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Seed Characteristics and Accelerating Method of Germination in *Bupleurum falcatum*

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시호 종자의 특성 및 발아촉진 방법 연구

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ABSTRACT : The experiment was conducted to determine the seed characteristics and preferable methods to enhance the seed germination rate in *Bupleurum falcatum*. The optimum temperature for the seed germination of *Bupleurum falcatum* is 20℃. Any significant promoting effects were not found in seed germination with hormone treatments and physical methods. At 15℃, prechilling combined with 50~200ppm of GA_3 treatment raised germination rate by 2 times of control ones. The most positive effect was observed in the treatment of 10^{-2}~10^{-3}M potassium nitrate only at 15℃ for 12 and 24 hours. The leachate of *Bupleurum falcatum* didn't inhibit the germination of *Lactuca sativa*, showing almost 100% of germination rate, which is suggested that no inhibitors contained in the seeds of *Bupleurum falcatum*. Observation of embryo conditions under stereoscopic microscope showed that the ratio of seeds with or without embryo is almost 50/50. The results suggested that the lower rate of germination in *Bupleurum falcatum* was caused by embryolessness of seeds.

Key words : *Bupleurum falcatum* L., Inhibitor, Embryolessness, Scanning electron microscope, Tetrazolium test.

The studies on the cultivation and biopharmaceutics of medicinal plants are rapidly increasing since the public announcement of strategies for the Traditional Korean Medical Insurance Coverage in Korea in 1987 and also the international cooperative research works between Korea and Japan from 1991 through 1996. In fact, the increasing stability of living in Korea is demanding the usage of medicinal plants to maintain good health. These plants not only increase the farmer's income but substitute agricultural products exported to mostly Japan. This indicates a prospective future in developing productivity and biopharmaceutics as well as exporting abroad and trading for domestic purposes.

Until now, most of the medicinal plants have been propagated by using rhyzome or root cutting. Vegetative reproduction shows high efficiency for sprout, while caring and selling are a little inconvenient, for example, large space and conditions for storage of

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rhyzome or root cutting are needed. If propagation by seeds is available, it will yield more crops and increase productivity as needed by demand. On the other hand, we are facing dormancy problems with certain medicinal plants and insufficient information for germinaton and growth of medicinal plants. Clarification of how and when dormancy can be broken and of which factors can accelerate germination is very important. It is our objective to elucidate what are the most effective conditions for breaking dormancy and accelerating germination.

Many seeds do not germinate when placed in conditions which are normally regarded as favorable to germination, namely, adequate water supply, a suitable temperature, and an atmosphere of normal composition. Nevertheless, seeds can be viable as they can be induced to germinate by various special artificial treatments or under specific external conditions.

It is reported that impermeability of water to seeds is responsible for germinating in legume[4,19,20]. Most reported works has proceeded from the assumption that a degree of dormancy was caused by the inhibitor.[2,20] It is associated with varnish-like deposit on the surface of seeds, with the cuticle, and with the densely packed palisade layer which envelops the whole seed except at the hilar area. For the conditions to enhance germination, chemicals such as potassium nitrate and sulfuric acid were applied[1~3,20] and also prechilling at 5℃ for 5 to 10 days.[21] To determine the effect of several different promoting substances for seed germination GA,[2,20,27] kinetine, indoleacetic acid, have been treated. The stratification is known to hasten the development of embryo after-ripening.

There are many problems for breaking dormancy in the family of *Umbelliferae*[5~10,16~19,25,26] in the medicinal plants. Even though *Bupleurum falcatum*[11,12,14,15,21~23] is out at market lately, there is selling some difficulty for germination. *Bupleurum falcatum* is an important medicinal species under the family of Umbelliferae. To acceralate the selling of seeds of *Bupleurum falcatum*, germination rate has to be improved and the characteristic of seeds has to be elucidated.

In this study, we have examined optimum condition and preferable methods to enhance germination in *Bupleurum falcatum*. Here we also explain one of the factors causing ungermination in *Bupleurum falcatum*.

Materials and Methods

1. Seed sampling

Bupleurum falcatum seeds were harvested from September 26th through October 20th in 1993. Seeds were collected from plants which were mostly 5 years old grown at experimental field at Rural Development Administration(RDA), some at Hamyang Medicinal Plant Experiment Station, and some at National Yeongnam Agricultural Experiment Station.

