A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Characterization of Microspore Development and Pollen Tube Growth Response to Self- and Cross-Pollination in Jeju Old Local Citrus Species

제주 재래귤에서 소포자 발달 특성과 자가 및 타가 수분에 대한 화분관의 신장 반응

Panha Pok

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Characterization of Microspore Development and Pollen Tube Growth Response to Self- and Cross-Pollination in Jeju Old Local Citrus Species

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Submitted in partial fulfillment of the requirements for the degree of Master of Science in Agriculture

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ABSTRACT

This study was conducted to characterize the microspore development and evaluate their self- and cross-compatibility in Jeju old local citrus species. Anthers from the flowers with different length of byungkyul, dangyooza, and sadoogam were collected at different developmental stages and then embedded, sectioned, and stained with Toluidine blue O. Pollens of ten citrus species were tested for their viability using Lugol's solution and fluorescein diacetate (FDA) staining method at open flower stage. Flowers of all species were emasculated and self- and cross-pollinated just before anthesis. Pistils of nine pollinating combinations among byungkyul, sadoogam, and dangyooza were collected at 1, 3, 5, 7, and 9 days after pollination for evaluating pollen tube behavior. Pistils of the rest combinations were collected at 9 days after pollination for self- and cross-compatibility evaluation. Pistils were fixed in FAA and prepared using squashing and staining method with aniline blue. Microspores developed normally in all eight developmental stages. All species had high percentages of viable pollen ranged from 76.6% to 89.1% in Lugol's solution staining and 69.6% to 81.5% in FDA staining. Pollen tubes behavior in style was distinguishable between compatible and incompatible pollinations. Many pollen tubes grew through style and reached ovaries and ovules in compatible pollinations. However, pollen tubes in the incompatible pollination were arrested in upper and lower style. Pollen tube performance largely depended on female-male combinations. Consequently, all of these Jeju old local species are male fertile and have ability to produce fertile pollens for self- and cross-pollinations. Nine of 10 citrus species used in this study were self- and cross-compatible to each other. However, dangyooza was cross-compatible and self-incompatible, exceptionally.

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INTRODUCTION

Citrus is a leading fruit crop which is grown in more than 100 countries in tropical, subtropical, and Mediterranean climates. The main producers were China, Brazil, and the United State of America with total production of 115.5 million tons or 48.6% of total world production in 2012. The main citrus types were oranges, tangerines, grapefruits, and lemons and limes (Food and Agriculture Organization, 2012). Citrus is well known for nutrients and medical values of some biochemical compounds such as Vitamin C, Vitamin B complex, ascorbic acids, and carotenoids. Fresh grapefruits, pomelos, and oranges also provided fiber and pectin which are known to reduce the risk of heart attacks. The flavonoids from citrus juices, particularly those from oranges and grapefruit, are effective for improving blood circulation and possess anti-allergic, anti-carcinogenic, and antiviral properties (Filatoya and Kolesnova, 1999). In Korea, citrus is a major fruit crop and has long cultivation history dating back to the Three Kingdoms period, the fifth centuries A.D. according to the literature record (Kim, 1988; Moon et al., 2007). Modern citrus industry was established in 1960s by introduction of satsuma mandarins and old local citrus species have been maintained just in backyard due to their several seed formation and low fruit quality.

During the last 30 years more than one hundred cultivars were released via different breeding channels. Among them, cross hybridization was the most important method in breeding new citrus cultivars (Deng, 2005). So, sexual reproduction is very important in citrus cultivar improvement. In contrast, citrus has unique and complicated reproductive biology such as male and female sterility, self- and cross-incompatibility, polyembryony, long juvenile stage, and large seedling population (Khan and Kender, 2007). Sterility could be divided into three types: female sterility, male sterility, and self-incompatibility (Ollitrault et al., 2007). Degree of female fertility/sterility should be rated on the basis of the average number of seeds per fruit obtained through hand pollination (Yamamoto et al., 1995). The degree of male sterility was variable in cultivated citrus species and usually produced seedless or few seedy fruits when cultivated in solid block or when cross pollination was prevented. However, low parthenocapy accessions need cross-

pollination for fruit set. In citrus, male and female sterility may be due to different genetic factors such as sterility genes and chromosomal aberration (Ollitrault et al., 2007). Self-incompatibility is an inheritable trait in woody plants. This trait was also reported in citrus (Ngo et al., 2001; Soost, 1969; Vardi et al. 2000; Yamamoto et al., 2006). Self-incompatibility and cross-incompatibility were controlled by S genes (Soost, 1969; Yamamoto et al., 2006). Citrus was gametophytic self-incompatible. Bicellular pollen germinated and grew into style, thereafter these tubes were arrested in various distances in style (Soost, 1969). The growth of pollen tube was dependent on concentration of pollen tube growth factor, which is exhausted at second mitosis without getting any support from stylar tissue (Brewbaker and Majumder, 1961).

