saikosaponin c showed the opposite result or no changes. There was a difference between cultivars in total saikosaponin content for two-year-old plants and the cultivar from Jeongseon was higher than the cultivar from Kumamoto. But, no difference was found for one-year-old plants. For the yield of total saikosaponin obtained per unit area, the cultivar from Jeongseon showed a higher value than the cultivar from Kumamoto for two-year-old plants , but for one-year-old plants it showed the opposite result.

Table 3 represents yield and various characteristics on one-year-old and two-year-old plants in *B. falcatum*. From sowing to germinating, it took about 1 month. Plants flowered at 113 ～ 1 17 days for one-year-old plants and 455 ～ 455 days for two-year-old plants after sowing. Germinating and flowering time of the cultivar from Kumamoto were a few days later than that from Jeongseon for one-year-old plants but no difference was found for two-year-old plants. The average of plant height was 70.9 cm for the cultivar from Jeongseon and 80.1 cm for the cultivar from Kumamoto for one-year-old plants, and 93.6 cm and 109.2 cm for two-year-old plants, respectively. The root diameter of the cultivar from Kumamoto was larger than that from Jeongseon, while it showed a reversal trend in 1000-seed weight. The cultivar from Kumamoto was higher in root eight than that from Jeongseon.

Table 2. Saikosaponin contents in the roots of one-year-old and two-year-old *Bupleurum falcatum*.

Cultivars from	Plant age	Sampling number	Saikosaponin content (mg g^{-1}) a	c	d	Total a+c+d	Root Yield (gm^{-2})	Yield of total saikosaponins* (mg m^{-2})
Jeongseon								
	One year	12	0.60 ± 0.02	0.24 ± 0.03	0.73 ± 0.05	1.58a ± 0.08	36.5	57.7 ± 2.72
	Two year	11	0.34 ± 0.03	0.39 ± 0.03	0.54 ± 0.10	1.26b ± 0.15	59.5	74.9 ± 8.92
Kumamoto								
	One year	12	0.65 ± 0.13	0.28 ± 0.11	0.73 ± 0.18	1.66a ± 0.41	39.2	65.1 ± 15.9
	Two year	11	0.30 ± 0.07	0.27 ± 0.10	0.38 ± 0.13	0.95c ± 0.30	63.4	60.2 ± 18.9

* Yield of the roots (gm^{-2}) x total saikosaponin content. Values with the same letter are not significantly different by LSD test at 5% level.

Table 3. Yield and various characteristics in one-year-old and two-year-old plants.

Cultivars from	Plant age	Sowing date	Germination date	Flowering date	Plant height (cm)	Maximum root diameter (mm)	1000-seed weight (g)	Yield of root (g m^{-2})
Jeongseon								
	One year	Apr.5	May 5	Jul.28	70.9	8.1	1.79	36.5
	Two year			Jul.13	93.6	13.2		59.5
Kumamoto								
	One year	Apr.5	May 8	Aug. 1	80.1	8.7	1.65	39.2
	Two year			Jul. 13	109.2	14.4		63.4

Discussion

DM production is characterized by a similar response to LAI. A comparison of both showed that DM production was a similar function of LAI. It revealed that the relationship between DM production and LAI was linear. Brougham（l956） found that both the percent of solar radiation interception and the rate of DM production increased with LA development and approached a maximum. The results obtained from the present study showed that the curve of growth showed three distinct phases. For approximately 2 and a half months in spring, the growth rate increased slowly, then the rate had a rapid increase for the subsequent 2 months, and thereafter it declined. Maximum DM production continued until flowering stage. Eu and the rate of DM production increased with increasing LA until LA reached a maximum. It suggested that Eu was related to LA and DM production and the direct correspondence between them.

Isoda et al.（1994） reported that the cultivar with sparse leaf distribution in the upper layers of the canopy or with smaller leaflets intercepted a larger amount of radiation in soybean. In peanut, small-leaved cultivars showed lower intercepted radiation per unit leaf area index（interception efficiency）（Aboagye et al., 1994）. But, Maeda（l993） reported that the erect type and less branching habits of subspecies *fastigita* had been introduced into ssp. *hypogaea*, to increase yield in peanut. Aboagye et al.（1995） suggested that high radiation interception efficiency through, particularly, the reduction of self and/or mutual shading would increase DM production in peanut. DM production was proportional to the amount of radiation intercepted by crop （Shibles and Weber, 1965）. Bhatt （1995） reported that there was a significant

relationship of DM yield with photosynthetically active radiation intercepted, LAI, CGR and plant population. In the present experiment, there were positive relations between NAR vs. LAI, DM production vs. LAI, Eu vs. LAI, and Eu vs. DM production for one-year-old plants. The plants of the cultivar from Kumamoto had relatively high LAI, NAR and CGR values than those from Jeongseon. It was also higher in growth parameters such as plant height, root diameter and yield, except 1000-seed weight. Eu showed higher values around flowering stage. All these facts suggest that the larger leaf and/or LA in earlier growth stages may attain greater Eu, and higher DM production or yield in the case of *B. falcatum.*

Yamamoto et a1.（1996） reported values of Eu, 1.09 % for barley, 1.l5 ～ 1.94 % for rice and 0.92 % for soybean. In the present experiment, similar results were observed. Eu was 1.02 % for the cultivar from Jeongseon and 1.23 % for that from Kumamoto in one-year-old plants and 1.16 % for the cultivar from Jeongseon and 2.09 % for that from Kumamoto in two-year-old plants at flowering stage. Eu of the cultivar from Kumamoto was higher than that from Jeongseon. Eu in two-year-old plants was higher than in one-year-old plants. Leaf arrangement and orientation have been suggested as a factor of influencing the distribution of solar radiation within the crop canopy（Kasanaga and Monsi, 1954; Saeki, l960）. Although the relationship of DM and Eu with a plant type has not yet been demonstrated in *B. falcatum*, it was classified as bolting and non-bolting type and Tani et al.（1987） reported that non-bolting type had more contents of saikosaponins compared to bolting type. Two-year-old plants were consisted of erectophile leaves compared to one-year-old plants, which might have led to an increase in photosynthetic

productivity and high En.

Relationship of accumulated DM and Eu had different tendency between one-year-old and two-year-old plants. Horikoshi et al.（1976） reported that there was no significant relationship between the first year's growth and the second year's growth. In the present experiment, there was no relationship between one-year-old plants and two-year-old plants in DM, Eu, yield of roots and content of saikosaponin. Generally, the harvest of two-year-old plants was not advantageous because DM increase of two-year-old plants was not rapid particularly in latter growth stage. Moreover, root dry weight did not increase so much in two-year-old plants.

In both of the cultivars from Kumamoto and Jeongseon, the saikosaponin content of one-year-old plants was higher than that of two-year-old ones. For the yield of total saikosaponin calculated per square meters in two-year-old plants, the cultivar from Jeongseon was higher than that from Kumamoto because of the high saikosaponin content of the cultivar from Jeongseon. In one-year-old plants, saikosaponin yield of the cultivar from Kumamoto was higher than that from Jeongseon because of the high root yield of the cultivar from Kumamoto.

B. falcatum is a perennial medicinal plant and usually a second-year crop is harvested for the material of Chinese crude drugs. When only yield of saikosaponin obtained per square meters is considered, it is more efficient to harvest one-year-old *B. falcatum*. From these results, the annual cultivation of the high root yield and high saikosaponin content cultivar is recommended from the economic view point of saikosaponin production.

3. Variation and Distribution of Saikosaponin in a Root

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., l995), effects of mineral fertilizers (Minami and Sugino, 1995), distribution in a root (Tani et al., l986), 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., l988) 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 thee 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, Mishima, Tsukuba, Yasato) were grown. Cultivation, sampling methods and saikosaponin analysis were described in materials and methods of the previous section.

From the outer layer, the roots of the cultivar from Jeongseon were sliced using a microtome to obtain the phloem and xylen 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.

