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Microspore Development According to the Floral Budsize in *Astragalus membranaceus* Bunge

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ABSTRACT: *Astragalus membranaceus* has flowers that are similar to that of the legume family, but shows poor bearing when self-pollination is induced. Thus, this study was carried out observing the ripening procedure of pistils and stamens and development stages of pollen in the context of the birth and growth of the flower. As to the bearing of the flower of *A. membranaceus*, few pod setting and 13% pod setting were observed when self-pollination is induced by paper-bag covering or artificial pollination treated respectively. The result indicates that *A. membranaceus* is a cross-pollination plant. A pistil grew faster than a stamen until just before blooming. The flower size was about 17.0 mm × 4.0 mm. Pistils and stamens had the same length after flowering. Pollen mother cells passed through meiosis and mitosis when its length reached around 3.5 mm, thus creating the tetrade when 4 mm long. Pollen attained full growth when the bud was about 10 mm long. An anther was found to tend to dehisce when the length of a bud reached around 12.0 mm. As to the shape of pollen, about 70% were normal. 1% and 30% were small or empty pollen respectively. The result indicates that pollen of *A. membranaceus* attains full growth just before anther dehiscence which occurs before blooming while pistils grow faster than stamens until before flowering.

Key words : astragali radix, *Astragalus membranaceus*, floral budsize, microspore, pollen, stamen.

"Huang-qi" (*Astragalus membranaceus*) is a perennial medical herb its roots being used as herb medicine. The roots contain a number of ingredients including saponin (astragaloside I~VIII), isoflavonoid, and an amino acid called γ-aminobutyric acid (Masaki *et al.* 1994). Especially, Korean *A. membranaceus* containing γ-aminobutyric acid is found to have a superior effect on balancing blood pressure compared to its Chinese and Japanese counterparts (Kim *et al.*, 2000). Astragalus is a wild growing herb that can be found throughout Korea and in the northeastern province of Heilongjiang, south the Shandong peninsula of China. Astragalus cultivated in Korea is known to have 16 (2n=16) vegetable cell chromosomes. Even with the roots as herbal medicine, they propagate by seed. If seeds are sown in spring, flowering begins in the middle or late of July and continues until October as an indefinite inflorescence. The flower is a member of the legume family in its shape, its axillary raceme, and 5~22 flowers that bloom with considerable distance from each other. The corolla is similar to that of leguminous plants with a posterior banner petal, two lateral wing petals, and two anterior keel petals with the shape of a butterfly. It is of light yellow and up to 16~18 mm long. A total of 10 stamens are arranged with 9 stamens gathered and one fell apart as a diadelphous patern. An ovary is covered by thin and soft hair with a long ovary stalk and a hairless style. Growing well in cool weather, mainly 2~3 year old roots have been produced in Jungseon, the Samchuk area in Kangwon province and Jecheon in Chungbuk province. However, a recent rise in demand led to a steady expansion of its cultivation areal into the plains in the central & south and the central & north area including Pocheon, Yeoju in Kyonggi province where 1 year old roots are produced. The previous study on *A. membranaceus* concerns the method of cultivation including sowing, manuring (Park *et al.* 1988, Masaki *et al.* 1995), and the harvest following the rise of demand and the expansion of the area under cultivation. However, only a few base studies on its breeding, have been performed yet. The roots which are easily damaged by wetness are vulnerable to rottenness in the rainy season during its growth period. Thus, the cultivation of a species with a resistance towards wetness is urgent. It is assumed to be a self-pollination plant from its flower but when artificial self-pollination with a paper-bag covering is induced, it shows poor bearing (Kim *et al.* 2000). Therefore, this study, aimed at finding the reason focused on the observation of the development process of its pistils and stamens and the development stages of pollen according to the growth of the flower bud using a microscope. The result is presented here.