Jeju old local citrus species are being preserved and a few studies have been reported on their usefulness and utilization as of present (Choi et al., 2012; Kim et al., 2009). These studies were mostly restricted on bioactive components and phylogenic analysis, but study on floral and fruit biology is still scarce. The information of reproduction system is very important in citrus breeding program. Old local citrus species have different fruit characteristics, and their fruits mostly contain many normal seeds. Seed formation of these old local citrus species are not fully understood yet and so complicated due to polyembryony, gamete sterility, self- and cross-incompatibility, and parthenocarpy in the reproduction system. Yamamoto et al. (1995) reported that female fertility was directly related to seediness. This means seedy species have high ovule fertility which is ready for seed development. Hence, this study was conducted to investigate seed formation with the evaluation of microspore development and self- and cross-compatibility through their pollen tube growth response to self- and cross-pollinations in Jeju old local citrus species.

MATERIALS AND METHODS

Plant materials

All trees of ten local cirus species were grown in an experimental area of Jeju special self-gerverning province agricultural research & extension services, Seogwipo, Jeju island. They were binkyul (*C. leiocarpa*), byungkyul (*C. platymamma*), dangyooza (*C. grandis*), dong-geongkyul (*C. erythrosa*), gamza (*C. benikoji*), gigak (*C. aurantium*), hongkyul (*C. tachiban*), jinkyul (*C. sunki*), pyunkyul (*C. tangerine*), and sadoogam (*C. pseudogulgul*). Flower and pollen collections, pollination, and pistil sample collections were conducted from April to the end of May 2014. Flowers at stage 59 (Agusti et al., 1995) just before anthesis were gathered for pollen collection. Anthers were gathered on filter papers in petri dish and were kept at 25^{o}C. Pollens were harvested after 48 hours, and were transferred into vials and were stored at -20^{o}C until use. Flowers and pistils were collected and proceeded in different methods as shown below depended on purposes.

Microspore development analysis and pollen viability

Microspore development analysis: flowers with different length of 3 citrus species, byungkyul, dangyooza, and sadoogam were collected and fixed immediately in fixative solution (Formalin: acetic acid: ethanol in the ratio of 1:1:18, v/v/v) over night at 4^{o}C. Anthers were separated from fixed flowers and were dehydrated in ethanol series and were embedded with Technovit 7100 as processes shown in Table 1. Sample blocks were cut using a microtome (Leica RM2165, Leica Co., CA, US) and were stained with 0.05% alkaline Toluidine blue O (Sigma Aldrich, USA). Sections were observed under the light microscopy (Leitz DMRBE, Leica Co., CA, US), and pictures were taken using microscope camera ProgRes® C5.

Table 1. Sample preparation method for histological observation.

	Solution	Vacuum duration	Storage duration (4°C)
Dehydration	30%	30 min	12 hrs
	50%		
	70%		
	90%		
	100%		
	100%	0 min	24 hrs
Infiltration	Ethnol:Technovit (2:1)		
	Ethnol:Technovit (1:1)		
	Ethnol:Technovit (1:2)		

Pollen viability: the methods were conducted by the methods of Liu et al. (2004) with some modifications. Twenty anthers from different flowers were placed in 200 μL of 1% Lugol's solution and Fluorescein Diacetate (FDA) solution (200 $\mu g \cdot mL^{-1}$ FDA and 0.5 M sucrose solution) in 1.5 mL tube before crashing the anthers by forceps and kept in dark for 5-10 minutes (for FDA staining). 10 μL samples were dropped on 10 glass slides and covered with cover glasses. These samples were observed under the light and fluorescein microscopy (Leitz DMRBE, Leica Co., CA, US), and pictures were taken by microscope camera ProgRes® C5. At least 2,000 pollen grains were counted for each species in each staining methods. Dark stained pollen was viable and the less stained one was inviable. Florescent intensity of pollen grain in FDA staining expressed the esterase activity and it was used to verify pollen viability (Liu et al., 2004).

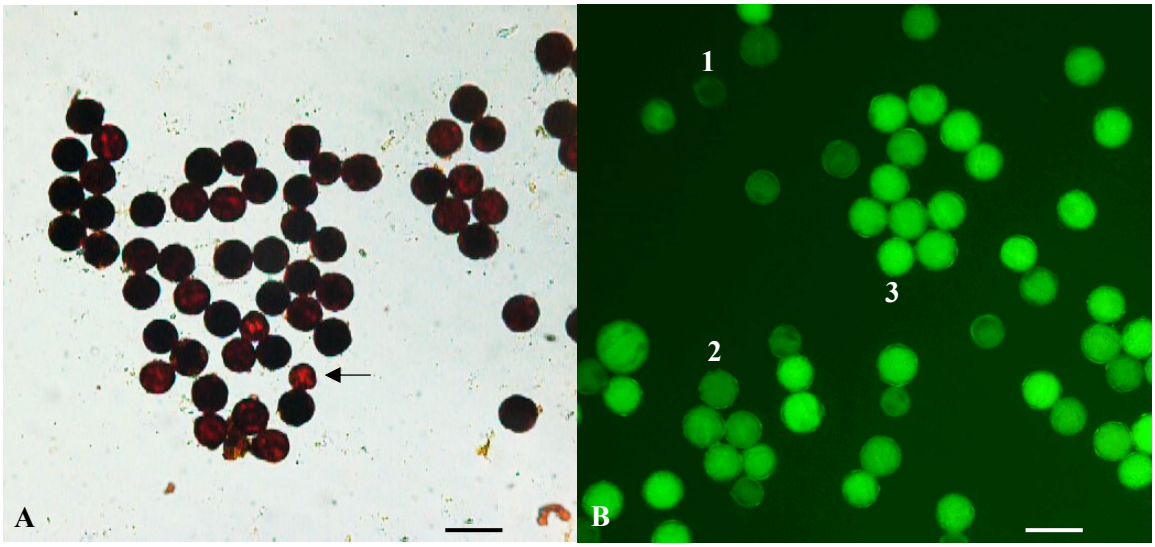

Fig. 1. Pollen viability test by Lugol's solution (A) and FDA (B). Black and solid dark stained pollen, viable pollen; less stained pollen (arrow head), inviable pollen; 1, inviable pollen; 2, low viable pollen; 3, high viable pollen.

