Studies on the male sterility of Satsuma orange (*Citrus unshiu* MARC.)

Effect of temperature on the recovery of pollen fertility concerning the metabolism of carbohydrates and enzyme activities in anther

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Summary

Pollen fertility of Satsuma orange could be increased about 14 to 23% by the temperature treatments (at 15°C and 20°C) as compared with the outdoor control. At 25°C temperature treatment, the increase of pollen fertility was a little higher than the outdoor control, but the flowers were comparatively smaller in size and feeble which dropped off within 2 or 3 weeks after anthesis. Therefore, it may be considered that the favorable minimum temperature during the growth and development of flower buds is 15~20°C, deliberating on the increase of pollen fertility and the development of flower buds.

The increase of pollen fertility caused by the temperature treatment would be attributed to the recovery of some of the vacuous starveling microspores at the vacuole enlarging stage and the starch accumulation in microspore at the vacuole disappearing stage.

These phenomena indicate the following: 1) Enzyme activities, such as hexokinase, invertase,

amylase and maltase in anther and activity of starch synthesizing enzyme system in microspore were increased by the high temperature treatments. 2) These high activities of the enzyme might decompose much more starch which reserved in anther walls and supplied rich nutrient to microspore. Furthermore, it might also release much more energy through the glycolytic pathway in anther. 3) The vacuous starveling microspores might have utilized the rich energy and absorbed the nutrient from periphery of the anther sac and excluded the large vacuole from the cytoplasm in microspore. 4) As a result, a great number of microspores recovered from the critical vacuole enlarging stage during the pollen development and deposited starch in the cytoplasm at the vacuole disappearing stage. 5) Consequently, those recovering microspores proceeded to the next growth cycle, became viable pollen grains and increased the pollen fertility at anthesis.

Introduction

Cytological and histological studies on the pollen sterility of *Citrus* plants have been carried out by many workers, concerning the influence of low- and high-temperatures^(4,10,16,18,30) YANG *et al.*⁽³⁰⁾ also described that the pollen fertility could be increased and the abnormal behavior of tapetum could be recovered partially by the temperature treatment (above 20°C) during the pollen development. However, the mechanism of its physiological bases is still obscure.

NAKAMURA⁽¹⁸⁾ stated that the nutrient supply to the starved microspores was decreased at vacuole stage and the reservation of starch in microspore after the vacuole disappearing stage was disturbed by the influence of low temperature. It is well known that under low temperature conditions the microspores fail to reserve starch at the vacuole disappearing stage and the starch content decreases markedly in anther wall at the initial stage of its development⁽²⁹⁾

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These phenomena may be related to the activities of enzymes, such as amylase, invertase and hexokinase.

The experiment reported herein was carried out to depict the influence of temperatures on the pollen sterility concerning the metabolism of carbohydrates and enzyme activities in anther at the vacuole stage of microspore and to make sure the mechanism of pollen abortion in Satsuma orange.

Materials and Methods

Materials used for this study were 5~6 years old Satsuma orange trees (*Citrus unshiu* MARC.), which were planted individually in 60 cm diameter unglazed pots containing loamy soil. Each plot consisted of nine plants. Vinyl chambers ($2 \times 2 \times 1.8$ m) were made in the glasshouse for the temperature treatments. Air temperatures for warming within the chambers were controlled electrically by using the lead heating cells. Furthermore, only the minimum temperatures were checked throughout the experiments in each chamber at 10, 15, 20 and 25°C, respectively. Temperature treatments were carried out from mid January to early Spring during 1968-1969.

For cytological studies fresh sample of flower buds were collected at 1~2 days intervals. Microspores and pollen grains were stained with aceto-carmine, and their sizes were measured at various developmental stages by micrometer. During the course of development of pollen grains, iodine potassium iodine solution was used for the observation of starch in microspores and pollen grains.

Analysis of sugars in anther was done with the thin layer chromatography (TLC). The development was carried out twice by dimensional ascending manner in the solvent mixture of ethyl acetate/iso-propylalcohol (70:30, v/v) and ethyl acetate/iso-propylalcohol/water (52:36:12, v/v). Sulfate anisaldehyde was used as color reagent.