（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） X 100

Phloem ratio（%） =（phloem tissues area /total area） X 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 4 represents root dry weigh, root diameter and saikosaponin content in *B. falcatum* originated from seven different regions. There was a difference among cultivars in total saikosaponin content for one-yew-old plants and the values were in the order of Tsukuba＞ Jeongseon＞ Mishima＞ Yasato＞ Kumamoto＞ Fukuoka＞ Suwon. The order of saikosaponin composition was saikosaponin d, saikosaponin a＞ saikosaponin c for all cultivars.

（2） Distribution of saikosaponin in a root

Fig. 11 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 5）.

Fig. 12 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. 13. The correlation was negative（r = -0.837） at 1 % level of significance.

Distribution of saikosaponin in different tissues of the root of *B. falcatum* is presented in Table 5. 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.

Table 4. Dry weight and saikosaponin content of *Bupleurum falcatum.*

Cultivars	Dry weight of	Root diameter	Saikosaponin content (%)			
from	a root(g)	(mm)	a	c	d	Total
Mishima	2.31	9.68	0.64±0.013	0.39±0.035	0.77±0.111	1.795±0.159
Yasato	3.27	12.l9	0.53±0.l28	0.41±0.l35	0.63±0.205	1.565±0.468
Tsukuba	3.06	0.69	0.62±0.091	0.58±0.0935	0.90±0.136	2.095±0.323
Kumamoto	3.02	10.90	0.37±0290	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±0.02l	0.18±0.018	0.32±0.052	0.820±0.090

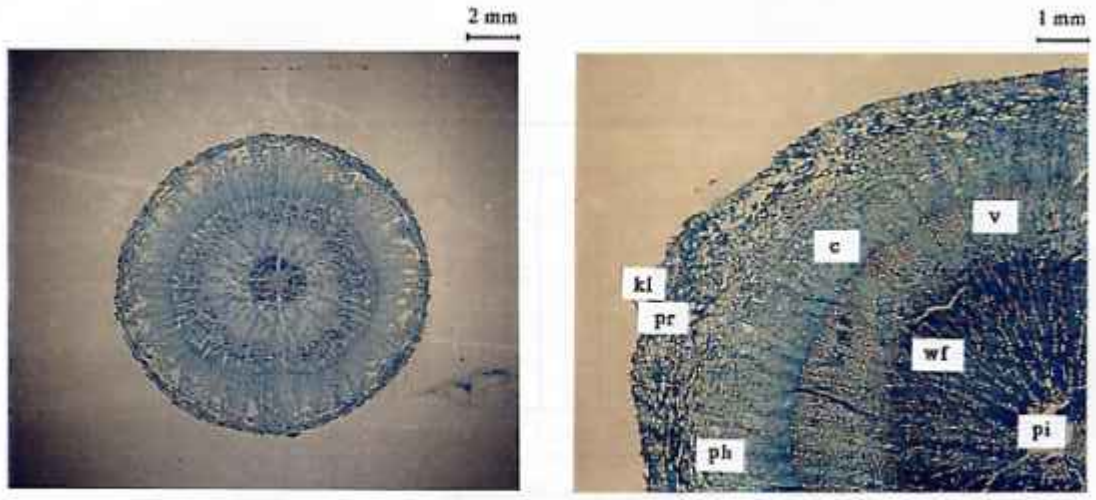


Fig. 11. A cross-section of one-year-old (left) and two-year-old (right) *B. falcatum* root.
kl, cork layer; pr, pericycle; c, cambium; v, vessel; wf, wood fiber; pi, pith; ph, phloem.

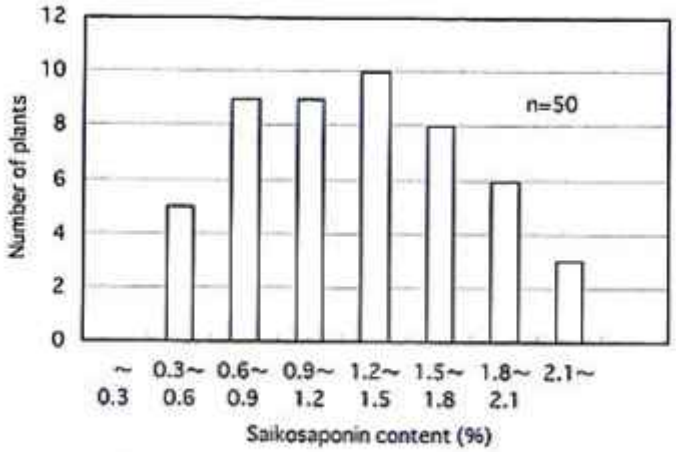


Fig. 12. Frequancy distribution of total saikosaponin content of one-year-old plants.

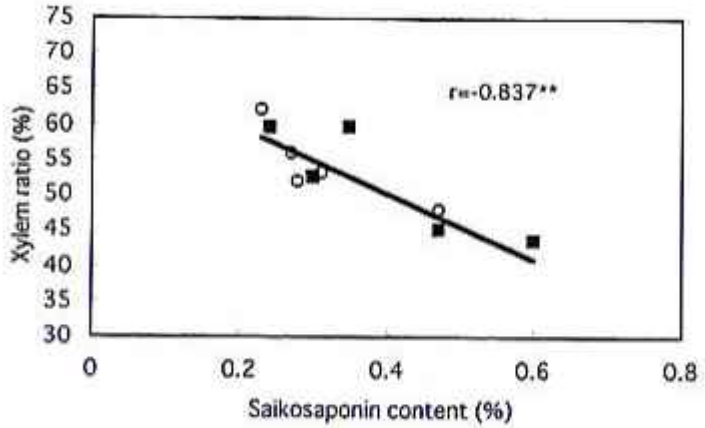


Fig. 13. Relationship between saikosaponin content and xylem ratio in *Bupleurum falcatum*. ○:one-year-old plants; ■:two-year-old plants; **:1% level of significance.

Table 5. Distribution of saikosaponin in different tissues of the root of *Bupleurum falcatum.*

Tissues	Area ratio	Saikosaponin content (%)				Distribution
	(%)	a	c	d	Total	rate (%) *
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	83.9 ± 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) X 100;
(Total saikosaponins content in xylem tissues/total saikosaponins content in phloem and xylem tissues) X 100.

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 6. 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 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.

Table 6. Saikosaponin content in different parts of the root of *Bupleurum 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	033 ± 0.042	0.23 ± 0.023	0.39 ± 0.051	0.94 ± 0.116
middle part of root	0.5 ± 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

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.（l995） 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.

II. HAPLOID INDUCTION

1. Introduction

ln addition to clarify the characteristics of DM and saikosaponin production and Eu, genetical improvement of *B. falcatum* is indispensable for obtaining high yield and quality of roots, especially to find the way of shortening the breeding intervals.

B. falcatum is a cross pollinating species and genetically heterozygous. Anther culture is usually used for production of haploid plants from pollen grains to promote a rapid large scale production of homozygous plants. Homozygous plants obtained by doubling their chromosome number can be used as inbred lines in hybrid breeding. The monoploids with only one set of homologous chromosome are useful for selecting plants with desirable characteristics. Also, using the complete homozygosity achieved makes easier the practice of genetic experiments（Collins and Sunderland, 1974）. In order to select promising genotypes effectively at the monoploid level, large quantities of androgenesis embryos must be produced and evaluated. Since the first haploid plant was obtained through pollen embryogenesis in the anther culture of *Datura innoxia*（Guha and Maheswari, l964）, many studies have been carried out using various crop species. Some studies have been conducted on Umbelliferae species using carrot anther （Collins and Sunderland, l974; Hu et al., 1993）. For *B. falcatum*, some studies on tissue culture were reported using another parts i.e.; root, leaf and shoot apex（Amano et al., 1989; Fujioka et al., 1987; Hiraoka et al., 1983, 1986; Lee et al., 1988; Park et a1., 1994, 1995; Xia et al., 1992）. However, there is no successful study on anther culture in *B. falcatum* reported.

Within the anther culture system, there is usually much variability for callus formation and embryo production. Efficiency in anther culture has been attributed to the genotypes（Collins and Sunderland, l974; Lu et al., 1991）, the temperature pretreatment（Yamaguchi et al., 1990）, the stage of anther inoculated（Collins and Sunderland, 1974; Corduan and Spix, l975; Oono, 1975; Tomes and Collins, 1976; Wang et al., 1973）, and combination of kinds and concentration of hormones in the medium（Hu et al., 1993; Matsubara et al., 1992）. Thus, evaluation of each condition is important for identifying the optimum procedure of anther culture.