Collected seeds were desiccated at room temperature for a month at Germplasm and Seed Technology Institute in Gyeongsang National University. The Vertical Blast Seperator(model # KSCOB 18) was used for seed sampling and selected seeds were stored at 5℃ until the germination tests.

Three or four replications of 100 seeds each were the basic unit for all treatments throughout the experiments in completely randomized design. Seeds were placed on two sheets of Whatman filter in 9 cm petri

dishes. Distilled water was added to each petri dish for the germination test.

2. Temperature treatment

To verify the proper temperature for germination of *Bupleurum falcatum* the experiments were conducted at 15℃, 20℃, 25℃ of growth chambers(16/8 hr, 670nm, 1,051 mol/m^2/sec).

The experiments were concluded 21 days after treatment.

3. Treatment of promoting substances

Gibberellic acid treatment: Seeds were placed on filter paper wet with 3 different concentrations of GA_3 (50 ppm, 100 ppm, and 200 ppm) and then were kept in petri dishes. For another experiment, seeds were pre-chilled at the 5℃ for 6 days before gibberellic acid treatment.

1) Kinetin treatment

The sampled seeds were treated with 10^{-4}M and 10^{-5}M of kinetin, and 10^{-4}M of kinetin combined with 2.5×10^{-4}M of GA_3 at 15℃, 20℃, and 25℃, respectively.

2) Indoleacetic acid

IAA treatment was made with the concentration of 1.0×10^{-4}M, 2.5×10^{-4}M, and 5.0×10^{-4}M. Germination test was conducted with the treated seeds at different temperatures of 15℃, 20℃, and 25℃, respectively.

4. Chemical treatment

Seeds were immersed at 10^{-2}M and 10^{-3}M of potassium nitrate for 12 and 24 hr, respectively. After treatment in potassium nitrate, seeds were throughly washed out with tap water and then dried for 12 hr at room temperature. Then they were tested at three different temperature conditions as mentioned previously.

5. Leaching and soaking method

Samples of 400, 500, and 1,000 seeds were placed in packets of folded gauze fastened with paper clips. These packets were placed in running tap water for 24, 48, 72, and 96 hr. In order to enhance germination of *Bupleurum falcatum*, seeds were soaked in distilled water for 24, 48, 72, and 96 hr at room temperature.

6. Inhibitor test

In order to verify whether any inhibitors exist or not in the seeds of *Bupleurum falcatum* and *Ostericum koreanum*, 400, 500, and 1,000 seeds of each species were soaked in 25ml, 50ml, and 100ml of distilled water for 24, 48, 72, and 96 hr, respectively. The leachate were applied to *Lactuca sativa* which is known to show high rate of germination. The final count of seed germination of *Lactuca sativa* was made at the 6th day after treatment.

7. Tetrazolium test

To determine the seed viability of *Bupleurum falcatum*, tetrazolium test was conducted. After samples were soaked in distilled water for up to 16 hr at 20℃ in the growth chamber, it was longitudinally bisected under the magnifying glass, followed by application of 0.5% and 1.0% tetrazolium chloride. Then seeds were kept at 35℃ growth chamber for 3 to 4 hr. The cut surface was examined for red-pink granular area. Embryo, embryolessness, and cavity[5] of *Bupleurum falcatum* were also observed.

8. Scanning electron microscopy

The seeds were cut to face micropyle and fixed with 2.5% buffered glutaraldehyde for 3 days, using 0.1M phosphate buffer, pH 7.5. Seeds were then washed several times with the same buffer. Postfixation was done with 1% OsO_4 in phosphate buffer for 1 hr at 4℃. Then the samples were dehydrated in the ethanol series, treated with acetone, critical point dried from CO_2, mounted, and sputer gold coated. Samples were observed using a JSM 6400 operated at 25KV.

Results and Discussion

Difficulty in obtaining satisfactory germination of *Bupleurum falcatum* seed is one of the main problems encountered in using *Bupleurum falcatum* which is used almost all the prescription for chinese medicine. Kawatani et al.[15] reported that the seeds required a long time for germination, and germination itself is very irregular. To clarify the factors related on the low germination, several treatments including promoting sunbstances, chemicals, and physical methods were carried out.