The contents of reducing sugar, non-reducing sugar and starch in anther at various stages were estimated according to the method of SOMOGYI⁽²⁶⁾.

Activity of starch synthesizing enzyme system in microspore: Fresh samples of anther at the vacuole disappearing stage of microspore were collected and the method of histo-chemical test was used^(13,22,27). All these anthers cut in two were incubated in the medium with glucose-1-phosphate. The number of microspore with starch were investigated by using the smear method with iodine potassium iodine solution. Preliminary works indicated that optimum condition for the activity of starch synthesizing enzyme system in microspore at the vacuole disappearing stage was at 37°C and sodium acetate buffer pH 5.0 for 24 hours.

Activities of various enzymes in anther: The anther at the vacuole enlarging stage were collected and stored in refrigerator at -20°C until the extractions were made. Enzymes (amylase, maltase, invertase and hexokinase) were extracted according to the method of KATSUMATA *et al*^(11,12,13).

Amylase activity: Amylase activity was expressed by the determination of the dextrinizing activity⁽¹⁾ and of the saccharogenic activity⁽²¹⁾. The optimum condition for dextrinization was at 40°C and sodium acetate buffer pH 6.5, and for saccharogen was at 40°C and phosphate buffer pH 6.0~6.5.

Invertase and maltase activities: The enzyme activity was determined following the methods of SOMOGYI⁽²⁶⁾-NELSON⁽¹⁹⁾. The optimum condition of maltase was at 40°C and sodium acetate buffer pH 5.0, and of invertase was at 40°C and McILVAINE's citrate-phosphate buffer pH 4.0.

Hexokinase activity: Hexokinase activity was expressed by the determination of the phosphorylations of glucose and fructose. The phosphorylation of glucose was estimated by the methods of SOMOGYI⁽²⁶⁾-NELSON⁽¹⁹⁾, and the phosphorylation of fructose was determined by the method of ROE⁽²³⁾. The optimum condition for hexokinase activity was at 37°C and Tris-HCl buffer pH 7.5.

Results

1. Effect of temperature on the growth and development of flower buds and pollen grains :

Preliminary experiments revealed that even at any time of dormancy in winter the Satsuma orange trees which were kept under high temperature conditions showed flower formation.

Fig. 1. Effect of temperature on the growth of flower buds and flowering date.

F : flowering (Stage VIII). M : young microspore (Stage I).

1st T. (—) : first treatment, 2nd T. (----) : second treatment.

T : treatment

Table 1. Effect of temperature on the flowering habit in Satsuma orange.

