〔韓作誌(Korean J. Crop Sci.) 42(4) : 435~445(1997)〕

Factors Involved in Promoting Seed Germination of *Foeniculum vulgare****

Eun Il Lee*, Chu Ho Choi**, Jong Ki Lee*** and Seok Hyeon Kim*

회향종자의 발아촉진에 관여하는 요인***

이은일* · 최주호** · 이종기*** · 김석현*

ABSTRACT : The effects of temperature, prechilling, chemicals such as GA_3, IAA, kinetin and KNO_3 on the germinability of *Foeniculum vulgare* seed of medicinal plant were examined.

In *Foeniculum vulgare*, the germination rate appeared to be around 50~60% or more in general, showing no differences in germination rate with different temperatures, promoting substances, physical or chemical treatments, and prechilling treatments.

The observation of embryo under stereoscopic microscope for *Foeniculum vulgare* in Umbelliferae showed that seeds with or without embryo was almost the same in number. This result suggests that the lower rate of germination in this species is caused by embryolessness of seeds. The straight-shaped embryos as well as Y-shaped embryos were also observed. *Foeniculum vulgare* of medicinal plants in Umbelliferae were observed under scanning electron microscope, and did not show any opening problem near micropyle area.

Final count should be made on 7th day of germination test.

Key words : *Foeniculum vulgare*, Embryolessness, Cavity, Germination rate

Against the increased demand for medicinal plants, the difficulties are low germinability of the seeds. It is necessary to find out a solution to propagate the plants by seed in order to meet the commercial demand for the plants. However, the phenomena of the low germinability of the medicinal plants are not clear but imminent solution to promote the germinability is very essential. Environmental conditions during seed development and the degree of maturation can influence the intensity dormancy. Seeds of some species are shed before they are morphogically mature. Momonoki et al.[20] reported that the endosperm of *Bupleurum falcatum* seeds might be physiologically immature at the time of shedding and gradually mature as the after-ripening is progressed during the storage period.

This results in dormancy because the immature embryo is unable to germinate. Further embryo maturation occurs following seed dispersal and may take a few days or several months[3]. Ahluwalla[2] reported that

본 연구는 1993년도 한국학술진흥재단의 공모과제 연구비에 의하여 수행되었음.

* 경상대학교 농과대학 농학과(Department of Agronomy, College of Agriculture, Gyeongsang National University, Chinju 660-701, Korea)

** 진주산업대학교 농학과(Department of Agronomy, Chinju National University, Chinju 660-758, Korea)

*** 농촌진흥청 연구관리국(Research Management Bureau, RDA, Suwon 440-707, Korea) 〈'97. 2. 28 接受〉

late germination of seeds is the primary and most serious difficulty in several medicinal plants including *Atropa belladonna*. That might be due to the fluctuation of ambient temperature and relative humidity. Also, the maximum germination rate for the seeds stored in the optimum condition was obtained in 6 months after storage. The disappearance of dormancy during storage at room temperature is widespread in cereal crops storage for 1 to 2 months at 15 to 20℃ suffices to allow maximum germination. Seeds that have been after-ripened in dry storage are not as responsive to exogenous gibberellin, presumably because the seed has had adequate time to synthesize this compound.

When dormancy is developed in seed embryos or buds in various species and organs, it is involved in some degree of hormonal control; gibberellins, cytokinins, inhibitors such as ABA, and possibly ethylene[5,14,16,25]. A balance between inhibitory and stimulatory substances seems to be involved in the control of germination[1,13,19,24]. Gibberellin(GA) is known to be very effective in promoting germination and also in weakening the inhibitory effect of high temperature[23].