The haploid plantlets originated from anther culture of *B. falcatum* should have a half of the diploid chromosome number （n=13） of their originated plant （2n=26）. Variation of chromosome number was reported for different cultivars（Amano et al., 1989; Chung et al., 1995; Ohta, 1991; Ohta et al., l986）. Regenerated plantlets might also have various kinds of aneuploidy （Amano et al., 1989; Kohda et al., 1990）. Therefore, it is necessary to observe chromosome numbers for roots from seeds of various cultivars and for plantlets regenerated through anther culture to confirm the haploidy.

Objectives in this chapter are;

-to investigate and determine the optimum condition for the callus formation in anther culture.

-to produce haploid plantlets through somatic embryogenesis by anther culture.

-to observe variation of chromosome numbers for plants and regenerated plantlets.

2. Optimum Condition for Callus Formation in Anther Culture

B. falcatum is a cross-pollinating crop and its genetic composition is heterozygous. Anther culture is used for production of haploid plants to conduct a rapid production of homozygous plants. The haploids, which have only one genome, are useful for selecting recessive characteristics because the genotype can be identified by the phenotype. Since the first haploid plant was obtained in the anther culture of *Datura innoxia* (Guha and Maheswari, 1964), many studies have been carried out using various crop species. But no publication has reported the production of haploid plants having a half (n=13) of the chromosome number of the originated plant (2n=26) through anther culture in *B. falcatum*.

Within anther culture system, there is much variability for callus formation and embryoid production efficiency. Objective of this study is to evaluate several factors such as stage of anther development, temperature pretreatment, and phytohormones which may affect callus formation from anther culture of *B. falcatum*. Materials originated from two different habitats were used and the difference between the genotypes in the response to these factors was also studied.

Materials and Methods

Two cultivars, originated from Jeongseon, Korea and Kumamoto, Japan, were grown in the field of Kyushu University in l995. Generally, flowers containing anthers of less than 200 μ m in length were collected in the morning.

They were immersed in 70% ethanol for ten seconds and 0.5% sodium hypochlorite of active chlorine with a drop of Tween 20 for 15 minutes for surface sterilization and then rinsed three times with sterile distilled water. After removal of the petals under microscope, anthers were transferred to a petri dish and then incubated at 25 ℃ in the dark. Culture medium contained 20 ml of MS medium （Murashige and Skoog, 1962）, supplemented with 30 g of sucrose, 1 mg of 2,4-D, 7 g of agar per liter and adjusted to pH 5.8 except for the studies on hormone combinations.

For the study of the optimum stage of anther, anthers to be inoculated were classified into three classes, i.e. less than 200 μ m, 200 ～ 300 μ m and more than 300 μ m in length and the developmental stage of microspores was determined by microscopic observation （Table 7）.

To investigate the effect of low temperature pretreatment, flowers containing anthers, were kept in a petri dish to prevent desiccation, and subjected to the low temperature pretreatment at 0, 5, 10, and l5 ℃ for 24, 72 and 120 hours in the dark and then anthers were inoculated.

To investigate the effect of phytohormones, eleven treatments were employed namely: 2,4-D, NAA, kinetine, zeatin, picloran, and hormone concentrations from 0.1 to 2 mg per liter. The rate of callus formation was represented by the percentage of the number of callus-forming anthers to that of the plated anthers.

Table 7. Developmental stage of anther of *Bupleurum falcatum*.

Length of anther (μm)	Developmental stage of pollen	Color of anther
~ 200	~ Uninucleate	Colorless
200 ~ 300	Uninucleate ~ Binucleate	Green
300 ~	Binucleate ~	Yellowish

Results

Callus formation started in some anthers from about 3 weeks after inoculating. But, almost all anthers formed callus about 5 weeks after inoculating. The effect of stage of anther on callus formation is shown in Table 8. For anthers of less than 200 μ m in length, which were very young and colorless and contained pollens of uninucleate or younger stage, the average of callus formation was 30.1 % for the cultivar from Jeongseon and 41.2 % for that from Kumamoto. However, for anthers of above 300 μ m, which were yellowish, very poor callus formation rate was observed in both of the materials.

Table 9 represents the effect of low temperature pretreatments on callus formation. Exposure of the anther at 5 or 10 ℃ for 72 or 120 hours was very effective for both cultivars. The cultivars from Jeongseon and Kumamoto showed 18.9 ～ 37.8 % and 36.8 ～ 40.5 % in frequency, respectively. Whereas exposuring anthers to 0 or l5 ℃ was not effective for both of the materials. No callus was induced or very low in frequency at 0 or 15 ℃ .

Table 10 shows the effect of concentrations of phytohormones on callus formation.

Table 8. Effect of stage of anther on callus formation.

Length of anther (μ m)	No. of anthers cultured		No. of calli obtained		Frequency (%)*	
	J**	K**	J	K	J	K
～ 200	259	296	78	122	30.1	41.2
200 ～ 300	370	222	63	28	17.0	12.6
300 ～	185	148	7	1	3.8	.007

* $\frac{\text{No. of calli obtained}}{\text{No. of anthers cultured}}$ X 100

** J: the cultivar from Jeongseon, K: the cultivar from Kumamoto.

Table 9. Effect of low temperature on callus formation.

Temp.	Hours	No. of anthers cultured		No. of calli obtained		Frequency (%)*	
		J**	K**	J	K	J	K
0	24	185	185	0	8	0	4.3
	72	37	185	0	0	0	0
	120	185	185	0	2	0	1.1
5	24	185	666	11	57	6.0	8.6
	72	185	407	68	157	36.8	38.6
	120	185	185	63	75	34.1	40.5
10	24	185	185	19	17	10.3	9.2
	72	148	185	28	68	18.9	36.8
	120	185	185	70	73	37.8	39.5
15	24	185	185	0	0	0	0
	72	185	185	1	0	.005	0
	120	185	185	9	0	4.9	0

$$\frac{\text{No. of calli obtained}}{\text{No. of anthers cultured}} \times 100$$

** J: the cultivar from Jeongseon, K:the cultivar from Kumamoto.

Table 10. Effect of phytohormones on callus formation.

2.4-D	NAA	Kinetin	Zeatin	Picloram	No of anthers cultured		No of calli obtained		Frequency (%) *	
($mg\ L^{-1}$)					J**	K**	J	K	J	K
0.1	0.1				111	9			8.1	
0.1				0.1	185	185	48	110	26.0	59.5
0.1				1	185	148	0	0	0	0
0.1		1			74		14		18.9	
0.1			1		37		7		18.9	
0.1		2			37		7		18.9	
0.1			2		74		14		18.9	
0.1	2				185	185	0	0	0	0
1		0.1			370	296	11	21	3.0	7.1
1	0.I				185	111	11	21	6.0	18.9
1				0.1	185	185	18	3	9.7	1.6
1				1	185		32			17.3
1		2			185	148	0	0	0	0
1					74	74	33	21	44.6	28.4
	1				185	185	0	1	0	.005
		1			185	370	1	0	.005	0
			1		185		0		0	
				1	370	370	83	64	22.4	17.3

* $\frac{\text{No. of calli obtained}}{\text{No. of anthers cultured}} \times 100$

** J: the cultivar from Jeongseon, K: the cultivar from Kumamoto

The medium containing 0.1 mg of 2,4-D + 0.l mg of picloram per liter was the most favorable for both cultivars. The frequency was 26.0 % for Jeongseon and 59.5 % for Kumamoto. For single hormone treatment, the medium containing 1 mg of 2,4-D per liter was the most favorable and the frequency was 44.6% for the cultivar from Jeongseon and 28.4 % for that from Kumamoto. No callusing or very low rate, however, was observed in the medium containing NAA only.