Treatments		Date of treatment	Date of changing the treatment	Average temperature from Stage I to Stage VIII ¹⁾			Date of bloom	Length of pistil mm	Length of stamen mm	LP-LS ²⁾ mm
				Maximum	Minimum	Average				
Control	-1	Jan. 15, '68	—	22.3°C	10.4°C	16.4°C	May 8, '68	16.4	12.1	4.3
	-2	Feb. 21, '68	—	22.3	10.4	16.4	"	16.1	12.0	4.1
	-3	Jan. 16, '69	—	25.4	9.7	17.6	May 10, '69	16.1	12.0	4.1
Glasshouse	-1	Jan. 15, '68	—	28.0	10.6	19.3	Mar. 28, '68	15.2	11.7	3.5
	-2	Feb. 21, '68	—	28.0	10.6	19.3	"	15.3	11.7	3.6
	-3	Jan. 16, '69	—	27.5	9.8	18.7	Apr. 19, '69	15.6	12.0	3.6
10°C	-1	Jan. 15, '68	—	25.8	10.0	17.9	Feb. 27, '68	16.1	12.2	3.9
	-2	Feb. 21, '68	—	25.6	10.0	17.8	Mar. 18, '68	16.1	12.1	4.0
	-3	Jan. 16, '69	—	26.1	10.0	18.1	Mar. 1, '69	15.9	12.1	3.8
15°C	-1	Jan. 16, '69	—	26.5	15.0	20.8	Feb. 15, '69	13.5	10.8	2.7
20°C	-1	Jan. 15, '68	—	31.4	20.0	25.7	Jan. 25, '68	13.1	11.6	1.5
	-2	Feb. 21, '68	—	31.4	19.7	25.6	Mar. 6, '68	12.8	11.4	1.4
	-3	Jan. 16, '69	—	32.2	20.0	26.1	Jan. 28, '69	12.9	11.4	1.5
25°C	-1	Feb. 21, '68	—	32.3	24.6	28.5	Mar. 2, '68	11.7	10.6	1.1
	-2	Jan. 16, '69	—	33.0	24.9	29.0	Jan. 23, '69	11.8	10.9	0.9
20°C $\xrightarrow{I}$ Glasshouse ³⁾		Jan. 15, '68	Feb. 5, '68	19.7	1.2	10.5	Mar. 9, '68	14.8	11.5	3.3
20°C $\xrightarrow{IV}$ Glasshouse ³⁾		"	"	17.2	0.5	8.9	Feb. 22, '68	13.0	10.5	2.5
Glasshouse $\xrightarrow{I}$ 20°C ⁴⁾		"	Mar. 26, '68	31.9	21.0	26.5	Apr. 3, '68	14.6	11.5	3.1
Glasshouse $\xrightarrow{IV}$ 20°C ⁴⁾		"	"	32.1	21.1	26.6	Apr. 1, '68	14.0	10.9	3.1

1) I, II, ... : Showing the respective stage of pollen grains development.

I : Soon after the liberation of microspores from the wall of pollen tetrads.

II : Vacuole appearing stage of microspore.

III : Vacuole enlarging stage of microspore.

IV : Vacuole disappearing stage of microspore.

V : Pollen mitosis stage.

VI : Young pollen grain stage.

VII : Mature pollen grain stage.

VIII : Blooming stage.

2) Length of pistil—Length of stamen.

3) When the plants having the flower buds either at Stage I or Stage IV grown under 20°C were transferred into the glasshouse without heat system.

4) When the plants possessing the flower buds either at Stage I or Stage IV grown under glasshouse without heat system were transferred into 20°C minimum temperature chamber.

within a few days and accelerated blooming time. As shown in Fig. 1, the plants treated with 20°C or 25°C in January and February bloomed within 2 weeks after treatments. The growth of flower buds under high temperature conditions was more rapid than that under low temperature conditions. To explain further, after the treatments it bloomed 7 to 10 days at 25°C, 10 to 14 days at 20°C, 30 days at 15°C and 43 to 44 days at 10°C.

The ultimate size of flower buds under high temperature was smaller than those under low temperature and the control. The flowers were smaller in size, closing stigma and anthers (Table 1, and Fig. 2) under high temperature conditions (25°C and 20°C). Those appeared feeble dropped off within 2 or 3 weeks after the anthesis at 25°C treatment. On the contrary, the flowers under low temperature (10°C) were as large in size as that in the case of control, and the stigma and anthers showed a tendency to remain at a long distance. Detailed

observations of the development of pollen grains under various temperature treatments were shown in Fig. 3. It took long time for the maturity of the pollen grains in low temperature in comparison to high temperature, namely, the number of days from the vacuole appearing stage (Stage II) to the vacuole disappearing stage (Stage IV), the most important period during the pollen development, was 6 days at 10°C as well as in the control, 4 days at 15°C, 2 days at 20°C and 1.5 days at 25°C. There were no marked differences in the size of pollen grains at anthesis of all the treatments.

2. Effect of temperature on the pollen sterility :

Variation of pollen sterility in Satsuma orange occurred under various temperature treatments is shown in Table 2. The pollen sterilities at 20°C and 15°C decreased about 23 and 14% respectively, when compared with the outdoor control, whereas at 10°C and 25°C decreased only 4 and 6% respectively than the control.