Khan[16] has advanced the inhibitor-promotor concept one step further by suggesting the participation of three hormones in the control of seed dormancy and ascribing a particular function to each hormone. He suggested that gibberellins have a primary promotivey function in regulative seed dormancy. Gibberellins must be present for germination to occur and only an inhibitor can prevent this expression. To account for breaking of dormancy in seeds, Khan suggests that another class of hormones, cytokinins, play a permissive role by selectively antagonizing the inhibition when they are present. If inhibitors are not physiologically active, cytokinins have no effect on the breaking of dormancy since this role is governed by the gibberellins.

Many studies have, in fact, shown that ABA can completely or partially reserve the promotive action of either the gibberellins or cytokines. A shift in the balance between inhibitors and promoters must occur before germination is realized.

This study was carried out to determine the effect of several different promoting substances for seed germination, gibberellic acid(GA_3), kinetin, indoleacetic acid(IAA), and potassium nitrate(KNO_3) have been tested. Tetrazolium test was also conducted to verify seed viability, a stereoscopic microscope observation for embryo, and scanning electron microscope for the establishment of the openings of the seed coat from which radicles could emerge. The low germination of seeds is not ascribed solely to either dormancy or the presence of inhibitors. Interest has, therefore, to go to detect factors inducing low germinability of medicinal plants in Umbelliferae.

MATERIALS AND METHODS

1. Seed sampling

Seeds of *Foeniculum vulgare* was harvested from September 26th through October 20th in 1993 and in 1994. Seeds were collected at the experimental fields at Gyeongnam Provincial Rural Development Administration(GPRDA). Collected seeds were desiccated at room temperature for a month at Germplasm and Seed Technology Institute at Gyeongsang National University. The Ver-

tical Blast Separator(Model# KSCOB 18) was used for seed sampling and the selected seeds were stored at 5℃ until the germination tests were given.

2. Temperature treatments

To verify the proper-temperature for germination of *Foeniculum vulgare* in Umbelliferae, the experiments were conducted either at a constant temperature of 15, 20, 25℃, or at alternative temperature of 15 to 20℃ in growth chambers(16/8hr, 670nm, 1,051m mol/m^2/sec). The experiments were concluded 21 days after treatment. Seeds were prechilled at 5℃ for 6 days. Also, to verify the effect of time period of prechilling, seeds were prechilled for 14, 21, 28, and 42 days and tested for germinability.

3. Treatment of promoting substances

For chemical treatments gibberellic acid (GA_3), indolacetic acid(IAA), kinetin, and potassium nitrate at different concentrations were applied.

1) Gibberellic acid

Seeds were placed on filter paper with 3 different concentrations of GA_3(50, 100 and 200 ppm) without prechilling. Effect of prechilling at 5℃ for 6 days combined with gibberellic acid was compared with that of GA_3 alone.

2) Indoleacetic acid

IAA treatment was made at 5.0×10^{-5}M, 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 15, 20 and 25℃.

3) Kinetin

Seeds were treated with 10^{-5} M and 10^{-4} M of kinetin, and as a comparison, 10^{-4} M of kinetin was combined with 2.5×10^{-4} M of GA_3. Seeds were germinated at 15, 20 and 25℃.

4) Potassium nitrate

Seeds were immersed at 10^{-3} M and 10^{-2} M of potassium nitrate for 12 and 24hrs, respectively. After the treatment in potassium nitrate, seeds were thoroughly rinsed with running tap water and then dried for 12 hrs at room temperature. Then seeds were germinated at 15, 20, 25 or 15~20℃.

4. 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 wet Whatman filter paper in 9 cm petri dishes. Distilled water was added to each petri dish for germination test. Because of the length of viability, the seeds of less than 1-year-old were used for germination test. The final counts were made after 21 days; abnormal and dead seeds are also observed. An abnormal seedling means a seedling that does not have the essential structures indicative of the ability to produce a normal plant under favorable conditions. A dead seeds are considered dead when they produce no part of a seedling at the end of the test period.