Evaluation of pollen tube behavior and self- and cross-compatibility

Flowers at stage 59 of ten citrus species were emasculated and self- and cross-pollinated in all possible combinations (Agusti et al., 1995). Ten pistils of 9 pollinations among byungkyul, dangyooza, and sadoogam were collected at 1, 3, 5, 7, and 9 days after pollination for pollen tube growth observation. Pistils of the rest combinations were collected at 9 days after pollination for self- and cross-compatibility evaluation. Pistils were fixed immediately in FAA solution (formalin:acetic acid:70% ethanol in the ratio of 1:1:18, v/v/v) and kept at 4ºC until use (Distefano et al., 2009). Fixed pistils were cleaned by placing in distil water for 60 minutes with 15 minutes water change interval. Then they were softened in 2 N NaOH at 60ºC for 60 to 90 minutes before cleaning again with distil water. Pistils were stored in 0.1% aniline blue (0.1% aniline blue powder in 0.1 N K_3PO_4) at room temperature for 24 hours. Pistil samples were cut 2 times as shown in Fig. 2. Then samples pieces were squashed under cover glasses and slide glasses before observing under microscope.

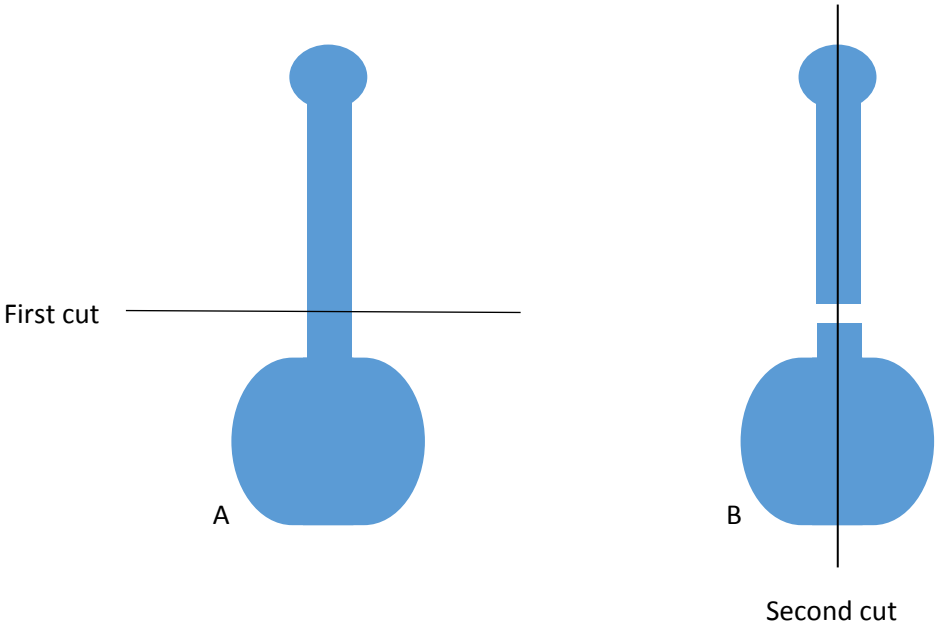

Fig. 2. Pistil cutting pattern. A: cut into 2 parts, stigma-pistil and ovary. B: cut those 2 parts again in vertical direction.

Statistical analysis

Statistical analysis was done using SAS program Version 9.1. Bar chart was drawn using Microsoft office Excel version 2013.

RESULTS

Microspore development and pollen viability

Microspore development was divided into eight stages with flower length and morphology by BBCH scale (Table 2 and Fig. 3). The earliest stage in our observation was sporogenous cell stage (SP) which was found in flower with less than 3 mm in length. All anther cell types developed fully and anther pattern was defined. Four anther layers were recognized in this stage (Fig. 3A). SP developed into microspore mother cell (MC) and then started to do meiosis. MC entered second meiotic stage while tapetum (TA) was enlarging (Fig. 3B). After meiosis, callose surrounding tetrad (TET) was degenerated. TA compressed together and the middle layer started to disappear (Fig. 3C). Microspores (MPs) were individually freed into pollen sac. These young MPs varied in shapes and started to generate cell wall. At this stage, TA cells kept increasing their size and clumped closely with each other (Fig. 3D). Anthers and endothecium expansion occurred when endothecium cells started to do mitosis (Fig. 3E-G). MPs enlarged their size and became round shape. TA cells changed into irregular shapes and reduced their contents. TA cells might be initially transferred their support for microspore development after TA cell enlargement (Fig. 3E). MPs with mature wall continued to enlarge and differentiated into vacuolated MP and vacuolated pollens (Fig. 3F-G). Pollen mitotic division occurred while TA cells were degenerating. Finally, TA completely disappeared from pollen sac. Septum cell degeneration and endothecium thickening initiated (Fig. 3G). Anther contained bicellular pollen grains and became bilocular after degeneration and breaking of septum. Mature pollen grains dehisced from sac through stomium (Fig. 3H).