1. Temperature treatment

Germination rate in *Bupleurum falcatum* showed 18.7~11.3% at 15℃, 33.3~36% at 20℃, and 28.7~27.8% at 25℃, respectively (Table 1, 2). These results suggest that optimum temperature for germination of *Bupleurum falcatum* is 20℃ for 21 days. Huang et al.[13] reported that the most proper temperature for germination of 24 medicinal plants ranges from 16℃ to 20℃, which is well accorded with our results.

2. Promoting substances on the germination

When seeds were germinated at 15℃, GA_3 treatment alone showed no improvement of germination (Table 1). Kinetine and IAA also showed no positive effect on germination of *Bupleurum falcatum*. Whereas prechilled lot with GA_3 treatment raised germination rate from 18.7% to 39.0% at 50 ppm, 41.3% at 100 ppm, and 40.0% at 200 ppm(Table 1).

Table 1. Germination percentage of *Bupleurum falcatum* after prechilling, leaching, and GA_3 treatment

	Treatment	Germination (%)
15℃	Control	18.7 $^{j\text{-}m**}$
	Prechilling*	11.0 $^{n\text{-}o}$
	Prechilling + GA_3 50ppm	11.0 $^{n\text{-}o}$
	Prechilling + GA_3 100ppm	16.7 $^{k\text{-}n}$
	Prechilling + GA_3 200ppm	15.0 ^{mn}
	GA_3 50ppm	25.3 $^{f\text{-}j}$
	GA_3 100ppm	22.0 $^{h\text{-}l}$
	GA_3 200ppm	14.7 ^{mn}
	Leaching 24 hrs	18.0 ^{lm}
20℃	Control	33.3 $^{b\text{-}e}$
	Prechilling	22.7 $^{h\text{-}k}$
	Prechilling + GA_3 50ppm	39.0 ^{ab}
	Prechilling + GA_3 100ppm	41.3 ^{a}
	Prechilling + GA_3 200ppm	40.0 ^{ab}
	GA_3 50ppm	11.4 $^{n\text{-}o}$
	GA_3 100ppm	23.3 $^{h\text{-}k}$
	GA_3 200ppm	20.6 $^{i\text{-}m}$
	Leaching 24 hrs	38.0 $^{a\text{-}c}$
25℃	Control	28.7 $^{e\text{-}h}$
	Prechilling	27.3 $^{e\text{-}i}$
	Prechilling + GA_3 50ppm	31.7 $^{c\text{-}f}$
	Prechilling + GA_3 100ppm	34.0 $^{b\text{-}e}$
	Prechilling + GA_3 200ppm	33.0 $^{b\text{-}e}$
	GA_3 50ppm	14.0 ^{mn}
	GA_3 100ppm	20.0 $^{j\text{-}m}$
	GA_3 200ppm	19.3 $^{j\text{-}m}$
	Leaching 24 hrs	15.0 ^{mn}

* Prechilling at 5℃ for 6 days

** Means in columns within each germination temperature were separated by DMRT at p=0.05

Table 2. Germination percentage of *Bupleurum falcatum* after kinetin, IAA and KNO_3 treatment