MATERIALS AND METHODS

This study was performed over 2 years from 1997 to 1998

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in the medicinal crop laboratory garden and microscope room of the Crop Experiment Station. Flowers of *A. membranaceus* cultivated in the laboratory crop garden according to the standard cultivation procedures were used for the study. And, to find the bearing capability of *A. membranaceus,* the pod setting rate was examined in 200 flowers which were left natural, treated with artificial pollination, and paper-bag covering respectively. Bearing resulted from the crossing in a natural state was examined for the flowers of open pollination. In the case of artificial pollination, oil paper-bag covering was performed on giving forms of the bud until 3 days before the blooming, around 3~5 PM when an anther was removed. Artificial pollination was carried out at about 10 AM on the next day and the pod setting was examined after 20 days since pollination. Lastly, the flowers treated with paper-bag covering, was covered by paper bags when the bud began shaping and was examined to observe the pod setting after 15 days since blooming. The growth stage of pistils and stamens by bud sizes was examined in the way that the peduncles of which the first flowers bloomed were picked and the size of their whole flowers was observed. Zoom stereo microscope (NIKON SMZ-10-TD) was used. For the development stage of pollens, peduncles were collected, fixed in a fixing fluid (95% Alcohol : glacial acetic acid = 3 : 1) for 24 hours, and then treated in 95% Alcohol and 85% Alcohol for 2 hours respectively which were stored in storing fluid (75% Alcohol) and used for microscopic examination. The dying reagent was prepared with 1% Iron acetocarmine. An Optical microscope (NIKON Diaphot) was used for examination. Pollens were classified into 3 shapes including normal pollen (N-pollen), small pollen (S-pollen) and empty pollen (E-pollen) and the proportion of each was investigated (Park *et al.* 1994).

RESULTS AND DISCUSSION

Bearing capability of *A. membranaceus*

The results from the induced self-pollination (autogamy) and cross-pollination (allogamy) for the purpose of investigating the fruition rate of *A. membranaceus* are shown in Table 1. When the flowers were covered by oil paper-bag, 99% of flower abscission was observed. When the flowers were induced to do self-pollination covered by gauze, they showed 100% flower abscission with no pod setting. Under artificial pollination, flower abscssion reached 87% with 13% pod setting. In case of open pollination, there showed the best result with 57% flower abscission and 43% pod setting. To sum up, *A. membranaceus* was found to do cross-pollination instead of self-pollination and to have low pod setting with 43% even by cross-pollination.

Wang *et al.* (1988) reported that *A. membranaceus (Fisch.) Bge. var. mongholicus (Bge.) Hsiao* was cross pollinated by entomophily and showed 2% bearing when covered by paper-bag. Therefore, acquisition of a pure bred through a self-pollination induction was regarded impossible. However, they said that the legumious plants with similar flower to that of *A. membranaceus* did self-pollination and their double fertilization was completed within 10 H after pollen reached a stigma (Fehr, 1980). Yet partial female sterility and lacking of self-pollination was observed in some mutation beans. It was considered due to their longer carpel, big torus, abnormally positioned calyx, and an anthar placed near the basal part of an ovary instead of near the stigma (Johns & Palmer, 1962).

As previously mentioned, most of the leguminous plants pass through self-pollination with flowers suitable for self-pollination while *A. membranaceus* does cross-pollination from unknown reason. So, the characteristics of a flower bud in its growing stages as well as the growth and formal characteristics of pollen were investigated. The result is presented in section 2 and 3.

Characteristics of pollen by the growth of flower bud

The development of pistils and stamens as a flower bud grows is shown as listed at Table 2. When a bud was 3.5 mm long and had a width of 1.0 mm or so after breaking out of the stalk, Pistils and stamens were both about 2.0 mm long. A pistil is positioned at the center and 10 stamens are arranged around it with 9 stamens gathered together and 1 stamen separated. When the bud was around 4.0 mm long and 1.3 mm wide, the pistil grew to a length of 2.3 mm while that of a stamen of remained at 2.0 mm. At this time an anther was about 1 mm long and 0.5 mm wide. Since then, the growth of a pistil surpassed that of stamens and an

Table 1. Effects of pod setting by pollination methods on in *A. membranaceus.*

Pollination methods	Self-pollination		Artificial pollination	Open pollination
	Wrapping of oil paper	Wrapping of gauze		
No. of crossed flower (%)	200 (100)	200 (100)	200 (100)	200 (100)
No. of flower abscission (%)	198 (99)	200 (100)	174 (87)	114 (57)
No. of pod setting (%)	2 (1)	0 (0)	26 (13)	86 (43)

Table 2. Characteristics of flower and microspore according to the floral budsize in *A. membranaceus*.