Viable pollen ranged from 76.6% to 89.1% in Lugol's solution staining. Data also showed high percentage of viable pollens in all species ranged from 69.6% to 81.5% in FDA staining (Table 2). Percentages of viable pollen in Lugol's solution were higher than in FDA. In overall comparison, there was no significant difference in pollen viability between species in this study (Table 2).

Table 2. Microspore development identified from flowers at the different sizes and developmental stages.

Species	Flower length (mm)	Flower stages[z]	Mirospore stages[y]
Byungkyul	<3	55	A
	3-5	55-56	B,C
	6-7	57	D
	8-9	57	E
	10-14	57-59	F
	15-18	59-60	G,H
Sadoogam	<3	55	A
	3-5	55-56	B,C
	6-11	56-57	D
	12-13	57	E
	14-15	57	F
	17-20	59-60	G,H
Dangyooza	<3	55	A
	3-5	55-56	B,C
	6-8	56	D
	9-12	57	E
	13-15	57	F
	16-19	59-60	G,H

[z]Flower stage from BBCH-scale (Agusti et al., 1995).
[y]Microspore stages from Fig. 3. A, sporogenous cell stage; B, meiotic microspore mother cell stage; C, tetrad stage; D, non-vacuolated microspore stage; E, early vacuolated microspore stage; F, vacuolated microspore stage; G, mitotic pollen stage; and H, mature pollen stage.