5. Tetrazolium test

Tetrazolium test was conducted by the Moor's method[21]. For regulation, at least 200 seeds should be tested. The seeds were randomly selected in replicates before conditioning. After seeds were soaked in distilled water for up to 16 hrs at 20℃ in the

growth chamber, they were longitudinally bisected under the magnifying glass, followed by application of 1.0% tetrazolium chloride. Adequately prepared seed samples completely covered with testing solution. Seeds were then kept at 35℃ for 3 to 4 hrs. The cut surface was examined for red-pink granular area. Embryo, embryolessness, and cavity were also observed.

6. Scanning electron microscopic observations

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

7. Statistical analyses

Least Significant Difference Test(LSD) was applied to data analyses. For these analyses, SAS program(Statistical Analysis System, Inc., Version 4.0 Cauy, NC, USA) was used.

RESULTS AND DISCUSSION

1. Temperature effect

To clarify the factors involved in low germination, several different temperature treatments were employed. Germination rate at day 7 in *Foeniculum vulgare* was approximately 40~60%. *Foeniculum vulgare* showed a broad optimum temperature for germination from 15 to 25℃(Tables 1 and 2). The effect of prechilling is shown in Tables 1 and 4. The result of treatment showed 56.0, 60.3 and 51.0%, whereas 61.3, 56.0 and 69.0% in control[1]. Prechilling treatment at this temperature was not affected, rather decreased slightly. At 20℃ prechilling treatment resulted only 4.3% increased. The treatment of alternating temperature of 15 and 20℃ was not effective for germination[3]. At treatments of different time period of prechilling it was effective at the range of 14 days of treatment, increased slightly from 58.6 to 62.6%.

2. Promoting substances on the germination

At 15℃ the percentages of germination with the treatment of GA_3 at different concentrations of 50, 100 and 200 ppm were 60.0, 48.7 and 58.0% whereas 61.3% at control. At 20℃ results were 51.3, 48.8 and 54.0% compared with 56.0% at control and at 25℃ 47.3, 62.0 and 54.6%, whereas 69% at control. Either the treatments of GA_3 50, 100 and 200 ppm by itself or with prechilling were not affected and significantly indifferent among treatments(Tables 1 and 3).

Takahashi & Ogawara[23] 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 did not. Ahn et al.[4] described that a higher concentration of GA_3 and lower levels of kinetin were effective in enhancing seed germination in *Actinidia arguta*.

The treatments of kinetin 10^{-5} and kinetin 10^{-4} showed 62.0 and 56.0% whereas 60% at control. Kinetin treatment by itself was not significantly different compared with control. The results of kinetin 10^{-4}M+GA_3 2.5×

Table 1. Germination percentage of *Foeniculum vulgare** as affected by prechilling, prechilling combined with GA_3, and GA_3 treatments

Temp(℃)	Treatment	Germination percentage on day			
		7	14	21	Total
15	Control**	58.0	3.3	0	61.3
	Prechilling***	55.3	0.7	0	56.0
	Prechilling + GA_3 50 ppm	57.5	0	0	57.5
	Prechilling + GA_3 100 ppm	54.0	0.7	0	54.7
	Prechilling + GA_3 200 ppm	55.3	3.3	0	58.0
	GA_3 50 ppm	58.7	1.3	0	60.0
	GA_3 100 ppm	48.0	0.7	0	48.7
	GA_3 200 ppm	56.7	1.3	0	58.0
	LSD.05****				7.4
20	Control	56.0	0	0	56.0
	Prechilling 5℃	58.0	2.3	0	60.3
	Prechilling + GA_3 50 ppm	56.3	1.0	0	57.3
	Prechilling + GA_3 100 ppm	51.5	0.5	0	52.0
	Prechilling + GA_3 200 ppm	50.8	0	0	50.8
	GA_3 50 ppm	50.0	1.3	0	51.3
	GA_3 100 ppm	48.0	0	0.8	48.8
	GA_3 200 ppm	54.0	0	0	54.0
	LSD.05				5.5
25	Control	68.5	0.5	0	69.0
	Prechilling 6 days	50.7	0.3	0	51.0
	Prechilling + GA_3 50 ppm	55.8	0	0	55.8
	Prechilling + GA_3 100 ppm	55.8	0	0	55.8
	Prechilling + GA_3 200 ppm	51.8	0	0	51.8
	GA_3 50 ppm	47.3	0	0	47.3
	GA_3 100 ppm	60.0	1.3	0.7	62.0
	GA_3 200 ppm	53.3	1.3	0	54.6
	LSD.05				6.0