Developmental stage of flower bud	Calyx length (mm)	Flower size		Stamen length (mm)	Pistil length (mm)	Development of microspore
		Length (mm)	Width (mm)			
Bud	3.5	3.5	1.0	2.0	2.0	Pollen mother cell
	4.0	4.0	1.3	2.0	2.3	Tetarde
	4.3	4.3	1.5	2.1	2.5	
	4.5	4.5	1.8	2.3	2.8	
	5.0	5.0	1.9	2.5	3.2	
	5.2	5.2	2.0	3.2	4.0	
Initial growth of petal	5.2	5.5	2.1	3.6	4.3	Uni-nucleate
	5.2	5.8	2.2	4.1	4.8	
	5.5	6.5	2.3	5.0	5.5	
	5.7	7.5	2.7	5.5	6.0	
	6.0	8.5	3.0	6.5	7.4	Bi-nucleate
	6.0	10.0	3.0	7.5	8.5	Matured pollen
	6.0	12.0	3.5	10.0	11.0	Anther dehiscence
	6.0	15.0	4.0	14.5	14.8	
Flowering	6.0	17.0	4.0	15.0	15.0	

anther showed no significant chnage in size. The calyxes stopped developing when they were about 6 mm. When the flower bud was 11 mm long a pistil was 1 mm longer than a stamen. An anther bursted when the length of a bud was around 12.0 mm and the stamens around 11.0 mm. A pistil was found to grow faster than a stamen until the time of blooming. Complete flowering was observed when the bud became 17 mm long. At this time a pistil and stamens were both found to be about 15 mm long (see Fig. 1).

Pamplin (1963) reports that a legume has typical butterfly shaped flowers with its corolla consisting of 1 banner petal, two wing petals, and two keel petals and the androecium consisting of 1 detached stamen and 9 gathered stamens as a diadelphous patern encircling the centrally positioned pistil. Moreover, Guard (1931) finds that leguminous petals are arranged in the way that keel petals are placed on the side of the hypocotyl followed by two wing petals, and finally the banner petal. Johns and Palmer (1982) say that while flowering, every part shows rapid growth, anthers apparently do not grow much until microsporangia become well developed. As such, the flower of *A. membranaceus* is considered to be similar to that of the legume family. Furthermore several facts about *A. membranaceus* such as that the growth of a pistil surpasses that of a stamen until just before the blooming, that anther dehiscence occurs before flowering, and that around blooming time. the pistil and the stamens both are of about the same length were observed.

According to Table 2 showing the development process of pollen as a flower bud grows, the pollen mother cell was observed when a bud was 3.5 mm long. Segmentation into a tetard appeared when 4.0 mm long. The uni-nucleate period and bi-nucleate period were observed when the bud had a length of 5.5 mm and 8.5 mm respectively. When buds reached a length of 10 mm, pollen was observed to have attained full growth and when 12 mm long, stamens were 10 mm long and the opening of anthers were found. However, reproductive nuclear division in microspores was not observed and the developing shape are shown in Fig. 2.

Bang (1999) indicates that for the pollen's growth, the pollen mother cell gets through meiosis and the tetrad releases microspores which after passing through mitosis generate small reproductive cells and big nourishing cells. The period of 2 sperm cells generated from nuclear fission is different from plants to plants. Usually it appears in the pollen in the case of some gramineae plants and cruciferae plants, or when pollen reaches to the stigma and the pollen corolla gets growing in the case of some buttercup family plants. Hwang and Miyazawa (1967) say that during the generation of microspores in American Ginseng, the division of archesporial cells lead to the generation of microspore mother cells and then meiosis occurs as an anther grows. After that, the resulting tetardes are separated to create single microspores with nuclei at their centers which go through nuclear division again to generate a nourishing nucleus and a reproductive nucleus, but reproductive nuclear division in microspores is not observed.

In case of microspores in *A. membranaceus*, the development of uni-nucleate microspore (see Fig. 2E), bi-nucleate microspore (Fig. 2F) through tetarde following the growth of the flower bud was clearly observed. However, a three-