Discussion

Study of factors affecting callus formation and haploid production will be needed for successful anther culture. For successful anther culture, the optimum developmental stage of anther or microspore to be cultured must be decided（Collins and Sunderland, 1974; Corduan and Spix, 1975; Oono, 1975; Tomes and Collins, l976; Wang et al., 1973）. Wang et al. （1973） suggested that anthers containing late uninucleate pollem were the optimum stage for wheat. Corduan and Spix （l975） also obtained affluent callus formation from anthers of *Digitalis purpurea* between the late tetrad stage and the early uninucleate stage of the micropore. Oono （l975） reported that pollen at uninucleate stage （or near to this stage） exclusively formed callus in the anther culture of *Oryza sativa*. Collins and Sunderland （1974） found the early uninucleate stage optimum for culturing *Nicotiana attenuata, N. raimondii*, and *N. knightiana* anthers. Tomes and Collins （1976） reported the same results with Collins and Sunderland （1974）. In the present study, high frequency callus formation was obtained at uninucleate stage. This result is in agreement with the case in rice.

Lu et al.（1991） observed in wheat anther culture that both genotype and environment of the donor plant had a significant effect on the embryoid yield. One of the most important factors for callus formation in environmental conditions is temperature pretreatments and the effectiveness of low temperature pretreatment was obtained for rice（Genovesi and Magill, 1979; Yamaguchi et al., 1990）. For *B. falcatum*, low temperature pretreatment at 5 or 10 ℃ for 72 or 120 hours was shown to be effective for callus formation. This stimulatory temperature shock might have activated cell division of pollen grains at uninucleate stage. Yamaguchi et al.（1990） reported differences among rice cultivars in callus-forming response to the temperature and duration of pretreatment, but both cultivars in this study showed a similar response to the temperature and duration and no difference was found between cultivars of *B. falcatum*.

The composition and concentrations of phytohormones in the medium are important factors determining not only the callus and embryoid formation from anthers but also the subsequent regeneration of the plantlets. For instance, eggplant and pepper of Solanaceae and carrot of Umbelliferae anthers require both auxins and cytokinins（Hu et al., 1993,; Matsubara et al., 1992）. Optimal concentrations of 2,4-D are different among species such as carrot, eggplant and pepper（Hu et a1., 1993; Matsubara et a1., 1992）. However, for *B. falcatum* in present study, the single treatment of auxin（1 mg of 2,4-D per liter） was effective and also complex treatment of 0.l mg of 2,4-D and 0.1 mg of picloram per liter was effective at the same level. Factors of inducing callus and embryogenesis might vary with cultivars, but no differences in materials originated from two different habitats were observed in the present study.

In conclusion, it was found that for *B. falcatum* anther culture, very young anther having pollens of uninucleate or younger stage, low temperature pretreatment of 5 ～ 10 ℃ for 72 ～ 120 hours and phytohormones of 1 mg of 2,4-D per liter or 0.1 mg of 2A-D + 0.1 mg of picloram per liter were most effective for callus formation. This optimum condition was used for the anther culture experiment described later in this thesis.

3. Induction of Haploid by Anther Culture

In the previous section, the optimum condition for callus induction in anther culture was determined. In this section, the production of haploid plants through somatic embryogenesis by way of anther culture for *B. falcatum* was attempted. Differences among genotypes for callus induction was also studied.

Materials and Methods

（1） Comparison of callus formation among genotypes

Three *B. falcatum* cultivars originated from Fukuoka and Kumamoto, Japan and from Jeongseon, Korea were grown in a field at Kyushu University. Anthers were obtained in July to October, l995. Anthers from one plant were cultured on the same day. Anthers at the uninucleate stage were collected in the morning, and were surface-sterilized in ethanol, and 2% sodium hypochlorite and rinsed three times with sterile distilled water. Following the method described in the previous section, the MS medium（Murashige and Skoog, 1962） containing 3% sucrose and 1 mg of 2,4-D per litter adjusted to pH 5.8 was used for callus induction. The anthers were placed in a petri dish containing 20 mL of the medium and then incubated at 25 ℃ in the dark.

（2） Induction of haploid plantlets

After 5 weeks of incubation one callus block of 3 mm in diameter was plated in a petri dish with 10 mL regeneration medium containing 3% sucrose without phytohormones, and cultured to induce plant regeneration at 25 ℃ under a 16 hour-photoperiod of 400 μ mol m^{-2} s^{-1} PAR

following the method used in carrot（Hu et al., l993）. For chromosome observation, root tips were pretreated with a solution of 0.1 % colchicine for 2 hours at 20 ℃ . After fixation in ethanol/acetic acid（3:1 v/v） for 30 min. at 5 ℃ , they were treated in 1N HCl for 5 min. and stained in an 1 % aceto-orcein solution. Chromosomes of plants grown from the seed as well as anther-derived plantlets were observed.

Results

（1） Comparison of callus formation among genotypes

The formation of callus was observed from about 3 weeks after inoculation. A total of 4736 anthers were cultured, and the obtained calli totaled 990. The frequency of callus formation of different cultivars, from July l5 to August 17, is shown in Table 11. The value of callus formation based on the average frequency was 22.6 %, 10.0 % and 23.1 % for the cultivars from Fukuoka, Kumamoto, Jeongseon, respectively. The values in Table 11 also vary with plants. The difference among plants on callus formation was large and in the case of the cultivar from Fukuoka, it varied from 1.8 % to 51.1 %. The frequency of callus formation was particularly high for the plants cultured on July 29. Callus formation frequency differed among individual plants as well as cultivars.

Table 11. Callus formation of *B. falcatum* from three cultivars during July 15 to August 12.

Date	No. of anthers cultured			No. of calli obtained			The mean of frequency (%)
	F**	J**	K**	F	J	K	
Jul. 15	111			2			
19	296			108			
22	333			20			
25	370			12			22.6
27	222			51			(1.8-51.1) *
28	148			7			
29	370			189			
31	185			80			
Aug. 4	185			32			
Aug. 1			222			14	10.0
3			259			30	(6.3-111.7) *
9			222			26	
10		518			94		
11		481			163		23.1
12		444			76		(17.1-33.9) *
17		370			86		
Total	2220	1813	703	501	419	70	

*The range of frequency of callus formation.

**F: the cultivar from Fukuoka, J:the cultivar from Jeongseon, K: the cultivar from Kumamoto.

（2） Induction of haploid plantlets

Figure 14 shows callus formation from a pollen of anther about 3 weeks after planting. The calli were transferred to the regeneration medium. Two weeks later, roots and leaves were induced from callus. A total of 27 plantlets were regenerated. The average frequency of plantlets obtained from anthers cultured was 0.6 %. The average frequency of plantlets obtained from calli was 2.7 %. For acclimatization, the plantlets with roots were transplanted in a jiffy pot filled with vermiculite and maintained in 90 % humidity for about 1 month. The plantlets are growing in the laboratory（Fig. 15）.

The chromosome number of *B. falcatum* was determined as three basic cytogenetic types, 2n=20, 26 and 32 （Amano et al., l989; Chung et al., 1995; Ohta, l991） though aneuploid variations were observed. The chromosome number of many plants in this experiment was 2n=26 （Fig. l6,B）. Fig. l6（A） shows that the number of chromosomes of an anther-derived plantlet was n= 13, showing that this plantlet was a haploid which might be derived from a plant having 2n=26 chromosomes.

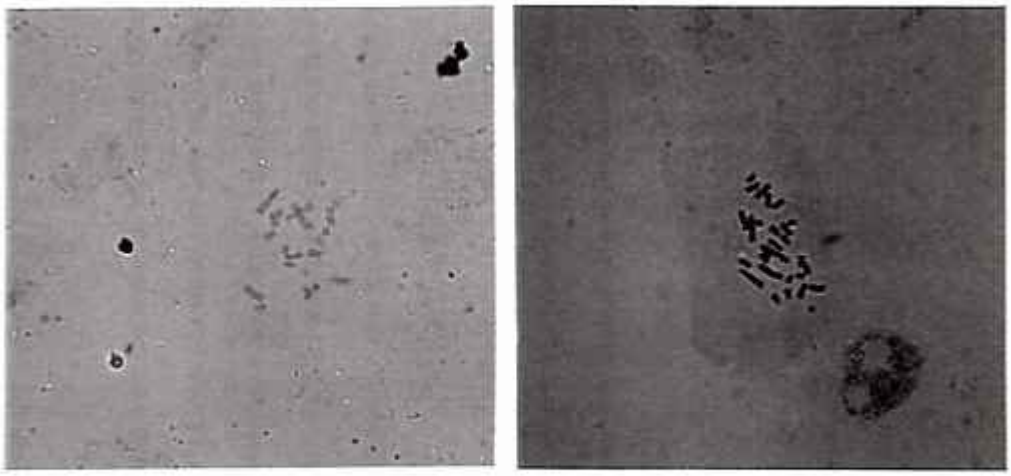
Discussion

Variations among genotypes for callus formation and plant regeneration were commonly observed in many species （Collins, l977）. In *B. falcatum*, variations among genotypes （among cultivars and among individual plants） were also observed in this study. Genotype identified having high callus inducing and regeneration ability can be used for future experiments very effectively.