* *Foeniculum vulgare*: 회향-The seeds were harvested in October, 1993 at Gyeongnam Provincial Rural Development Administration.
** Control: Not treated with any.
*** Prechilling: at 5℃ for 6 days.
**** Means separation in each column by LSD at $P=0.05$.

10^{-4}M were 51.3 at 15℃, 64.6 at 20℃, and 62.7% at 25℃, whereas 60, 58.7 and 60.1% at control. Both at 20 and 25℃ slightly increased but at 15℃ it was rather decreased. To the result kinetin treatment either with GA or without was not recommandable for enhancing germination rate of *Foeniculum vulgare*.

At 15℃ among IAA treatments, at 15℃ IAA 5.0×10^{-5} was 69.3% compared with 60.0% at control, at 20℃ it was 60.0% with treatment and 58.7% without, and at 25℃ was 63.4 with treatment and 60.1% without. Other concentrations of IAA treatments were no possitive effects on germination of *Foeniculum vulgare* (Table 2).

Almost all treatments including GA_3 alone, kinetin, and IAA did not promote the germi-

Table 2. Germination percentage of *Foeniculum vulgare** as affected by kinetin, IAA, and KNO_3

Temp(℃)	Treatment	Germination percentage on day			
		7	14	21	Total
15	Control**	60.0	0	0	60.0
	Kinetin 10^{-5} M	60.7	1.3	0	62.0
	Kinetin 10^{-4} M	56.0	0	0	56.0
	Kinetin 10^{-4} M + GA_3 2.5×10^{-4} M	51.3	0	0	51.3
	IAA 5.0×10^{-5} M	69.3	0	0	69.3
	IAA 1.0×10^{-4} M	54.0	2.0	2.0	58.0
	IAA 2.5×10^{-4} M	58.0	0	0	58.0
	IAA 5.0×10^{-4} M	57.3	0.7	4.0	62.0
	KNO_3 10^{-3} M, 12 hr	56.0	0	0	56.0
	KNO_3 10^{-3} M, 24 hr	52.0	1.3	0	53.3
	KNO_3 10^{-2} M, 12 hr	55.3	0	0	55.3
	KNO_3 10^{-2} M, 24 hr	48.7	0.7	0	49.4
	LSD.05***				6.4
20	Control	58.0	0.7	0	58.7
	Kinetin 10^{-5} M	59.3	0	0	59.3
	Kinetin 10^{-4} M	58.0	0	0	58.0
	Kinetin 10^{-4} M + GA_3 2.5×10^{-4} M	63.3	1.3	0	64.6
	IAA 5.0×10^{-5} M	60.0	0	0	60.0
	IAA 1.0×10^{-4} M	58.7	0	0	58.7
	IAA 2.5×10^{-4} M	66.0	2.0	0	68.0
	IAA 5.0×10^{-4} M	58.0	0	0	58.0
	KNO_3 10^{-3} M, 12 hr	53.3	0	0	53.3
	KNO_3 10^{-3} M, 24 hr	58.0	0	0	58.0
	KNO_3 10^{-2} M, 12 hr	61.3	0	0	61.3
	KNO_3 10^{-2} M, 24 hr	68.7	1.3	0	70.0
	LSD.05				7.2
25	Control	58.7	0.7	0.7	60.1
	Kinetin 10^{-5} M	56.7	2.7	0	59.4
	Kinetin 10^{-4} M	46.0	2.0	0	48.0
	Kinetin 10^{-4} M + GA_3 2.5×10^{-4} M	58.0	4.7	0	62.7
	IAA 5.0×10^{-5} M	62.7	0.7	0	63.4
	IAA 1.0×10^{-4} M	55.3	0	0	55.3
	IAA 2.5×10^{-4} M	55.3	0.7	0	56.0
	IAA 5.0×10^{-4} M	64.7	0	0	64.7
	KNO_3 10^{-3} M, 12 hr	61.3	2.0	0	63.3
	KNO_3 10^{-3} M, 24 hr	54.0	1.3	0	55.3
	KNO_3 10^{-2} M, 12 hr	48.7	4.7	0	53.4
	KNO_3 10^{-2} M, 24 hr	57.3	2.7	0	60.0
	LSD.05				8.2