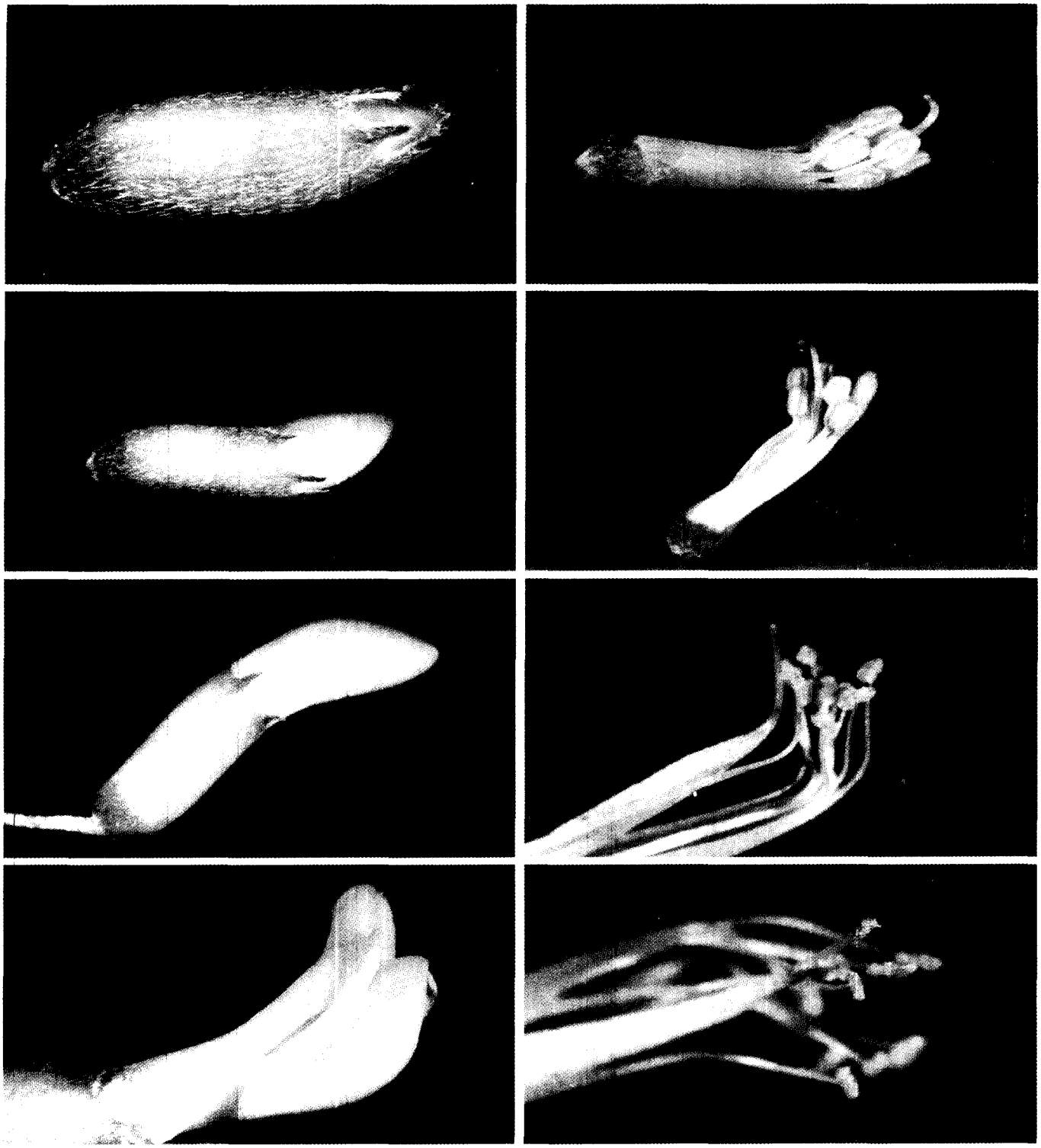

Fig. 1. Flower developmental stages from floral buds in *A. membranaceus*. A : Fully covered sepal buds, B : 1/3 petal developed bud, C : Half petal developed buds, D : Opened floral buds.

nucleate stage in pollen of full growth was not identified, which is considered due to the completion of starch accumulation in microspores.

Thus, apparently a pistil grows faster than stamens, but the development of both, fertilization after pollination, and the development process of the embryo need further investiga-

