Short Report

Some Factors Affecting the Callus Formation in Anther Culture of *Bupleurum falcatum* L.*Tae-Kwon SHON, TOTOK Agung Dwi Haryanto and Tomohiko YOSHIDA
(Faculty of Agriculture, Kyushu University, Fukuoka 812-81, Japan)

Received August 22, 1996

ミシマサイコの葯培養におけるカルス形成率に及ぼす要因：孫太権・TOTOK Agung Dwi Haryanto・吉田智彦（九州大学農学部）

キーワード：植物体再分化，ホルモン，ミシマサイコ，葯培養。

Key words: Anther culture, Anther stage, *Bupleurum falcatum* L., Phytohormone, Temperature pretreatment.

We reported the production of the haploid plant with half ($n=13$) of the chromosome number of the original plant ($2n=26$) through anther culture in *B. falcatum*¹⁾.

The objective of this study is to evaluate several factors as the stage of anther development, temperature pretreatment, and phytohormones, which may affect the variability in callus formation by anther culture of *B. falcatum*.

Materials and Methods

B. falcatum populations, originated from Jeongseon, Korea and Kumamoto, Japan, were grown in a field at Kyushu University in 1995.

To study the optimum stage of anther, anthers for inoculation were classified into the three classes, i. e. less than 200 μm , 200–300 μm and more than 300 μm in length, and the developmental stage of microspores was determined by microscopic observation.

To investigate the effect of low temperature pretreatment, flowers containing anthers were kept in a petri dish to prevent desiccation, and subjected to low-temperature pretreatment at 0, 5, 10, and 15°C for 24, 72 and 120 hours in the dark. The anthers were then inoculated.

Eleven treatments were employed to investigate the effect of phytohormones, namely: 2, 4-D, NAA, kinetine, picloram, 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 anthers with calli to that of the plated anthers. Other details are shown in Shon et al¹⁾.

Results and Discussion

Almost all anthers formed a callus about 5 weeks after planting. The effect of the stage of anther on callus formation is shown in Table 1. For anthers of less than 200 μm , which were very young and colorless and contained a pollen of an uninucleate or younger stage, the

Table 1. Effect of stage of anther on callus formation.

Length of anther (μm)	No. of anthers cultured		% of anthers with calli	
	J ^a	K ^b	J	K
~200	259	296	30.1	41.2
200~300	370	222	17	12.6
300~	185	148	3.8	0.0

^a: Jeongseon, ^b: Kumamoto.

Same for Tables 2 and 3.

Table 2. Effect of low temperature on callus formation.

Temp.	Hours	No. of anthers cultured		% of anthers with calli	
		J	K	J	K
0	24	185	185	0.0	4.3
	72	37	185	0.0	0.0
	120	185	185	0.0	1.1
5	24	185	666	6	8.6
	72	185	407	36.8	38.6
	120	185	185	34.1	40.5
10	24	185	185	10.3	9.2
	72	148	185	18.9	36.8
	120	185	185	37.8	39.5
15	24	185	185	0.0	0.0
	72	185	185	0.0	0.0
	120	185	185	4.9	0.0

* This report was presented at the 201st meeting of the Crop Science Society of Japan in April, 1996.

Table 3. Effect of phytohormones on callus formation.

2,4-D	NAA	Kinetine (mgL ⁻¹)	Picloram	No. of anthers cultured		% of anthers with calli	
				J	K	J	K
0.1			0.1	185	185	26.0	59.5
0.1			1	185	148	0.0	0.0
0.1	2			185	185	0.0	0.0
1		0.1		370	296	3.0	7.1
1	0.1			185	111	6.0	18.9
1			0.1	185	185	9.7	1.6
1		2		185	148	0.0	0.0
1				74	74	44.6	28.4
	1			185	185	0.0	0.0
		1		185	370	0.0	0.0
			1	370	370	22.4	17.3

average callus formation was 30.1% in Jeongseon and 41.2% in Kumamoto. However, for anthers of above 300 μ m, which were yellowish, very low callus formation was observed in both materials.

Table 2 represents the effect of low temperature pretreatment on callus formation. Exposure of the anther at 5 or 10°C for 72 or 120 hours was very effective for both materials. Jeongseon showed 18.9–37.8%, and Kumamoto showed 36.8–40.5% in frequency, respectively. Whereas, exposure of anther at 0 or 15°C was not effective and it was similar for both materials.

Table 3 shows the effect of concentrations of phytohormones on callus formation. The medium containing 0.1 mg of 2, 4-D + 0.1 mg of picloram per liter was the most favorable for both materials. 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 Jeongseon and 28.4% for Kumamoto. However, no callusing, or very low frequency of callus formation, was observed in the medium containing NAA only. Factors of callus induction and embryogenesis might vary among cultivars, but no difference in materials originated from two different habitats was observed in this study.

In conclusion, for *B. falcatum* anther culture, very young anther containing pollens of an uninucleate or younger stage, that received a low temperature pretreatment of 5–10°C for 72–120 hours, and phytohormones of 1 mg of 2, 4-D per liter, or 0.1 mg of 2, 4-D + 0.1 mg of picloram per liter, were most effective for inducing callus formation.

References

1. Shon, T.K. et al. 1996. Jpn. J. Crop Sci. 66 : 137–138.