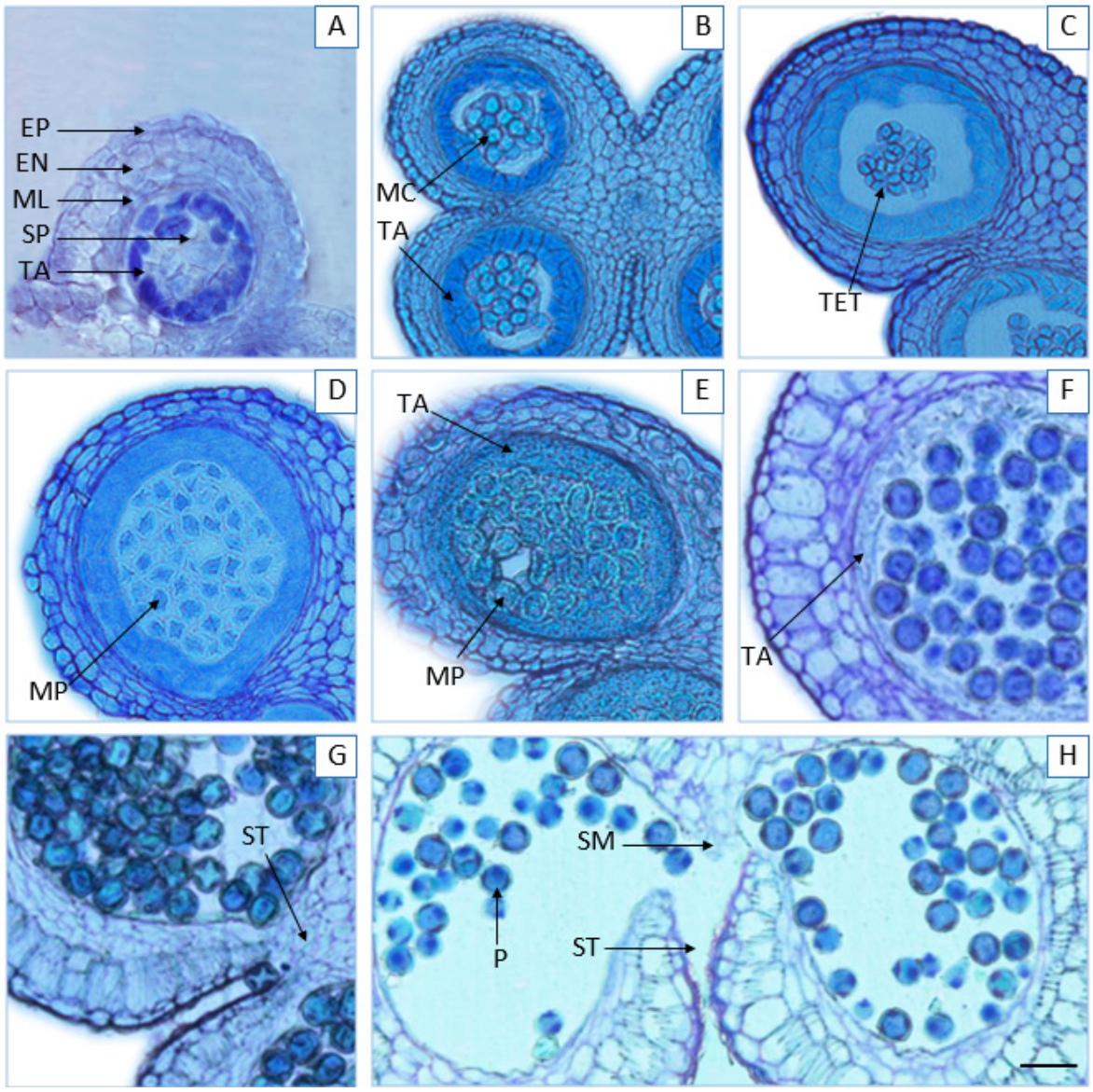

Fig. 3. Microspore development in old local citrus species in Jeju, Korea. A, sporogenous cell stage; B, meiotic microspore mother cell stage; C, tetrad stage; D, non-vacuolated microspore stage; E, early vacuolated microspore stage; F, vacuolated microspore stage; G, mitotic pollen stage; H, mature pollen stage. EP, epidermis; EN, endothecium; ML, middle layer; MC, meiotic cell; MP, microspore; SP, sporogenouos cell; TA, tapetum; TET, tetrad; P, pollen grain; Sm, septum; and ST, stomium. Scale bar indicates 50 μm**.**

Table 3. Percentages of viable pollen estimated by Lugol's solution and FDA.

Species	Lugol's solution (%)	FDA staining (%)	Mean
Byungkyul	83.7±0.6[z]	71.6±1.0	77.6
Sadoogam	84.9±0.3	75.4±0.9	80.2
Dangyooza	82.5±1.5	79.0±0.7	80.8
Dong-geongkyul	82.3±1.3	69.6±1.0	75.9
Binkyul	89.1±0.8	71.2±0.6	80.1
Gamza	76.6±1.7	74.7±1.2	75.7
Gigak	82.9±0.5	74.9±0.6	78.9
Jinkyul	87.3±0.5	72.5±0.9	79.9
Pyunkyul	87.2±0.5	81.5±0.8	84.4
Hongkyul	87.7±1.1	73.4±0.9	80.6

[z] values were indicate means ± standard error.

Evaluation of pollen tube growth patterns and self- and cross-compatibility

Byungkyul, sadoogam, and dangyooza were self- and cross-pollinated with each other to analyze the pattern of pollen tube growth shown in case of self- and cross-incompatibility or self- and cross-compatibilty. Most pollens of all species well germinated and pollen tubes grew into stigma in all pollinating combinations including self-pollinations (Fig. 4). Despite self-pollination in sadoogam, pollens germinated and pollen tubes grew faster than other pollinations and reached upper part of pistil 1 day after pollination. Pollen tubes in byungkyul and sadoogam species reached the lower part of pistils at 3 days and then got into ovaries and ovules at 5 and 7 days after pollination, respectively. However, pollen tubes in dangyooza were just in the upper part of pistils at 3 days, reached ovaries at 5 to 7 days, and later got into ovules 7 to 9 days after pollination except self-pollination (Fig. 4). In the case of self-pollination of dangyooza, there was not any pollen tube reaching ovule even at 9 days after pollination (Fig. 4 and Table 4). In addition, pollen tubes of byungkyul showed slower growth than the others, having pollen tubes in 37.5% of pollinated flowers reached in ovules at 7 days after pollination (Fig. 4). The growth patterns of pollen tubes including pollen tube behavior and growth speed were very similar in all pollinating combinations just except self-pollination of dangyooza (Figs. 4 and 5). In case of dangyooza self-pollination, most of pollen tubes were arrested in upper part of pistil and then the number of pollen tubes kept decreasing in lower part of pistil because most tubes ceased their growth and then completely disappeared just near ovary (data not shown). Besides pollen tubes growth, the shape of stubby end was observed in abnormal pollen tubes (Fig. 5E).

Compatibility in self- and cross-pollinations was evaluated by the number of pollen tubes reached ovaries at 9 days after pollination (Table 4). There were totally 59 pollinating combinations. The number of pollen tubes reached ovaries was noticeably different between pollinating combinations. Twenty-nine combinations had more than 50 pollen tubes in ovary and the other 25 combinations had pollen tubes between 20 and 49. Only 5 combinations had less than 20 pollen tubes reached ovary of their mating partners. Sadoogam × sadoogam had the highest average number of pollen tubes reached ovaries. In contrast, dong-geonkyul × gikak, gikak × gikak, and dangyooza × dong-geonkyul had less than 10 pollen tubes. Dangyooza × dangyooza had an average number of 0.9 pollen tubes

in ovary (Table 4). Most pollinated combinations were considered as self- and cross-compatible to each other because their pollen tubes could grow into ovaries and reached ovules of their mates. In the case of dangyooza, it was cross-compatible, but not self-compatible because less than 1 tube reached ovary and no tube met ovules at 9 days after self-pollination (Table 4).

In addition, with the same data we tried to analyze more critically to evaluate pollen performance. There was not a significant effect of pollen or pistil genotype on pollen tube performance (Table 4). However, we could find the effects of specific female-male combinations on pollen tube growth. For instance, 77.5 pollen tubes of byungkyul presented in ovaries of hongkyul, but only 29.4 in dangyooza. Pistils of dangyooza allowed pollen tubes of sadoogam and dong-geongkyul to reach ovaries with very different number (Table 4).