	Treatment	Germination(%)
15℃	Control	11.3 $^{g-i*}$
	Kinetin 10^{-5}M	22.0 ^{f}
	Kinetin 10^{-4}M	8.0 $^{i-l}$
	Kinetin 10^{-4}M + GA_3 25×10^{-4}M	20.0 ^{f}
	IAA 1.0×10^{-4}M	1.3 ^{m}
	IAA 2.5×10^{-4}M	2.0 ^{m}
	IAA 5.0×10^{-4}M	12.6 $^{g-i}$
	KNO_3 10^{-3}M 12 hr	36.0 ^{a}
	KNO_3 10^{-3}M 24 hr	33.3 $^{a-c}$
	KNO_3 10^{-2}M 12 hr	35.3 ^{a}
	KNO_3 10^{-2}M 24 hr	32.3 $^{a-c}$
20℃	Control	36.0 ^{a}
	Kinetin 10^{-5}M	27.3 $^{d-e}$
	Kinetin 10^{-4}M	30.7 $^{b-d}$
	Kinetin 10^{-4}M + GA_3 25×10^{-4}M	22.7 ^{ef}
	IAA 1.0×10^{-4}M	6.0 $^{j-m}$
	IAA 2.5×10^{-4}M	10.7 $^{g-j}$
	IAA 5.0×10^{-4}M	13.4 $^{g-k}$
	KNO_3 10^{-3}M 12 hr	3.3 ^{lm}
	KNO_3 10^{-3}M 24 hr	–
	KNO_3 10^{-2}M 12 hr	–
	KNO_3 10^{-2}M 24 hr	5.3 $^{k-m}$
25℃	Control	27.8 ^{cd}
	Kinetin 10^{-5}M	20.7 ^{f}
	Kinetin 10^{-4}M	22.0 ^{f}
	Kinetin 10^{-4}M + GA_3 25×10^{-4}M	29.3 $^{b-d}$
	IAA 1.0×10^{-4}M	0.7 ^{m}
	IAA 2.5×10^{-4}M	2.0 ^{m}
	IAA 5.0×10^{-4}M	6.7 $^{j-l}$
	KNO_3 10^{-3}M 12 hr	–
	KNO_3 10^{-3}M 24 hr	4.0 ^{lm}
	KNO_3 10^{-2}M 12 hr	–
	KNO_3 10^{-2}M 24 hr	–

* Means in columns within each germination temperature were separated by DMRT at p=0.05

At 20℃, almost all treatment including prechilling combined with GA_3, GA_3 alone, kinetin, and IAA rather decreased the germination rate. Under this temperature, the treatment of prechilling combined with GA_3 decreased the germination rate by half.

At 25℃, IAA has toxic effects, showing the germination rate of 0.7~8.7%, compared to 27.8% of control. No other promoting effects was found with several treatments.

Takahashi[28] reported that the treatment of 5×10^{-4}M of GA_3 combined with 10^{-5}M kinetin increased the germination rate in *Coptica japonica*, whereas 10^{-5}M and 10^{-4}M of kinetin alone didn't affect on germination. In our results, kinetin treatment combined with GA_3 improves the germination rate only at 15℃, while there is little positive effect at 20℃ and 25℃. This difference between two reports might result from different species, climate, and soil condition.

3. Physical and chemical treatment

With prechilling treatment, germination rate of *Bupleurum falcatum* showed almost little effect at both 15℃ and 25℃(Table 1). Instead, the significant decrease(11.0%) was observed at 20℃, compared to 33.3% of control.

When seeds were treated in potassium nitrate, some interesting result was obtained. At 15℃, potassium nitrate improved the germination rate by 3 times. While at 20℃ and 25℃, potassium nitrate showed very toxic effect on germination, inducing almost ungermination. The explanation for this inhibiting effect at 20℃ and higher temperature is not clear now. But potassium nitrate might diffuse through cell wall to embryo and affect the germination at higher temperature.

Leaching in the running tap water for 24 hr showed slightly positive effect on germination only at 20℃(Table 1). This result suggests that there might be no inhibitor in the seeds of *Bupleurum falcatum*.

4. Verification of inhibitor in *Bupleurum falcatum* seed

In order to verify the seed germination inhibitors in *Bupleurum falcatum* and *Ostericum koreanum* which are representative seeds having low germination rates, seeds were soaked for 24, 68, 72, and 96 hr in distilled water, respectively(Tables 3, 4). The leachates were applied to *Lactuca sativa*

Table 3. Germination percentage of *Lactuca sativa* moistened with leachate of *Bupleurum falcatum* and *Ostericum koreanum*

Temp.	Leachate	Number of seeds	Soaking time	Germination(%)
20℃	Control			100.0
	Bupleurum falcatum	400	24hr*	98.3
		400	48hr*	99.3
		400	72hr*	98.7
		400	96hr*	99.3
		500	24hr**	99.3
		1,000	24hr***	99.3
		1,000	48hr***	99.0
		1,000	72hr***	100.0
		1,000	96hr***	100.0
	Control			100.0
	Ostericum koreanum	400	24hr*	98.0
		400	48hr*	99.3
		400	72hr*	98.7
		400	96hr*	98.0
		500	24hr**	98.7
		1,000	24hr***	100.0
		1,000	48hr***	99.3
		1,000	72hr***	99.4
		1,000	96hr***	99.4

* Soaked in 100 ml distilled water. ** Soaked in 50 ml distilled water.
*** Soaked in 25 ml distilled water. **** Abnormal seeds were not shown.