When the plants having the flower buds either at Stage I or Stage IV grown under 20°C were transferred into the glasshouse without heat system (hereafter referred to as 20°C $\xrightarrow{\text{I or IV}}$ glasshouse) on Feb. 5, 1968, the former flower buds (Stage I) showed very high pollen sterility, *i. e.*, 90%, and the latter ones (Stage IV) showed as low sterility as that at 10°C, *i. e.*, 78%. Furthermore, when the plants possessing the flower buds either at Stage I or Stage IV grown under glasshouse without heat system were transferred into the 20°C-

Fig. 2. Photographs showing the flower styles in Satsuma orange under different temperature conditions.

A : Control, B : 15°C, and C : 20°C.

Different lengths between stigma and anthers are notable.

minimum temperature chamber (hereafter referred to as glasshouse $\xrightarrow{\text{I or IV}}$ 20°C) on March 26, 1968, the flower buds (Stage I) showed a little decrease in their pollen sterility about 7% and the flower buds (Stage IV) showed only about 3% decrease as compared with the control of the glasshouse. The decrease in pollen sterility at 20°C and 15°C were associated with the decrease in the number of large and medium sized abortive pollen grains, which, indeed, affected the sterility.

Furthermore, the increase in pollen sterility at 20°C $\xrightarrow{\text{I}}$ glasshouse was also associated with the increase in the number of medium sized abortive pollen grains. It may be said that the pollen grains ceased their growth at Stage III (the critical period of the degeneration of pollen grain) under unfavorable low temperature conditions. A large number of small sized pollen grains at 25°C were due to the abnormality of meiotic division and sporads at pollen tetrads (Table 3). There were no marked differences in the number of abnormal pollen grains

Fig. 3. Effect of temperature on the development of pollen grains.

Table 2. Effect of temperature on the pollen sterility in Satsuma orange.

Treatments	No. of pollen grains observed	Number of abortive pollen grains				Pollen sterility (%)	Pollen fertility (%)
		Large (>27.5 μ)	Medium (27.5—22.5 μ)	Small (22.5 μ >)	Total number		
Control	1000	284	498	52	834	83.4	16.6
Glasshouse	1000	260	470	44	774	77.4	22.6
10°C	1000	256	488	50	794	79.4	20.6
15°C	1000	230	411	49	690	69.0	31.0
20°C	1000	197	352	55	604	60.4	39.6
25°C	1000	260	415	102	777	77.7	22.3
20°C $\xrightarrow{I}$ Glasshouse	1000	162	693	53	908	90.8	9.2
20°C $\xrightarrow{IV}$ Glasshouse	1000	267	468	50	785	78.5	21.5
Glasshouse $\xrightarrow{I}$ 20°C	1000	221	438	43	702	70.2	29.8
Glasshouse $\xrightarrow{IV}$ 20°C	1000	241	462	45	748	74.8	25.2

Table 3. Effect of temperature on the abnormalities in meiotic division and at pollen tetrads stage.

	Control	Glass-house	10°C	15°C	20°C	25°C	20°C $\downarrow$ I Glass-house	20°C $\downarrow$ IV Glass-house	Glass-house $\downarrow$ I 20°C	Glass-house $\downarrow$ IV 20°C
No. of PMCs or sporads observed	500	500	500	500	500	500	500	500	500	500
Abnormality in meiotic division in PMCs (%)	4.2	4.0	3.8	3.6	4.0	4.7	3.9	4.0	3.8	4.0
Abnormality at pollen-tetrads stage (%)	4.5	4.6	4.5	4.4	5.1	7.6	4.8	4.7	4.5	4.8

Table 4. Effect of temperature on the frequency of various types of pollen grains in Satsuma orange.