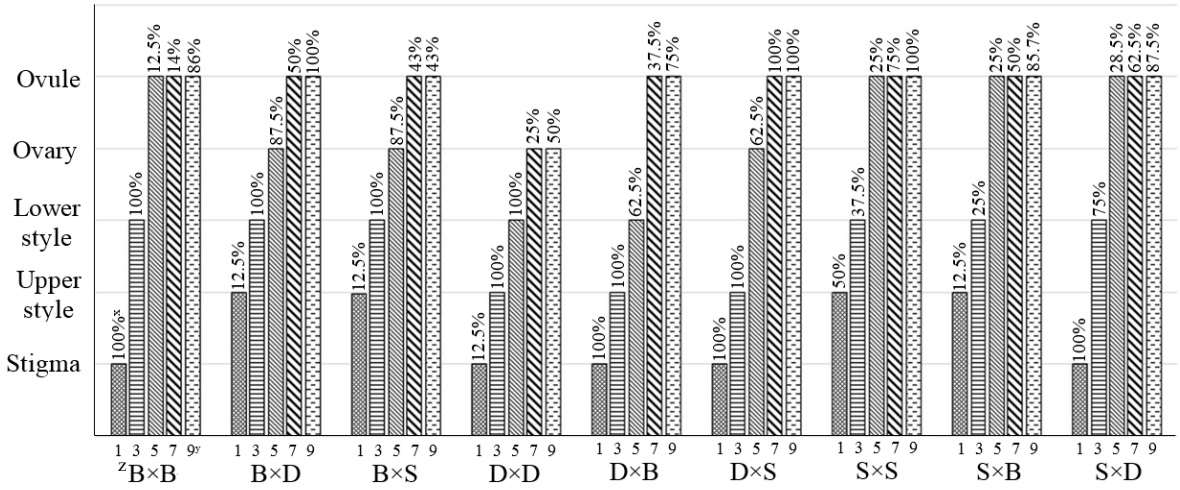

Fig. 4. Pollen tube growth in self- and cross-pollinations among three citrus species.
[z]B, byungkyul; D, dangyooza; S, sadoogam.
[y]Days after pollination.
[x]Flowers with pollen tube reaching different parts of their pistil (%).

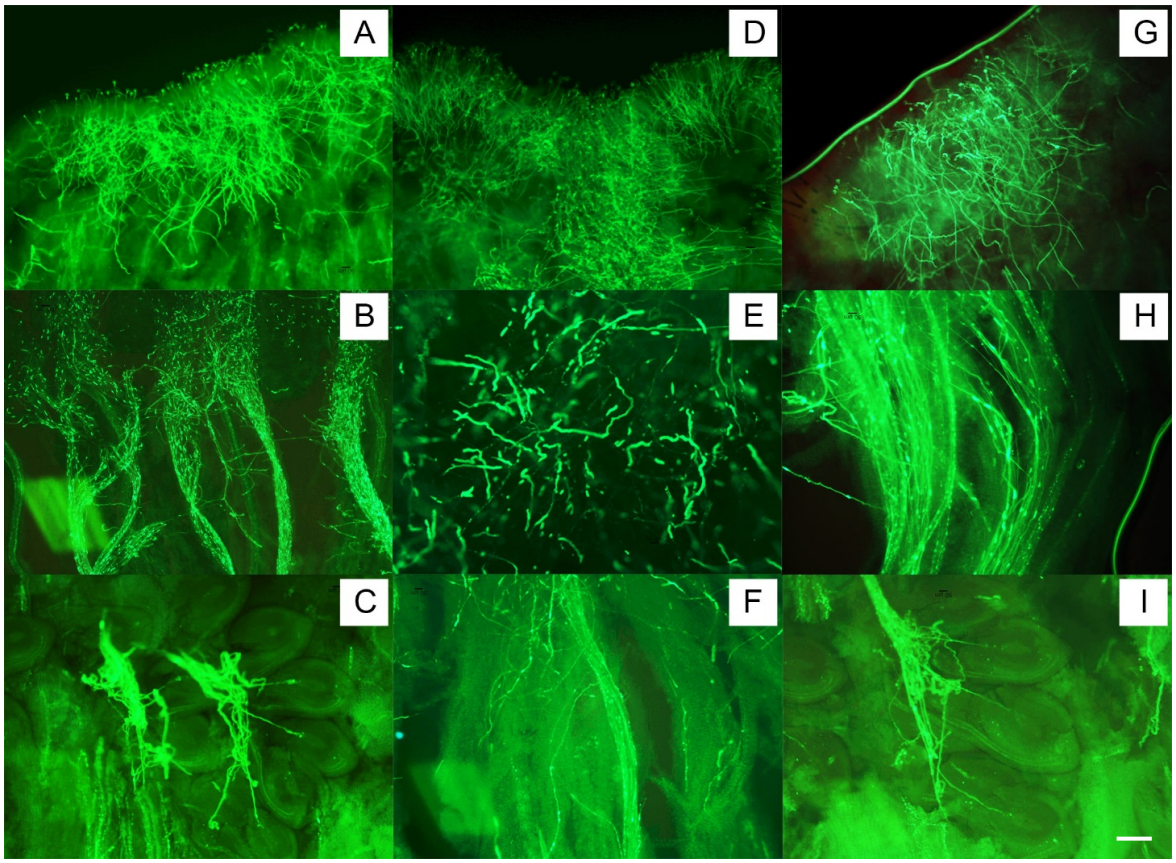

Fig. 5. Pollen tube behavior in compatible and incompatible pollinations. Pollen tubes of byungkyul self-pollination (A, stigma; B, upper and lower style; C, ovary); pollen tubes in dangyooza self-pollination (D, stigma; E, abnormal pollen tube at upper style; F, upper and lower style); pollen tubes of sadoogam self-pollination (G, stigma; H, upper and lower style; I, ovary). Scale bar indicates 200 μm.