* *Foeniculum vulgare*: 회향-The seeds were harvested in October, 1993 at Gyeongnam Provincial Rural Development Administration.

** Control: Not treated with any.

*** Means separation in each column by LSD at $P=0.05$.

Table 3. Germination percentage of *Foeniculum vulgare** as affected by the several different methods at an alternating temperature of 15 and 20℃

Treatment	Germination percentage on day			
	7	14	21	Total
Control**	54.6	0	0	54.6
Prechilling***	45.3	0	0	45.3
Prechilling + GA_3 50 ppm	50.6	0	0	50.6
Prechilling + GA_3 100 ppm	50.0	0	0	50.0
Prechilling + GA_3 200 ppm	48.0	0	0	48.0
GA_3 50 ppm	36.7	0	0	36.7
GA_3 100 ppm	50.0	0	0	50.0
GA_3 200 ppm	45.3	0	0	45.3
KNO_3 10^{-2}M, 12 hr	42.0	0	0	42.0
KNO_3 10^{-2}M, 24 hr	38.6	0	0	38.6
KNO_3 10^{-3}M, 12 hr	41.3	0	0	41.3
KNO_3 10^{-3}M, 24 hr	46.0	0	0	46.0
LSD.05****				6.6

* Seeds were harvested in October, 1994 and the experiment was conducted in March, 1995.
** Control: Not treated with any.
*** Prechilling at 5℃ for 6 days.
**** Means separation within column by LSD at $P= 0.05$.

Table 4. Germination percentage of some Umbelliferae as affected by prechilling at 5℃ for several different time periods

Species☆	Prechilling (days)	Germination percentage on day				
		5th	7th	14th	21st	Total
Foeniculum vulgare†	Control	44.7	6.7	–	–	58.6
	14	38.0	23.4	–	–	62.6
	21	50.6	1.4	–	–	52.0
	28	44.3	5.3	–	–	50.0
	42	38.3	–	–	–	38.6
	LSD.05					11.3
LSD.05						8.4

†: Germinated at 20 ℃, ∓: germinated at 25℃.
NS, ***: nonsignificant or significant F at 0.001.
☆: Seeds were harvested at National Yeongnam Agricultural Experiment Station in 1994.

Table 5. Percentage of embryo vs embryolessness in *Foeniculum vulgare* by application of tetrazolium test

Source of seeds	Harvested year	With embryo(%)	Embryolessness(%)	Dead seed(%)
GPRDA*	1993	48.0	47.0 (21.0**)	5.0
		44.0	44.0 (27.0**)	12.0
	1994	50.0	47.0 (27.0**)	3.0
		47.0	40.0 (26.0**)	13.0

* GPRDA: Gyeongnam Provincial Rural Development Administration.
** Percentage of cavity.

nation rate.