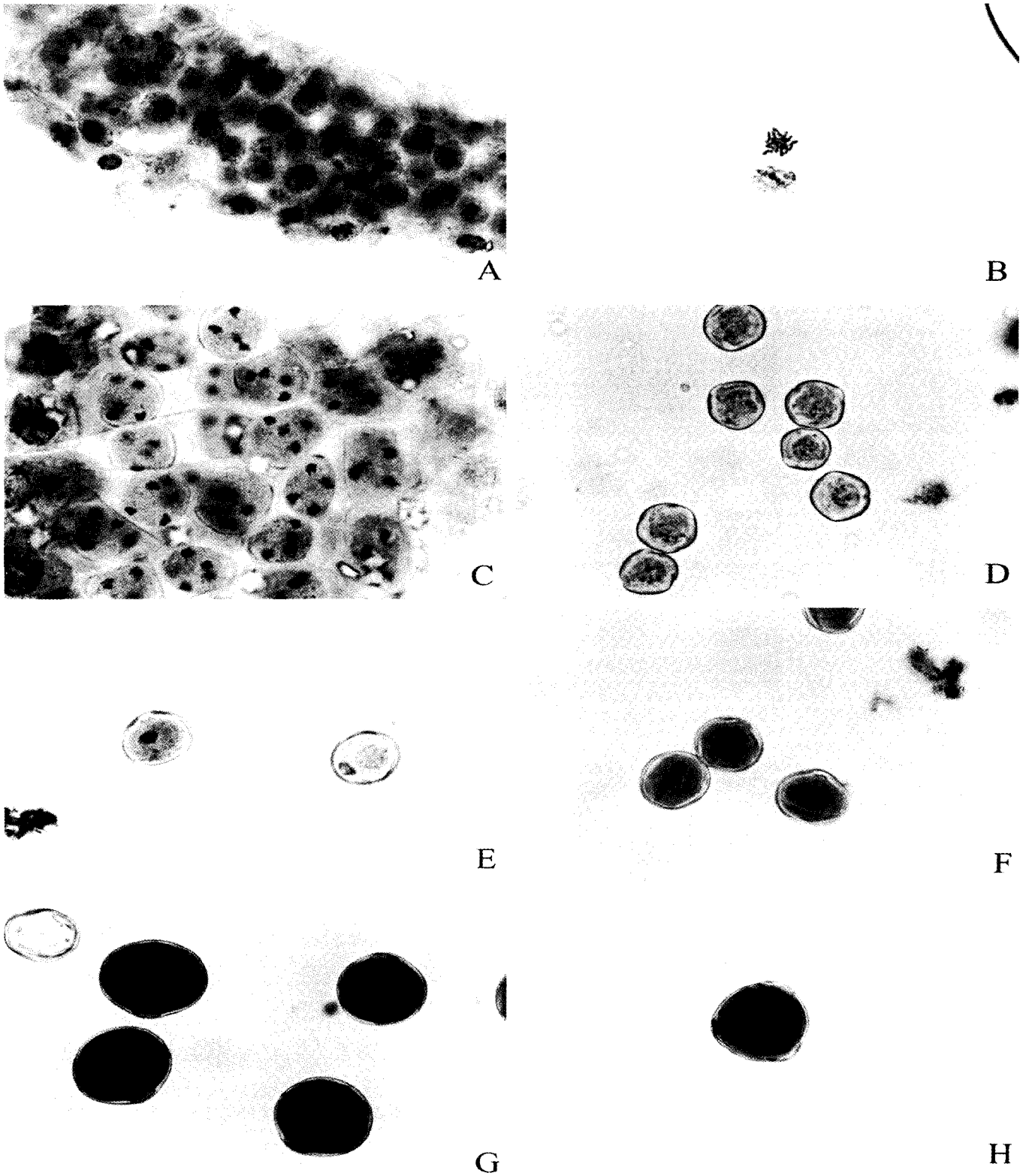

Fig. 2. Developmental stages of pollen in *A. membranaceus*. A : pollen mother cell, B : metaphase of meiosis, C : tetrade stage, D : early microspore phase just release from tetrade, E : uni-nucleate stage, F : bi-nucleate stage, G : matured pollen, H : fully matured pollen grain with three germ pore.

Table 3. Pollen polymorphism of collected regional lines *A. membranaceus*.

Strains	No. of pollen type (%)			
	N†	S	E	Total
Jacheon	1196 (68)	19 (1)	544 (31)	1759 (100)
Suwon 1	1282 (72)	14 (1)	487 (27)	1783 (100)
Suwon 2	1123 (67)	21 (1)	528 (32)	1672 (100)
Suwon 3	1343 ((67)	13 (1)	639 (32)	1995 (100)

†N : Normal pollen grain, S : Small pollen grain, E : Empty pollen.

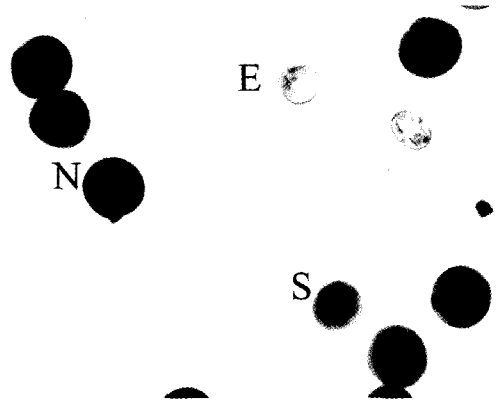

Fig. 3. Pollen polymorphism of *A. membranaceus*. N : normal pollen grain, S : small pollen grain, E : empty pollen.

tion to find the exact reason for sterility from self-pollination.