Fig. 14. Anther before callusing (left), callus formation from a pollen (middle) and callus from a filament (right).

A;After 1 month from transferred to a regeneration medium B;Regenerated plantlets in a flask C;Regenerated plantlets in a pot

Fig. 15. Plantlets regenerated through somatic embryogenesis.



A;n=13 B;2n=26

Fig. 16. Chromosomes in a root tip cell of a regenerated plantlet (A) and a plant from a seed (B).

Haploid plantlets, which were conformed by chromosome observation as haploidy, were successfully induced. The author did have callus from an anther filament（Fig. 14）, which was discarded and not counted as a callus-forming anther. A regenerated plant from filament-derived callus might have 2n=26 chromosomes, because the filament was originated from sporophytic tissues of chromosomes, 2n=26.

This is the first report on the production of a haploid plantlet through the anther culture of *B. falcatum*. Haploid-production may offer a tool for more efficient breeding programs and genetic studies in *B. falcatum*.

4. Variation of chromosome number for plants and regenerated plantlets

B. falcatum has variation of chromosome number in different geographical origins as well as in the same cultivar with 2n=20, 26, 32 of three basic types（Amano et al., 1989; Chung et al., 1995; Ohta et a1., 1986; Ohta, 1991）. Furthermore, variation of chromosome number was observed in the regenerated plants from tissue culture（Amano et al., l989; Kohda et a1., 1990）.

The objective of this study is to obtain more information on the chromosome number variation for different cultivars and regenerated plantlets through anther culture.

Materials and Methods

Three cultivars, originated from Jeongseon, Korea and Fukuoka, Kumamoto, Japan, were used for somatic chromosome observation. Seeds were germinated for l month in a jiffy pot at 20 ℃ and the root tips were used for chromosome observation. Root tips were pretreated with a solution of 0.1 % colchicine for 2 hours at 20 ℃. After fixation in ethanol/acetic acid（3 : 1 %）for 30 min. at 5 ℃ and macerated with 1N HCl for 5 ～ 7 min. at 60 ℃, they were stained in an 1 % aceto-orcein solution. For chromosome observation of regenerated plantlets, root tips, obtained from regenerated plantlets through anther culture, were treated with 2M 8-hydrooxyquinline for 4-5 hours at 20 ℃, fixed in 45% glacial acetic acid for 5 to 10 min. at 5 ℃, macerated with a mixture solution of 1N HCl and 45% glacial acetic acid（2: 1） for 10 seconds at 60 ℃ and stained with 1 % aceto-orcein.

Results

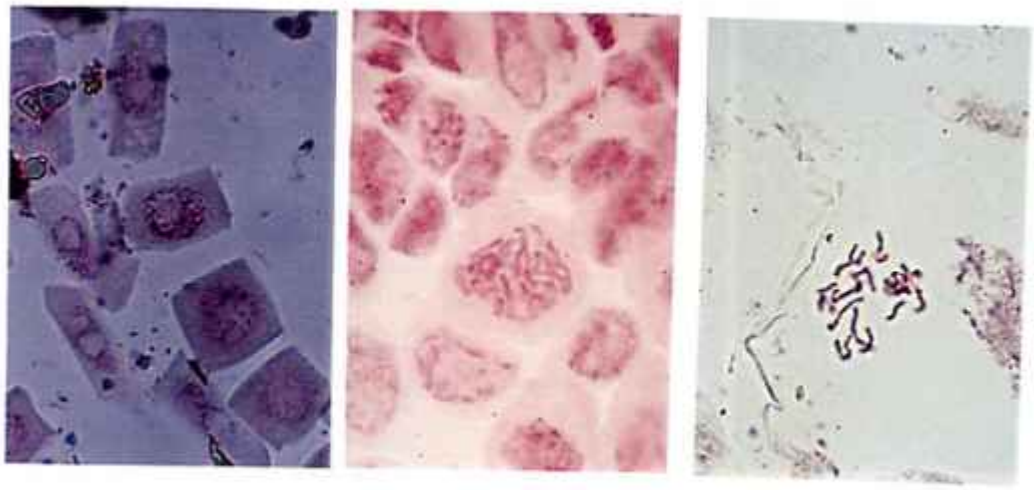
ln *B. falcatum*, the chromosome formed dark stained chromatin threads and spread over the nucleus uniformly at interphase（Fig. l7, A）. At prophase, all chromosomes were concentrated homogeneously along with their long axis, thus early and late concentrated segments were not distinguishable（Fig. 17, B）. At metaphase, all chromosomes were observed very clearly （Fig. 18）.

Fig. 18 shows somatic chromosomes of *B. falcatum* of different cultivars. Chromosome number was determined to be 2n=20 （the cultivar from Fukuoka）, and 2n=26 （the cultivars from Kumamoto and Jeongseon）.

Variation of somatic chromosomes of *B. falcatum* from seeds is represented in Fig. 19. Anuploids of somatic chromosome were found as follows; 2n=23, 25 for the cultivar from Jeongseon, 2n=18 for that from Fukuoka, and 2n=22, 24, 48 for that from Kumamoto（Fig. 19 and Table 12）.

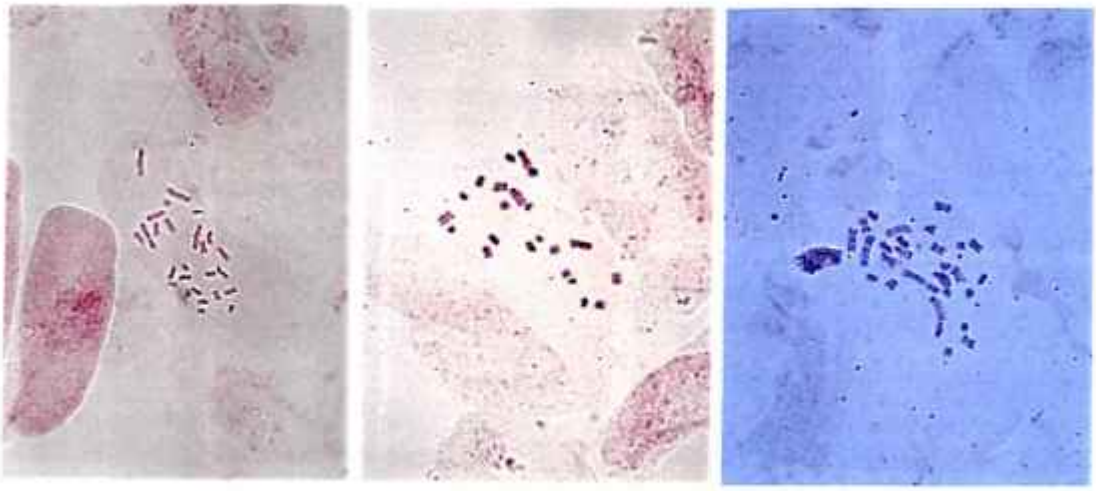
Discussion

In this study, somatic chromosome numbers of 2n=20, 26 （the cultivars from Fukuoka and Kumamoto） were observed. This number confirms previous reports of Ohta et al.（1986） and Ohta（199l）.