Treatments	No. of pollen grains observed	No. of normal pollen grains	No. of abnormal pollen grains			
			Empty	With one and more than two nuclei	With the disintegration of cytoplasm	Total number
Control	1000	166	593	77	164	834
Glasshouse	1000	226	527	80	167	774
10°C	1000	206	541	85	168	794
15°C	1000	310	455	79	156	690
20°C	1000	396	395	59	150	604
25°C	1000	223	532	73	172	777
20°C $\xrightarrow{I}$ Glasshouse	1000	92	723	54	131	908
20°C $\xrightarrow{IV}$ Glasshouse	1000	215	519	79	187	785
Glasshouse $\xrightarrow{I}$ 20°C	1000	298	451	73	178	702
Glasshouse $\xrightarrow{IV}$ 20°C	1000	252	508	77	163	748

in all the temperature treatment, except at 20°C $\xrightarrow{I}$ glasshouse. It can be emphasized that the sterility of the pollen grains depends on the variation of the number of empty pollen grains at the various temperature conditions during the flower bud development (Table 4).

3. Effect of temperature on the contents of carbohydrates in anther :

Carbohydrates, such as starch and sugars, in anther at the vacuole enlarging stage of microspore (Stage III) at various temperature treatments were determined (Fig. 4). The amount of starch content in anther was the least at 25°C and it gradually increased with the decrease in the temperature under all treatments. The amount of total sugars and non-reducing sugar in anther above 15°C temperature treatments were comparatively higher than that at 10°C and that of outdoor control.

Fig. 4. Effect of temperature on the contents of sugar and starch in anther at the vacuole enlarging stage of microspore.

In Fig. 5, the effects of temperature on the changes in starch and sugar contents in anther at various stages of pollen development under 20°C temperature condition and outdoor control were graphically demonstrated. Total sugar contents in anther both under 20°C and outdoors increased at the early stages of pollen development from Stage I to III, remained constant from stage IV to VII and increased again. The sugar contents at 20°C, however, were always higher than the outdoor control throughout all stages of pollen development. On the contrary, the fluctuation of starch contents in anther occurred during the pollen development both under 20°C and outdoor control as shown in Fig. 4-D. It decreased at the early stages of pollen development, followed by a little increase and sharp drop. The fluctuation under 20°C was wider than the outdoor control throughout the growing stages of microspore development.

Fig. 5. Changes in sugar and starch contents in anther at various stages of pollen development as affected by the temperature treatment (20°C).

Fig. 6. Comparison of sugar constituents in anther at the vacuole enlarging stage of microspore and mature pollen grain stage among various Citrus plants.

4. Effect of temperature on the constituent of sugars in anther :

No differences of free sugar constituents in anther were observed between temperature treated and outdoor control in Satsuma orange and among various *Citrus* species, as shown in Fig. 6. Several kinds of free sugars, such as glucose, fructose, sucrose, maltose, xylose, two UV-light spots and one unknown spot were detected from the ethanol extraction of the anther in all the examined plots by means of TLC. However, relatively small spots of all free sugars, especially sucrose, glucose, and fructose on TLC from the anther extraction of male sterile species were observed. Moreover, it was found that the spots of sugars on TLC from the anther extraction of 20°C temperature treated Satsuma orange were recovered as large as that of the male fertile species.

5. Effect of temperature on enzyme activities in anther at the vacuole enlarging stage and in microspore at the vacuole disappearing stage :

Accumulation of starch in microspores of Satsuma orange occurred in a few fertile ones at the vacuole disappearing stage but it was lacking in most of the sterile ones. When the trees were treated with high temperature treatments (above 15°C) during the bud growing period starch contents in microspores increased (Fig. 7). It is well known that starch accumulation is associated with an activity of starch synthesizing enzyme system. Preliminary experiment showed that the activity of starch synthesizing enzyme system in microspore of Satsuma orange was found at the vacuole disappearing stage, although the activity was not detected at the vacuole enlarging stage. Temperature treatments increased the activity of starch synthesizing enzyme system in microspores as shown in Fig. 8. The activities at 25°C and 20°C were about 2~3 times higher than that under the outdoors and at 10°C.

Starch accumulation in microspores at the vacuole disappearing stage might be closely related to the various enzyme activities in anther just at the vacuole enlarging stage. Therefore, the

Fig. 7. Percentage of microspores with starch at the vacuole disappearing stage of microspore as affected by the temperature treatments.