Table 4. Variations of electrical conductivity and pH balance according to several different soaking period for *Bupleurum falcatum* and *Ostericum koreanum* at the room temperature

Species	Soaking time	ECW(dsMP^{-1})	pH
Bupleurum falcatum	24hr	2.43	8.05
	48hr	2.96	7.65
	72hr	2.78	7.74
	96hr	2.85	7.95
Ostericum koreanum	24hr	8.37	8.48
	48hr	9.13	8.35
	72hr	7.01	8.18
	96hr	11.12	8.42

* ECW: Electrical conductivity

seeds having almost 100% of germinability. The results showed no abnormal sprout and no decrease of germination rate(Table 3). This result indicates that there are no inhibitors in seeds of *Bupleurum falcatum* as well as *Ostericum koreanum*.

Kim et al.[18] reported that there is phenol-like inhibitor in *Onanthe stolonifera*. When seeds were moistened with *Coronilla varia* leachate, the growth of timothy was inhibited[20]. Brant et al.[2] observed the significant difference of germination rate between control and seeds treated with *Coronilla varia* leachate. Huang et al.[13] also mentioned the presence of a kind of germination inhibitor in *Bupleurum falcatum*. Mononoki et al.[22] reported that ether fraction from the seed coats of *Bupleurum falcatum* inhibited the germination of lettuce seeds.

In contrast to other reports, our results showed no trace of germination inhibitors in the seeds of *Bupleurum falcatum*.

5. Observation of embryo and micropyle

To elucidate the factors causing low germination rate in *Bupleurum falcatum*, embryo and micropyle were observed by stereomicroscope and scanning electron microscope, respectively(Figs. 1, 2). Also viability was checked with tetrazolium test[24]. The seeds of *Bupleurum falcatum* from several sources were examined(Table 5).

The ratio between embryo and embryoless seeds ranged mean value of almost 50/50. This result shows good accordance with the report by Flemion and Uhlmann[8]. The notoriously poor germination of *Umbelliferous* seeds is evident by low minimum germination standards set by US Federal Government in 1945~55% for carrot and celery, and 60% for parsley and parsnip. Even now in Korea, the germination requirement for carrot seed is allowed to 65%. The difficulty in germination is not only for *Bupleurum falcatum* but also for some of *Umbelliferae* like *Daucus carota* and *Anethum graveolens*.

Kawatani et al.[15] reported that the embryo size of the freshly harvested seeds is the smallest, attaining to the largest 5 months after seed harvest of *Bupleurum falcatum*. They also mentioned that the characteristics of germination in *Bupleurum falcatum* L. is due to the after-ripening of the seeds, i.e., the embryo in freshly harvested seed is yet

Table 5. Percentage of embryo vs embryolessness in *Bupleurum falcatum* harvested in 1993 through 1994 and seed were applied with tetrazolium test through 1994~1996

Source of seeds	Harvested year	Embryo	Embryolessness	(Cavity)	Dead seed
			%		
Bupleurum falcatum	1993	64.0	34.0	(8.0)	2.0
Japanese variety	1994	54.0	34.0	(14.0)	6.0
GPRDA* (large seeds)	1994	54.0	41.0	(15.0)	3.0
GPRDA (small seeds)	1994	38.0	50.0	(34.0)	11.0
HMPES** (large seeds)	1994	49.0	51.0	(26.0)	–
HMPES (small seeds)	1994	34.0	65.0	(38.0)	1.0
NYAES*** (large seeds)	1994	47.0	53.0	(23.0)	–
NYAES (small seeds)	1994	46.0	54.0	(35.0)	–

* GPRDA: Gyeongnam Provincial Rural Development Administration
** HMPES: Hamyang Medicinal Plant Experiment Station
*** NYAES: National Youngnam Agricultural Experiment Station