Pollen of *A. membranaceus* is divided by its shape into 3 kinds, N-pollen, S-pollen and E-pollen (see Fig. 3). According to Table 3, Suwon 1 is the strain with the largest proportion of N-pollen with 72% and smaller proportions of S-pollen and E-pollen with 1% and 27% respectively. There was no significant difference between strains with 67~72% N-pollen, 1% S-pollen and 27~32% E-pollen.

Formal characteristics of pollen

Park *et al.* (1994) found that from observation of the microspore of *Bupleurum falcatum*, differences existed among collection areas and strains and N-pollen accounted for 76.6% with 23.4% for S-pollen. Also, Kim *et al.* (1996) reported that from microspore of a *Paeonia lactiflora*, the proportion of N-pollen was 71% and 29% for Non-N-pollen. The abnormal pollen contains different forms of pollen caused by swelling of the spindle axis at the at microspore membrane due to an abnormality during the 1st somatic cell division in some microspores. Polymorphism originated from abnormal microspores, not from normal tetrad. It resulted from an abnormality in the cleavage of cytoplasm after the 1 st or 2 nd reduction division, or from stagnation pollen generated by the delayed formation of some microspores (Harn, 1985; Kim *et al.*, 1984).

For *A. membranaceus*, around 70% were found to be normal pollen, 1% small pollen, and 30% empty pollen. However, much of the normal pollen is expected to be well pollinated with the full growth of microspores.

REFERENCES

Bang J. W. 1999. Plant embryology. Plant cytogenetics laboratory, Chungnam National University : 75-78.

Fehr, W. R. 1980. Soybean. In Fehr, W. R. and H. H. Hadley (eds.) Hybridization of crop plant. American society of agronomy. Madison Wis. pp. 589-611.

Guard, A. T. 1931. Development of floral organs of the soybean. *Bot. Gaz.* 91 : 97-102.

Harn C. Y. 1985. Anther culture and pollen dimorphism. *Korean J. Plant Tissue Culture.* 12(1) : 1-34.

Hwang J. K. and Y. I. Miyazawa. 1967. Morphalogical and embryological studies on the American ginseng (*Panax quinquefolium* L.). *Jeonbuk University.* 9 : 177-194.

Johns C. W. and R. G. Palmer. 1982. Floral development of a flower structure mutant in soybeans, *Glycine max* (L.) Merr. (Leguminosae). *Am. J. Bot.* 69 : 829-842.

Kim J. C. and J. H. Shin, J. H. Kim, and S. D. Park. 1996. Pollen morphology of *Paeonia lactiflora* Pallas and its germination. *Korean J. Medicinal Crop Sci.* 4(3) : 231-235.

Kim M. Z. and Y. R. Kim. 1984. Basic Studies on the induction of microspore-originated calluses or Embryos in the anther culture of *Capsicum annuum* L. *Korean J. Plant Tissue Culture.* 11(2) : 75-112.

Kim Y. G., S. Y. Son, N. S. Seong, and B. H. Lee. 2000. Flowering habit, pollination patterns and seed setting in *Astragalus membranaceus* Bunge. *Korean J. Crop Sci.* 45(3) : 171-175.

Masaki A., E. Katsura, Y. Katoh and T. Yamagishi. 1994. Chemical evaluation of Astragali radix. *Natural Medicines.* 48(4) : 244-252.

Masaki A., E. Katsura, Y. Minamiyama, T. Miura, H. Kaneshima, and T. Yamagishi. 1995. Effects of manurial elements on growth of *Astragalus membranaceus* Bunge, yield and glycoside contents of Astragali radix. *Natural Medicines.* 49(3) : 284-287.

Pamplin, R.A. 1963. The anatomical development of the ovule and seed in the soybean. Ph. D. diss. Univ. of Illinois, Urbana. (Diss. Abstr. 63-5128).

Park C. H., N. S. Seong, T. Shibata, K. Nishi, and E. Sakai. 1994. Morchological characteristics of pollen grain in *Bupleurum falcatum. Korean J. Breed.* 26 (2) : 182-188.

Park N. K., D. U. Choi, S. M. Chang, and J. Choi. 1988. Effects of N, P_2O_5, and K_2O application on the yield and quality of *Astragalus membranaceus* Bunge root. *Korean society of soil science and fertilizer.* 21(4) : 450-454.

Wang T. S. *et al.* 1988. Chinese Medicinal herb cultivation. Agriculture Publishing. 691-696.