Table 4. Number of pollen tubes in ovaries, measured at 9 days after self- and cross-pollinations.

Female/Male	Byungkyul	Dangyooza	Sadoogam	Gigak	Dong geongkyul	Pyunkyul	Hongkyul	Binkyul	Gamza	Jinkyul
Byungkyul	67.1±6.2[z]	82.2±8.6	84.5±10.7	41.8±11.4	69.8±8.1	-	80.7±6.0	77.3±6.2	40.1±12.4	74.3±9.7
Dangyooza	29.4±8.2	0.9±0.3[x]	85.0±5.8	20.7±6.9	9.8±2.4	-	30.6±5.4	35.5±10.4	68.5±10.6	48.2±11.9
Sadoogam	58.1±12.6	69.1±9.8	97.5±5.3	35.4±14.5	66.5±5.4	40.2±11.5	53.5±9.3	44.2±8.8	45.0±9.3	38.5±11.7
Gigak	77.5±7.7	71.6±4.7	42.8±9.2	9.8±3.7	28.6±7.1	43.0±7.4	25.5±6.9	57.0±3.6	35.1±11.2	22.4±8.8
Dong-geongkyul	52.8±7.6	44.0±6.0	11.0±4.2	8.6±2.2	58.8±8.5	32.0±8.3	29.8±9.0	50.2±8.8	61.2±9.8	71.0±9.1
Pyunkyul	71.2±5.7	66.2±2.6	67.0±5.1	-	-	56.8±4.8	-	-	-	-
Hongkyul	77.5±4.0	41.0±8.2	85.0±4.1	-	-	-	35.6±4.4	-	-	-
Binkyul	-[y]	-	-	-	-	-	-	29.2±10.4	-	-
Gamza	-	-	-	-	-	-	-	-	76.3±8.8	-
Jinkyul	-	-	-	-	-	-	-	-	-	49.3±13.5

[z] Value indicates means ± standard error.
[y] Dash sign indicates the pollinating combinations waw not done.
[x] Less than 5 pollen tubes reaching ovary indicate incompatible pollination (Yamamoto et al., 2006).

DISCUSSIONS

Microspore development and pollen viability

Anthers containing reproductive and non-reproductive tissues were responsible for producing and releasing pollen grains. These tissues and cell types had their own specialized tasks (Vinod et al., 2005). In our study, we recognized 8 microspore developmental stages based on differentiation of anther tissues such as tapetum, microspore, endothecium, and others. The early microspore development of male sterile mutant and male fertile 'Ougan' mandarin (*C. suavissima* Hort. ex Tanaka) was identical. The differences were noticed from the tetrad stage until mature pollen stage (Hu et al., 2007). In abnormal microspore development, variable size microspores developed from tetrad cells and then became less dense and shrunken. Tapetal cells degenerated and disappeared earlier, leaving various size aborted microspores without cytoplasm (Hu et al., 2007). In contrast, anthers of byungkyul, sadoogam, and dangyooza contained similar-size tetrad cells and microspores. Tapetum has many important functions as nutrients for microspore development, pollen wall components, and enzymes for microspore release from tetrad cells (Lersten et al., 2008; Vinod et al., 2005). Tapetum developed subsequently and provided its support for microspore development. It degenerated in pollen mitotic stage and completely disappeared just before septum degeneration and breakage (Hu et al., 2007; Sanders et al., 1999). Similar tapetum behavior and normal mature pollen grains were found Jeju old local citrus species. Pollen viability, pollen germination, and pollen tube growth ability in our study were the main evidences supporting our conclusion.

Pollen viability test is critical for crop improvement, breeding program, and other aspects of pollination biology (Stanley and Linskens, 1974). Chae et al. (2012) mentioned about high pollen viability in Jeju old local citrus species. Our results showed a good agreement with their results. Viable pollens were described as pollen grains capable of germinating on stigma. Prevailing environmental conditions, particularly temperature and humidity, affect the pollen viability (Shivanna and Tandon, 2014). Even a small proportion of viable pollen could yield full seed set, particularly in species with lower number of ovules (Shivanna and Tandon, 2014). Mash (*C. paradisi* Macfad) and Allen eureka [*C.*

limon (L.) Burm. f.] had only 12.0% and 54.5% of viable pollen. However, they were found to be self-compatible because some of their pollen tubes grew and reached base of style (Yamamoto et al., 2006). Thus, we could conclude that Jeju old local citrus species in this study were male fertile and they produced enough viable pollen for self- and cross-pollination and compatibility evaluation.