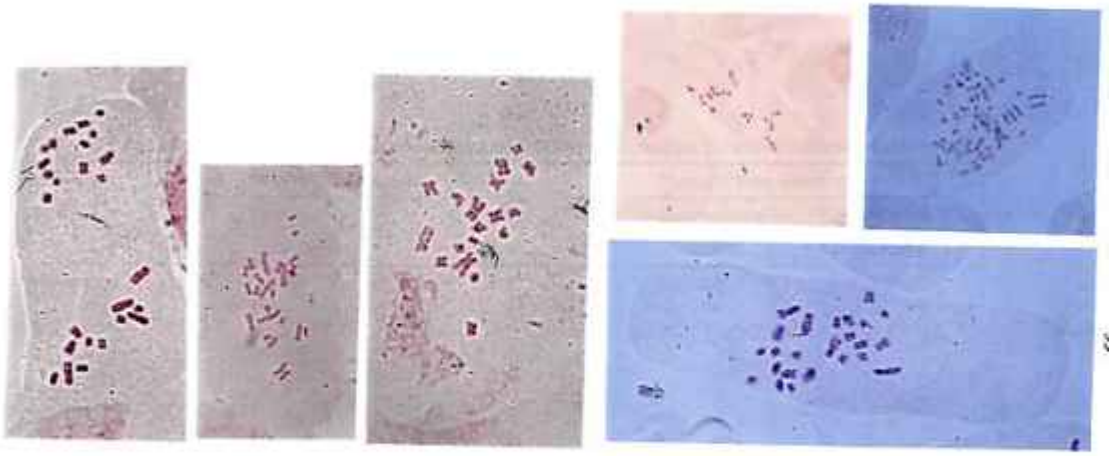
Interphase (A) Early prophase (B) Late prophase (C)

Fig. 17. Somatic chromosomes at interphase and prophase in *B. falcatum*.



Jeongseon (2n=26) Fukuoka (2n=20) Kumamoto (2n=26)

Fig. 18. Somatic chromosomes of *B. falcatum* of three cultivars from Jeongseon, Fukuoka and Kumamoto.



Jeongseon (2n=25,23) Fukuoka (2n=18) Kumamoto (2n=48,24,22)

Fig. 19. Variation of somatic chromosomes of *B. falcatum* from seeds of three cultivar from Jeongseon, Fukuoka and Kumamoto.

Table 12. Chromosome numbers of the plants from seeds and regenerated plantlets by anther culture of *B. falcatum*.

Cultivars from	Plant from seeds		Plantlets from anther culture	
	chromosome number	Number of plants	Chromosome number	Number of plantlets
Kumamoto	48	1	13	5
	26	22		
	24	1		
	22	1		
Jeongseon	26	17	13	4
	25	1		
	23	1		
Fukuoka	20	6		
	18	1		

The chromosome number of the cultivar from Jeongseon was determined for the first time as 2n=26. Most of the chromosome numbers was the same as the basic type with a few variation in this study. Ohta (1991) reported that *B. falcatum* showed polymorphic variation in external morphology such as plaint size, shapes of stem and basal leaves, size of florets, and had various chromosome numbers, i.e. 2n=19, 20, 21, 22-23, 24, 25, 27, 27-28, 27-29, 29-31, 30, 30-31, 32-34, 33, 34, 37, 40.

In regenerated plants by tissue culture, the variation in chromosome number differed among different cultivars; large frequency of chromosome number variation for the cultivars from Hiraodai (93.9 %) and that from Kirishima (94.1 %) and no variation for that from Mishima (Amano et al., 1989). Kohda et al. (1990) reported that propagations of the shoot primordia of *B. falcatum* were highly stable in the chromosome number as less than 1 % in frequency of chromosome number variation. In the present study, all regenerated plantlets by anther culture were stable in chromosome number as n=13, though a few materials were counted for regenerated plantlets.

B. falcatum was found to have a lot of intraspecific variation in external morphology as well as internal cytology. Thus, for classification and identification of cultivars and production of high yield and quality cultivars, homozygous plants through tissues culture are needed.

III. CONCLUSIONS

In this thesis, the author revealed the characteristics of dry matter（DM） production, growth pattern, efficiency for solar energy utilization （Eu）, and yield of saikosaponins and the differences in these factors, using different cultivars of *B. falcatum*. Also haploid plantlets were successfully created through anther culture for the first time in the world in this particular species.

Generally, the production of medicinal plants depends on both of environmental and genetical conditions. However, restricted information is available for the growth, development, DM and saikosaponin production and Eu in *B. falcatum* except for the works by Hiraoka et al.（1986）, Horikoshi et al.（1976）, Katano et al.（l989）, Kimata et al.（1979）, and Shimokawa et al.（1980）. Further, as *B. falcatum* has wide variations in growth, yield, quality, and so on, genetically uniform cultivars with high yield and quality are urgently needed.

In this study, root yield as well as saikosaponin content showed the differences between cultivars. The cultivar originated from Kumamoto, Japan showed higher mean vaules of DM production parameters measured than that from Jeongseon, Korea regardless of plant age. This was interpreted to suggest the difference among genotypes for the potential crop productivity in *B. falcatum*. Eu values varied between genotypes and the maximum values were 1.02 %～ 1.23 % for one-year-old plants and 1.16 %～ 2.09 % for two-year-old plants at flowering stage, which were nearly the same as other crop plants reported previously（Yamamoto et a1., 1996）. The cultivar from Kumamoto showed higher Eu values than that from Jeongseon. It was shown that

Eu related to LA and DM production and directly correspondent to DM production rate. But, the roots of the cultivar from Jeongseon had higher saikosaponin content than those from Kumamoto. It is proposed, therefore, that for the higher yield of saikosaponins per unit area in a year of *B. falcatum*, high root yield of the cultivar from Kumamoto and high saikosaponin content of that from Jeongseon must be combined by cross-breeding between the two cultivars.

Since haploid plants were first obtained in the anther culture of *Datura innoxia*, in 1964, many studies on anther culture have been carried out using various plant species. *B. falcatum* is a Umbelliferae species. For Umbelliferae, haploid plants were induced only in carrot (*Daucus carota*), using anther culture (Hu et al., 1993). But the formation of haploids by anther culture has not been reported in *B. falcatum*. Within the anther culture system, there was much variability for callus formation and embryo production. Variation in anther culture has been attributed to the developmental stage of anther, the kind of the medium, low temperature pretreatment, genotype and so on (Lu et al., 1991; Matsubara et al., 1992; Oono, 1975; Tomes et al., l976; Yamaguchi et al., l990). The results obtained in the present showed that exposure of anthers on 5 ℃ or 10 ℃ for 72 or 120 hours, the size of less than 200 μ m anther length, and 0.1 mg of 2,4-D and 0.1 mg of picloram in the MS media was the best combination of the treatment. For the best single hormone, 1 mg of 2,4-D in MS median was recommended. These findings enabled the future anther culture work in *B. falcatum* to be very efficient

It was also found that the differences in callus formation were observed among cultivars as well as individual plants. Variation among genotypes for callus formation and plant regeneration

was widely observed in many plant species （Collins 1977）. Though the details about the mechanism and the genetics about the differences among genotypes were not studied in this thesis, plants which were found having high callus formation ability could be used for the future experiments effectively.

The formation of somatic embryogenesis from pollen of anther was observed after 5 weeks of culture. It is known that *B. falcatum* has a wide variation in chromosome number depending on geographical origins as 2n=19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 3l, 32, 33, 34, 37, 40 （Ohta, 1991; Amano et al., 1989; Chung et al., 1995）. Using diploid plants with 2n=26 chromosomes, the plantlets obtained through anther culture were confirmed as haploid with n= 13 chromosomes. On the basis of the haploid production technic through anther culture established by the author, further studies about pure line selection, hybrid breeding, genetics especially about the phenotypic expression of recessive characters are expected to accelerate greatly.

Recently a lot of experiments for saikosaponin production have been attempted through biotechnological methods including tissue culture for secondary metabolite production （Jo et al., 1990; Kim et a1., l995）. Jo et al.（1990） reported that one-year-old roots from somatic embryos and three-month-old adventitious roots from callus had remarkably higher saikosaponin content than one-year-old root from seeds. Hiraoka et a1. （l986） reported that morphological characteristics and selected secondary metabolite contents of *in vitro* propagated plants were remarkably uniform compared to those of the plants propagated by seeds and the asexually propagated plants were higher in total saikosaponin content than those of sexually propagated

plants. Further studies about saikosaponin production *in vitro* are also expected.