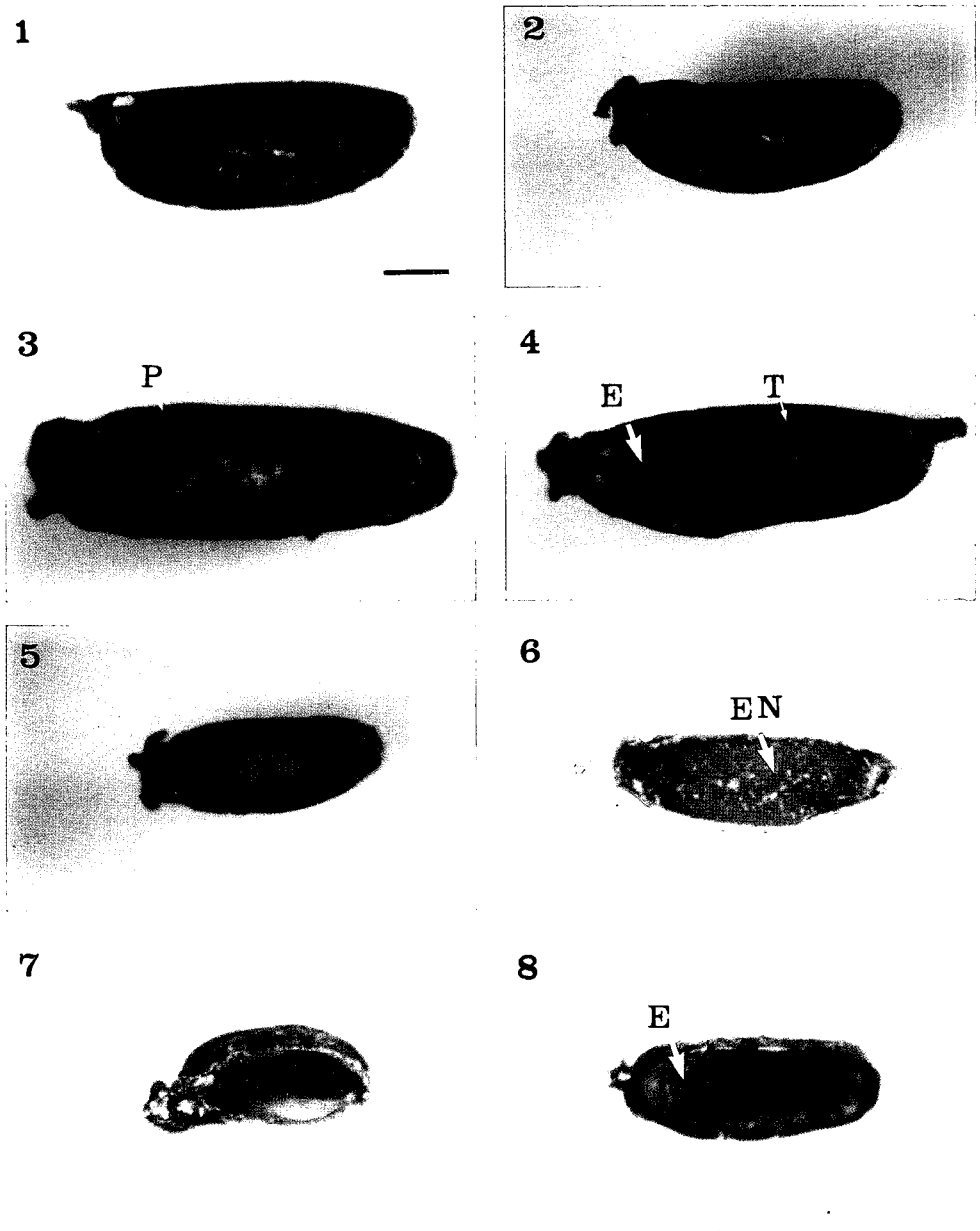

Fig. 1. Longitudinal section of a *Bupleurum falcatum* seed. Viability was confirmed by tetrazolium test.
1. Dead seed, 2, 3. Non viable, embryolessness, 4, 5. Heat damage, 6, 7. Embryolessness seed, 8. Viable seed
E, embryo; EN, endosperm; P, pericarp; T, testa; Bar scale=0.25μm.