Huang & Liu[15] indicated that the treatment of 0.2 or 1.0% of potassium nitrate to the germination showed no positive effect on the germination of *Bupleurum falcatum* seeds. Seung & Seo[22] also indicated that the treatment of 0.2% potassium nitrate for 10 minutes to *Bupleurum falcatum* showed 52% at 15℃(control, 50%), 40% at 25℃(control, 30%), and 0% at 35℃ either control or the treated. When seeds were treated in potassium nitrate, some interesting results were obtained(Table 2). Both at 15 and 25℃ the treatments of potassium nitrate did not increase germination percentage except at 20 ℃. The treatment of KNO_3 10^{-2} M, 24hr was resulted at 70.0%.

Generally, promoting substances, physical or chemical treatments show no effect for the improvement of germination in *Foeniculum vulgare*. It showed rather decreasing tendency of germination rate(Table 1). Therefore, in this species might have no problem to be germinated at 20℃.

3. Observation on embryo and micropyle

To clarify he factors causing low germination rate in *Foeniculum vulgare* embryo and micropyle area were observed by stereomicroscope and scanning electron microscope(Fig. 1).

In our results with *Foeniculum vulgare*, the ratio between embryo and embryoless seeds were about 1 to 1(Table 5). Even if *Foeniculum vulgare* showed an average of 61% germination rate, embryolessness could have had an effect on germination rate. Some seeds in the medicinal plants of Umbelliferae mainly as in *Bupleurum falcatum* and *Foeniculum vulgare* have an embryolessness problem, and some others have a little. The observation under the stereoscopic microscope in this study showed that sometimes the bigger one contained no embryo. In embryoless seeds there was a cavity in the endosperm at one end of the seed where the embryo would normally be found(Fig. 1). Such seeds have apparently normal endosperm while the embryos either aborted at an early stage of growth or never developed. Without embryo germination is impossible. Under the observation of stereoscopic microscope, one interesting factor was observed; the shape of embryo appeared to be Y-shaped or sometimes straight(Fig. 1). Embryolessness was caused by late season flowered seeds and by *Lygus oblineates*[8~12].

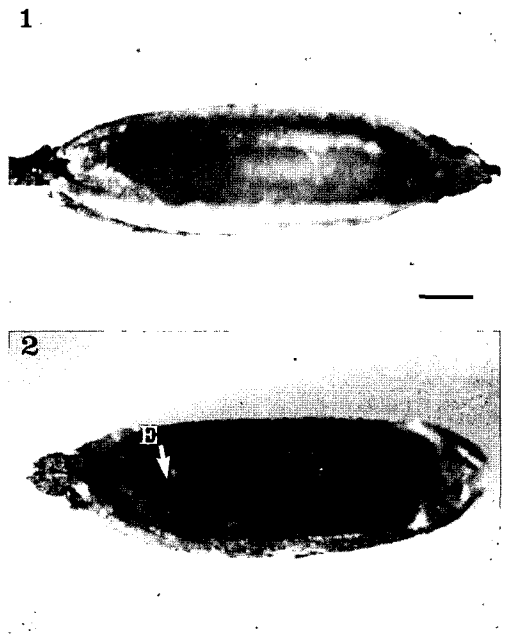

Fig. 1. Longitudinal section of *Foeniculum vulgare* seed. Viability was confirmed by tetrazolium test.
1. Embryolessness; 2. Viable seed with Y shaped embryo, E, Embryo; Bar scale=0.8mm.