In conclusion, this thesis not only revealed the fundamental characteristics of biomass and saikosaponin production, and efficiency for solar energy utilization in *B. falcatum* but also opened the new way for genetical improvements by anther culture for the species which was a valuable crop but had received relatively little scientific attention so far. For the future progresses of the agronomical and genetical improvements of this crop, more detailed information is required for growth, production physiology, genetics, etc. through the research works in the field and laboratory.

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References

Aboagye, L.M., A. Isoda, H. Nojima, Y. Takasaki, T. Yoshimura and T. Ishikawa 1994. Plant type and dry matter production in peanut（*Arachis hypogaea* L.） cultivars. I. Varietal differences in dry matter production. Jpn. J. Crop Sci. 63:289-297.

Aboagye, L.M., A. Isoda, H. Nojima, Y. Takasaki, T. Yoshimura and T. Ishikawa 1995. Plant type and dry matter production in peanut（*Arachis hypogaea* L.） cultivars. I. Varietal differences in radiation interception. Jpn. J. Crop Sci. 64:73-77.

Akahori, A. and K. Kagawa 1974. Quantitative determination of saikosaponin in *Bupleurum* extract. I. Colorimetric determination of saikosaponins. Shoyakugaku Zasshi 28: 116-121.

Akahori, A., K. Kagawa and A. Shimaoka 1975. Quantitative determination of saikosaponin in *Bupleurum* extract III. Extraction of saikosaponins from *Bupleurum* root. Shoyakugaku Zasshi 29:9-l05.

Amano, A., K. Fujimoto, H. Ohashi, K. Matsunaga and H. Mizukami 1989. Chromosome number variation in *Bupleurum falcatum* plants regenerated through somatic embryogenesis of callus cultures. Shoyakugaku Zasshi 43:13-l8.

Amano, A., K. Fujimoto and H. Ohashi l989. Geographical variation in somatic

chromosome numbers of *Bupleurum falcatum* L.. Shoyakugaku Zasshi 43:192-194.

Bhatt, R.K. 1995. Light interception, leaf area index and dry matter yield in fodder sorghum at different plant populations. J. Agronomy & Crop Sci. 174:287-290.

Brougham, R.W. 1956. Effect of intensity of defoliation on regrowth of pasture. Aust. J. Agric. Res. 7: 377-387.

Chung, S.H., J.W. Bang and H.W. Choi 1995. Cytogenetic analysis of *Bupleurum falcatum* L. cultivated in Korea. Korean J. Medicinal Crop Sci. 3:61-65.

Collins, G.B. and N. Sunderland 1974. Pollen-derived haploids of *Nicotiana knightiana, N. raimondii,* and *N. attenuata.* J. Exp. Bot. 25:1030-1039.

Collins, G.B. 1977. Production and utilization of anther-derived haploids in crop plants. Crop Sci. 17:583-586.

Corduan, G. and C. Spix 1975. Haploid callus and regeneration of plants from anthers of *Digitalis purpurea* L.. Planta（Berl.） l24: 1-11.

Fujioka, N., A. Tanaka, A. Koyama, Y. Hamamoto, A. Namera, K. Yamasaki and H. Kohda 1987. Propagation of *Bupleurum falcatum* by shoot tip culture. Shoyakugaku Zasshi 41:308-312.

Genovesi, A.D. and C.W. Magill 1979. Improved rate of callus and green plant

production from rice anther culture following cold shock. Crop Sci. 19:662-664.

Guha, S. and S.C. Maheswari 1964. *In vitro p*roduction of embryos from anthers of *Datura*. Nature 204: 497.

Hiraoka, N., T. Kodama and Y. Tomota 1983. *In vitro* propagation of *Bupleurum falcatum*. Shoyakugaku Zasshi 37: 62-67.

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

Hirota, O. and T. Takeda 1982. Studies on utilization of solar radiation by crop stands. IV. Estimated absorptivities of solar energy in each leaf layer of rice and soybean canopies. Jpn. J. Crop Sci. 51: l51-158.

Horikoshi, T., N. Homma and S. Ishizaki 1976. Studies on the cultivation of medicinal plants. VII. On the growth and yield of *Bupleurum falcatum* L.. Eisei Shikenjo Hokoku, 94: 166-l69.

Hu, K.L., S. Matsubara and K, Murakami 1993. Haploid plant production by anther culture in carrot（*Daucus carota* L.）. J. Japan. Soc. Hort. Sci. 62:561I -565.

Ishii, H., M. Nakamura, S. Seo, K. Tori, T. Tozyo and Y. Yoshimura l980.

Isolation, characterization, and nuclear magnetic resonance spectra of new saponins from the roots of *Bupleurum falcatum* L.. Chem. Pharm. Bull. 28:2367-2383.

Isoda, A., T. Yoshimura, T. Ishikawa, H. Nojima and Y. Takasaki 1994. Solar radiation penetration and distribution in soybean communities. Jpn. J. Crop Sci. 63:298-304.

Jo, P.H., R.S. Seong, H.H. Bae, W.Y. Soh and D.Y. Cho 1990. Saikosaponin contents in *Bupleurum falcatum* root produced by tissue culture. Kor. J. Pharmacogn. 21 :205-209.

Kasanaga, H. and M. Monsi l954. On the light transmission of leaves and its meaning for the production of the dry matter in plant communities. Jap. Jour. Bot. 14:304-324.

Katano, M., T. Sasahara, K. Sawa and H. Kurihara l989. Cultivation of *Bupleurum falcatum* L.. I. Leaf emergence rate of one-year -old seedlings. Kyushu Br. Crop Sci. Soc. Japan 56:97-101.

Kawashima 1969. Studies on the leaf orientation-adjusting movement in soybean plants. I. Fundamental pattern of the leaf orientation-adjusting movement and its significance for the dry matter production. Proc. Crop Sci, Soc. Japan 38:730-742.

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. Pham. Bull. 27:1836-l841.

Kim, K.S., N.S. Seong, Y.H. Chang, S.T. Lee, J.I. Lee, H.C. Oak and Y.A. Chae 1995. Variation of plant characters and correlation analysis of its in *Bupleurum falcatum* L.. Korean J. Medicinal Crop Sci., 3: 71-76.

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

Kohda, H., A. Namera, Y. Hamamoto and T. Okamoto l990. Propagation of *Bupleurum falcatum* by shoot tip culture. Shoyakugaku Zasshi 44:38-41.

Kokubun, M. and K. Watanabe 1981. Analysis of the yield-determining process of field-grown soybeans in relation to canopy structure. I. Effect of plant type alteration on solar radiation interception and yield components. Jpn. J. Crop Sci. 50:311-317.

Lang A.R.G. , Z. Yuequin and J.M. Norman 1985. Crop structure and the penetration of direct sunlight. Agric. Forest Met. 35:83-101.

Lee, S.Y., T.S. Kim, H.S. Kim and Y.T. Lee 1988. Somatic embryogenesis of *Bupleurum falcatum* L. I. Effects of growth regulator, glutamine and activated charcoal. Korean J. Breed. 20: 191-l98.

Lu, C.S., H.C. Sharma and H.W. Ohm 1991. Wheat anther culture: effect of genotype and environmental conditions. Plant Cell Tiss. Org. Cult. 24..233-236.

Maeda, K. 1993. Groundnut ideotype : Its agrophysiological characteristics and contribution of subsp. *fastigiata*. Jpn. J. Crop Sci. , 62: 21 1-221.

Matsubara, S., K. Hu and K. Murakami l992. Embryoid and callus formation from pollen grains of eggplant and pepper by anther culture. J. Japan. Soc. Hort. Sci. 61:69-77.

Minami, M. and M. Sugino l995. Effects of mineral fertilizers on growth and saikosaponins content of *Bupleurum falcatum* L.（1） Effects of different levels of nitrogen, phosphoric acid and potassium on growth and saikosaponins contents of one-year-old plant. Natural Medicines 49:230-239.

Minami, M., M. Sugino, M. Sadaoka, K. Ashida and K. Ogaki l995. Seasonal variation on growth and saikosaponin contents of *Bupleurum falcatum* L.. Yakugaku Zasshi 115: 145-155.