Evaluation of pollen tube growth patterns and self- and cross-compatibility

There were many investigators who reported about pollen tube behavior and self- and cross-compatibility of many citrus accessions (Distefano et al., 2009; Hu et al., 2007; Ngo et al., 2001; Yamamoto et al., 2010, 1995; Ye et al., 2009). Some scientists evaluated self- and cross-compatibility based on pollen tube behavior in style and the presence of pollen tube at the base of style and ovary (Distefano et al., 2009; Ngo et al., 2001; Yamamoto et al., 2006). We also considered on the same criteria to evaluate all pollinations. We could observe pollen tubes reached the ovules in all self- and cross-compatible pollinations. In the case of dangyooza self-incompatibility, lots of pollens were germinated on stigma and penetrated into style. Few tubes could pass through middle style, lower part of style, and got into ovary at 7 days after pollination. On the 9th day, pollen tubes still did not reach any ovules. These evidences led us to conclude that it was self-incompatible. This result was also supported by a previous study reporting that 71 pummelo accessions were self-incompatible and suggesting that all pummelos (*C. maxima* or *C. grandis*) were self-incompatible (Ngo et al., 2001). There were 2 possible reasons which could explain this phenomenon. First, there are complex cellular responses from the ovary such as calcium fluxes, actin rearrangements, and programmed cell death occurring in the incompatible pollen tube that prevent pollen tube growth (Silva and Goring, 2001). Second, there are the complex cellular responses from the style. Lipow and Wyatt (2000) suggested that in some taxa, OSI may be due to late acting mechanism. Therefore, further studies must be required to determine the reasons in self-pollinating dangyooza species.

We also found that pollen performance depended on pollen-pistil interaction. Pollen and pistil genotype did not show a clear effect on pollen tube growth. Several mating pairs also showed more clearly about the effect of pollen-pistil interaction when they were reversed their male/female roles (e.g. dangyooza and byungkyul, gigak and dangyooza,

and sadoogam and dong-geongkyul). There were many works reported about pollen performance in different plant species. They revealed that it depended on pollen genotype (Snow and Spira, 1991), pistil genotype (Hormaza and Herrero, 1999), and environmental conditions during flowering and pollination (Delph et al., 1997; Hedhly, 2011). Differences in pollen performance can result in significant selective advantages if some genotypes are favored over other leading to gametophytic selection (Mulcahy, 1979). In cherry (*Prunus avium* L.) and citrus, the main factors affecting pollen tube growth largely depended on male-female combination and the interaction with the prevailing environmental conditions, mainly temperature (Distefano et al., 2012; Hedhly et al., 2005). But, above reports were not related to reciprocal crosses as in this study. Therefore, further studies need to explain the different levels of compatibility in reciprocal pollinations.

Overall, these results showed that these ten Jeju old local citrus species were male fertile and produced enough viable pollens for self- and cross-pollination. Nine of them were self- and cross-compatible with each other, but their male-female interactions showed difference in pollen tube growth. Dangyooza could be used for cross-pollination with other nine species, but it could not be used for self-pollination.

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ABSTRACT IN KOREAN

본 연구는 제주 재래귤에 있어서 소포자 발달 특성과 자가 및 타가 화합성을 평가하기 위하여 수행되었다. 소포자 발달 특성 분석을 위하여 병귤, 당유자 및 사두감의 발달단계별 꽃에서 꽃밥을 채취하여 포매한 후 절편을 내어 Toluidine blue O 로 염색하였다. 10 개의 종에 대한 화분 발아력 측정은 Lugol's solution 과 fluorescein diacetate (FDA) 염색법을 이용하여 수행되었다. 모든 종에 대해 개화 직전에 화판과 수술 등을 제거 한 후 자가 그리고 타가 수분하였다. 병귤, 사두감 및 당유자 간의 9 개 교배조합에서 교배 1, 3, 5, 7 및 9 일 후에 암술을 채취하여 화분관 신장 양상을 평가하였다. 나머지 교배조합에 대해서는 교배 9 일 후에 암술은 채취하여 자가 또는 타가 화합성을 평가하였다. 채취한 암술은 FAA 액으로 고정한 후 압착하고 aniline blue 로 염색하여 조제되었다. 소포자 발달은 8 개 발육단계에서 정상적으로 진행되는 것으로 관찰되었다. 재래귤의 화분 활력은 Lugol's solution 분석에 대해 76.6-89.1%, 그리고 FDA 에 대해 69.6-81.5% 정도로 높은 활력을 가지고 있었다. 암술에서의 화분관 신장 양상은 화합성과 불화합성 간에 구분될 수 있었다. 화합성의 경우 많은 화분관들이 암술대에서 신장하여 자방 및 배주에 도달하였다. 그러나 불화합의 경우 화분관은 암술대의 상부 및 하부에서 신장이 정지되었다. 화분관 신장 반응은 교배조합의 자웅관계에 따라 크게 달라졌다. 따라서 모든 제주 재래귤은 정상 임성의 화분을 생산하여 자가 및 타가 교배가 가능하였다. 본 연구에 사용된 10 개 재래귤 중 9 개는 상호간에 자가 및 타가 화합을 나타내었다. 그러나, 당유자는 타가 화합 및 자가 불화합성을 나타내었다.

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