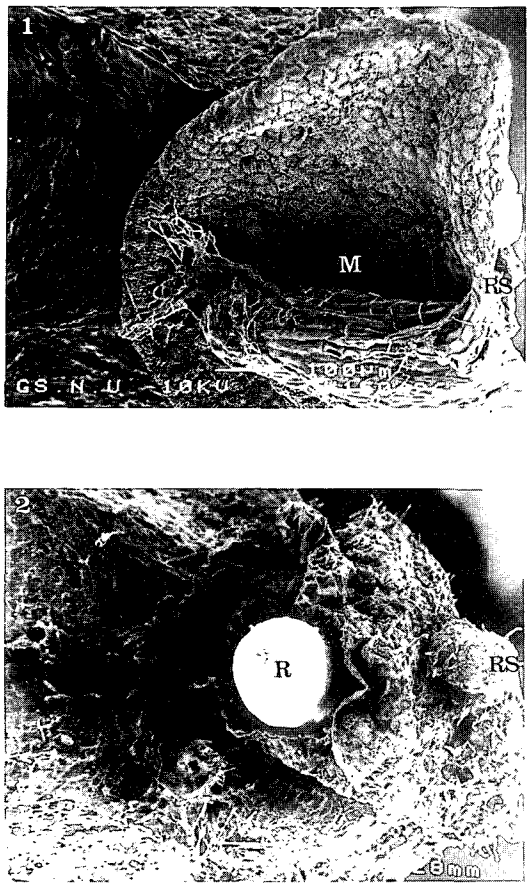

Fig. 2. Scanning electron micrograph of micropyle in germinated and ungerminated seeds of Japanese *Bupleurum falcatum*.
1. ungerminated seed, 2. germinated seed, M, micropyle; R, radicle; RS, remain of style.

immature and underdeveloped morphologically. Physiological immature embryo in the seeds of this family generally require an after-ripening period for germination. Flemion et al.[5~9] indicated that dill (*Anethum gravulens* L.) seed which failed to germinate had no embryos. The embryoless seeds had apparently normal endosperm. They also mentioned that embryolessness in this family has been found to occur at random from year to year with no correlation in regard to source of variety, yield, soil type, climatic conditions, genetical influence, and position on the plant. They also stated that there was a tendency that seeds produced out-of-doors late in the season showed a higher percentage of embryolessness. Another factor causing embryolessness is that *Lygus oblineates* bug may destroy the ovary at early stage of seed development. These insects pierce the tissue with their mouth parts and suck out juice, resulting in inhibition of embryogenesis.

Without embryo formation, germination is almost impossible. There is no problem with opening of micropyle in seeds of *Bupleurum falcatum* (Fig. 2). In our results, high rate of embryolessness can explain the low germination rate in seeds of *Bupleurum falcatum*.

Any hormone, and physical and chemical treatments won't improve the germination rate in *Bupleurum falcatum* seeds unless a better strand of seeds are cultivated. Maybe some other factors remain to be determined.

적 요

시호 종자의 발아율을 증가시키기 위한 방법과 종자의 특징을 연구하기 위하여 본 실험을 수행하였다.

시호의 발아에 유리한 온도는 20℃이며, 호르몬 처리나 기타 물리화학적 처리에 의해 발아율은 크게 증가되지 못했는데, 15℃에서 발아시킬 경우 50~200 ppm의 GA_3을 처리했을 때 발아율이 2배 이상 증가하였다. 또한 같은 온도에서 10^{-2}~10^{-3}M의 KNO_3을 처리한 경우에도 발아율은 3배 이상 증가하였다. 그러나 20℃ 이상에서는 강력한 저해 효과를 나타내었다.

시호의 leachate를 상추의 종자에 처리했을 경

우 발아율의 변화가 거의 없었으므로 시호의 종자에는 발아저해제가 거의 없는 것으로 생각된다.

해부현미경과 주사전자현미경으로 종자의 배와 주공을 관찰한 결과 배가 있는 것과 없는 것의 비율이 거의 50/50이며 주공 자체에는 문제가 없으므로 시호의 종자 발아율이 낮은 것은 근본적으로 배가 결여된 종자가 많기 때문인 것으로 생각된다.

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