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 Zhsshi 45: 342-344.

Murashige T. and F. Skoog 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physio1. Plant. 15:473-479.

Murata, Y. 1981. Dependence of potential productivity and efficiency for solar energy utilization on leaf photosynthetic capacity in crop species. Jpn. Jour. Crop Sci., 50: 223-232.

Ohta S. 199l. Cytogenetical study on the speciation of *Bupleurum falcatum* L. （Umbelliferae）. J. Sci. Hiroshima Univ., Ser. B, Div. 2. 23:273-348.

Ohta, S., H. Mitsuhashi and R. Tanaka 1986. Aneuploidal variation in *Bupleurum falcatum* L. from Japan. Journ. Jap. Bot. 61 :212-216.

Oono, K. l975. Production of haploid plants of rice（*Oryza sativa*） by anther culture and their use for breeding. Bulletin of the National Institute of Agricultural Sciences Series D 26: 139-222.

Park, C.H., C.Y. Yu, D.W. Kim, J.S. Seo, S.D, Ahn and B.H. Chang 1994. Direct somatic embryogenesis and plant regeneration from root suspension culture of *Bupleurum falcatum* L.. Korean J. Breed. 26..41-45.

Park, C,H., J.S. Seo, K.S. Kim, S.D. Ahn and B.H. Chang l995. Induction of somatic embryos and RAPD analysis in regenerated plantlets of *Bupleurum falcatum* L.. Korean J. Medicinal Crop Sci. 3:50-55.

Saeki,T. 1960. Inter-relationship between leaf amount, light distribution and total photosynthesis in a plant community. Bot. Mag. Tokyo 73:55-63.

Shibata, M., R. Yoshida, S. Motohashi and M. Fukushima 1973. Pharmacological studies on *Bupleurum falcatum* L. IV. Some

pharmacological effects of crude saikosides, saikogenin A and syrupy residue. Yakugaku Zasshi 93: l660-1667.

Shibles, R.M. and C.R. Weber 1965. Leaf area, solar radiation interception and dry matter production by soybeans. Crop Sci. 5:575-577.

Shibles, R.M. and C.R. Weber l966. Interception of solar radiation and dry matter production by various soybean planting patterns. Crop Sci. 6:55-59.

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

Shimokawa, Y. and H. Ohashi l980. Cultivation and breeding of *Bupleurum falcatum* L.（V） Relation among cultivation years, root growth and saikosaponin content. Shoyakugaku Zasshi 34:235-238.

Sinclair T.R. and T. Horie 1989. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: A review. Crop Sci. 29: 90-98.

Sinclair, T.R., T. Shiraiwa and G.L. Hammer l992. Variation in crop radiation use efficiency with increased diffuse radiation. Crop Sci. 32: 1281-l284.

Tanaka, T., E. Sakai, M. Mizuno, T. Kawamura, Y. Hisata, K. Okuda, Y. Noro, X.Z. Zheng and F. Ding l988. Cultivation and saikosaponin contents of Guangxi *Bupleurum*. Shoyakugaku Zasshi 42:236-239.

Tani, T., T. Katsuki, M. Kubo and S. Arichi 1986. Distribution of saikosaponins

in *Bupleurum falcatum* root. Journal of Chromatography 360:407-416.

Tani, T., T. Katsuki, M. Kubo, Y. Okazaki and S. Arichi l987. Histochemistry. XI. Histological and chemical characteristics of bolting and nod-bolting roots of cultivated *Bupleurum falcatum* L.. Chem. Pharm. BtIll. 35:4530-4536.

Tani, T., T. Katsuki, M. Kubo, Y. Okazaki and S. Arichi 1988. Morphological, histological and chemical evaluation of roots of *Bupleurum falcatum* L. cultivated at eighteen producing districts in Japan. Jpn. J. Medical and Pharm. WAKAN-YAKU 5: 13-20.

Tomes D.T. and G.B. Collins 1976. Factors affecting haploid plant production from in vitro anther culture of *Nicotiana* species. Crop Sci. 16:837- 840.

Wang C.C., C.C. Chu, C.S. Sun, S.H, Wu, K,C. Yin and C. Hsu 1973. The androgenesis in wheat（*Triticum aestivum*） anthers cultured *in vitro*. Scientia Sinica 16:21I 8-225.

Xia G.M., Z. LI, G.Q. Guo and H.M. Chen 1992. Direct somatic embryogenesis and plant regeneration from protoplasts of *Bupleurum scorzonerifolium* Willd. Plant Cell Reports 11 : 155-158.

Yamaguchi M., A. Yomoda and K. Hinata 1990. Varietal differences in the response to low temperature treatment for callus formation in anther culture of rice. Japan. J. Breed. 40:193-198.

Yamamoto, H., H. Honjo, S. Hayakawa, Y. Suzuki and N. Kawada 1996.

Seasonal changes in efficiency of solar energy utilization and solar energy conversion in two-rowed barley of warm regions in Japan. Jpn. J. Crop Sci. 65:207-213.

Yoshida, T. 1979. Growth analysis of late-summer sown barely. Jpn. J. Crop Sci. 48:495-501.

Abstract

1. Difference in dry matter （DM） production among cultivars originated from Jeongseon, Korea and Kumamoto, Japan was observed for one-yew-old plants as well as two-year-old plants. Accumulated DM and efficiency for solar energy utilization （Eu） was correlated significantly for one-year-old plants regardless of cultivars. There were significant correlations between leaf area index （LAI） and DM produced for one-year-old plants and two-year-old plants in both of two cultivars.

2. Maximum Eu was 1.02 %～ 1.23 % for one-year-old plants and 1.16 ～ 2.09 % for two-year-old plants. The cultivar from Kumamoto was higher than that from Jeongseon. High Eu was obtained at flowering stage regardless of cultivars and plant age.

3. Total saikosaponin content of one-year-old plants was higher than two-year-old plants, Total saikosaponin content was higher for the cultivar from Jeongseon than that from Kumamoto in two-year-old plants, but it showed no difference in one-year-old plants. For the yield of total saikosaponin obtained per unit area, the cultivar from Jeongseon showed higher values than that from Kumamoto for two-year-old plants. One-year-old plants was efficient than two-year-old plants for saikosaponin production in a year.

4. The saikosaponin content in roots of one-year-old plants for the cultivar from Tsukuba was higher than for the cultivars from Mishima, Yasato, Fukuoka, Jeongseon and Kumamoto.

5. Saikosaponins were predominantly produced in phloem tissues. 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.

6. Callus formation differed among genotypes. The average percent of callus formation in anther culture was 22.6 %, 10.0 %, and 23.1 % for the cultivar from Fukuoka, Kumamoto, Jeongseon , respectively.

7. The highest callus formation was 30.1 % for the clultivar from Jeongseon and 41.2 % for that from Kumamoto at uninucleate stage of anther. However, in case of the anther length above 300 μ m, very low callus formation was observed in all of the cultivars.

8. In the complex of phytohormones, the frequency of callus formation was the highest on the medium containing 0.1 mg of 2,4-D and 0.1 mg of picloram per liter and the value was 26% for the cultivar from Jeongseon and 59.5 % for that from Kumamoto. For single hormone, the medium containing 1 mg of 2,4-D per liter was the best for the callus formation, that induced the frequency of 44.6 % for the cultivar from Jeongseon and 28.4 % for that from Kumamoto.

9. Exposure of anther to 5 and 10 degree for 72 hours and 120 hours was very effective for callus formation. Whereas, exposure of anther to O ℃ or 15 ℃ was not effective. No callus was induced or very low at the O ℃ or 15 ℃ in all of the cultivars.

10, The somatic embryogenesis from pollen was observed after 5 weeks of culture on the MS medium containing 3% sucrose and 1 mg of 2,4-D per liter.

11. From 2 weeks after transferring to the regeneration medium, multiple roots and leaves were induced from callus. They were identified as haploids by cytogenetical observation.

12. The chromosome number from plants of different cultivars was shown as 2n=26, 2n=26 and 2n=20 for the cultivars from Kumamoto, Jeongseon, and Fukuoka, respectively.