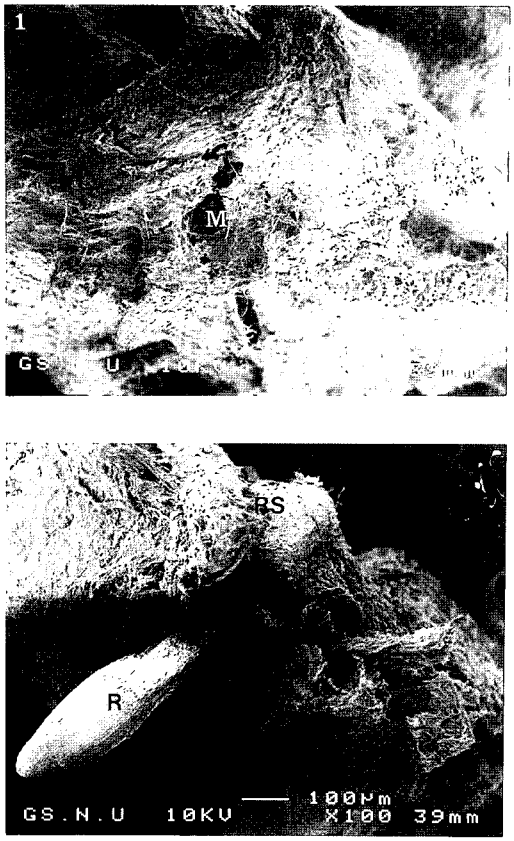

Fig. 2. Scanning electron microscope of micropyle in germinated and ungerminated seeds of *Foeniculum vulgare*.
1. Ungerminated seed, 2. Germinated seed, M, Micropyle; R, Radicle; RS, Remain of style.

Flemion et al.[8~12] also mentioned that embryolessness in this family has been found to occur at random from year to year with no correlation with the sources of variety, yield, soil type, climatic conditions, genetical influence and position on the plant, etc.

Some seeds can not be germinated because of impermeability to water which may be due to the pressure of the cuticle and a well developed layer of palisade cells or both heavy deposit of sublin, lignin or cutin are common in the integuments of many legume seeds as well as those of other hard coated species. In other seeds, the impermeability to water may be related to the fine structure of the hilum. For example the entry and exit of water is often controlled by a small opening in the seed coat near hilum, the strophiolar cleft, which is filled with a cork-like substances of sublin-the strophiolar plug. Water can only enter these seeds if the strophiolar plug is loosened. Strophiolar is usually located near raphe area. Other impermeable seeds do not possess a strophiolar plug and the seed coat must be abraded or punctured to make the seed permeable. The cuticle of bean seeds can effectively limit water penetration, leaving only the micropyle for imbibition. It has been demonstrated that Great Northern bean seeds with the micropyle sealed off gain only 0.25% of their weight while controlled seeds gain 79%. Thus, different parts of the seed are important in the control of water entry during the initial stages of imbibition[3].

In order to verify the openings of seed of medicinal plants in Umbelliferae, the seed coat was observed by scanning electron microscope. The appearance of micropyle area showed no opening problems for water enter or exit for germination(Fig 1).

In our results, high rate of embryolessness can explain the low germination rate in the seeds of *Foeniculum vulgare*. Any promoting substances, physical and chemical treatments could not improve the germination rate in *Foeniculum vulgare* seeds. This study should continue to make it possible to establish which species could reasonably be expected to germinate without special treatments, and which treatments could be most effective to promote germination of "difficult" species.

적 요

최근 생활수준의 향상과 보건에 대한 관심의 증가로 약용식물 재배와 한약에 대한 연구가 증가되고 있다. 그러나 대량번식을 위한 종자번식에 있어서 많은 문제점을 안고 있다. 특히 산형과 약용식물에 있어서 시호(*Bupleurum falcatum*)나 회향(*Foeniculum vulgare*)은 발아율을 증가시키는데 있어서 만족할만한 발아율을 얻지 못하고 있기 때문에 본 연구를 수행하였다.

회향은 60%의 발아율은 나타내며 15, 20, 25, 15~20℃의 발아온도 처리간의 별다른 차이가 없었다. 호르몬 처리나 물리화학적 처리에서도 발아를 증진시킬 수 없었다. 이러한 발아불량의 원인은 시호에서와 같이 결국 무배종자가 많은 것으로 추정된다. 해부현미경과 주사현미경으로 종자의 배와 주공을 관찰한 결과 배가 있는 것과 없는 것의 비율이 1/1이며 주공자체내에는 문제가 없었다. Y자형과 일자(一字)형의 배를 가진 종자가 동시에 관찰되었다.

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