Fig. 8. Activity of starch synthesizing enzyme system in microspores at the vacuole disappearing stage (at pH 5.0 and 37°C) as affected by the temperature treatment.
[Activity = % of microspore with starch (incubated) - % of microspore with starch (control)]

Fig. 9. Amylase activity in anther at the vacuole enlarging stage of microspore (at pH 6.5 and 40°C) as affected by the temperature treatments.

○—○ Control ●—● Glasshouse △—△ 10°C
 ▲—▲ 15°C □—□ 20°C ■—■ 25°C

various enzyme activities, such as amylase, maltase, invertase and hexokinase activities in anther at the vacuole enlarging stage were compared among the various temperature treatments. The results are presented in Figs. 9~12. The increase in activity of amylase was much greater for the high temperature treatments than for the low temperature treatments and the control during 3 to 6 hours after incubation. The rates of diminution in blue value from amylose were the same as that from soluble starch. Whereas the reducing value (as maltose) of amylose was higher than that of soluble starch. Therefore, it may be suggested that both α -amylase and β -amylase are contained in anther of Satsuma orange. Similar tendencies were obtained in the case of maltase and invertase activities between high and low temperature treatments. The activities of maltase and invertase at 25°C and 20°C were about 2.3

Fig. 10. Maltase activity in anther at the vacuole enlarging stage of microspore (at pH 5.0 and 40°C) as affected by the temperature treatments.

Fig. 11. Invertase activity in anther at the vacuole enlarging stage of microspore (at pH 4.0 and 40°C) as affected by the temperature treatments.

times and 4 times greater respectively than that of the control. The hexokinase activity in anther was expressed as the rate of phosphorylation of glucose and fructose. The rate of phosphorylation of fructose was greater than that of phosphorylation of glucose. Among the various temperature treated anther extracts, the rate of phosphorylation of glucose and fructose was the highest at 25°C, followed by 20°C, 15°C, glasshouse, 10°C and the outdoor control.

Fig. 12. Hexokinase activity in anther at the vacuole enlarging stage of microspore (at pH 7.5 and 37°C) as affected by the temperature treatments.

Discussion

The facts that male sterility could be influenced by temperature have been reported in many crops^(3,5,9,15,16,17,18,20,25). It has been known in Satsuma orange that the pollen fertility increases under warmer subtropical region of Taiwan, in certain warmer climatic regions of Japan and under glasshouse and the temperature controlled chambers (above 15°C)^(6,8,16,18,30,31).

From the results of the present experiments, the increase in pollen fertility at 20°C, 15°C

and glasshouse $\xrightarrow{I}$ 20°C were related to the decrease in the number of large and medium sized abortive pollen grains. On the contrary, the decrease in pollen fertility under 20°C $\xrightarrow{I}$ glasshouse was also related to the increase in the number of medium sized abortive pollen grains. In the abortive pollen grains, majority of them were medium sized ones and, therefore, it may be considered that the medium sized abortive pollen grains at anthesis were formed during the vacuole disappearing stage of microspore owing to cessation of its growth.

The period of the pollen development from the start of microspore formation to the maturity of pollen under high temperature was shorter than the low temperature. The number of days during the vacuole stage of microspore (from Stage II to Stage IV) was 2 and 4 days at 20°C and 15°C respectively and 6 days at 10°C as well as in the control. In these case, the former showed higher pollen fertility than the latter. Therefore, the number of days during the vacuole stage of microspore might be concerned with the pollen fertility in Satsuma orange.

Cytological observation showed that the abnormalities of meiotic division in PMCs and mitosis in pollens were inconsiderable regardless of the temperature treatments. Moreover, among the temperature treatments the disintegration of cytoplasm in pollen after mitosis stage was at the rate of 15 to 18%. On the other hand, the starch test reaction was negative in most of the microspores during the vacuole disappearing stage which indicated lack of starch. This may be suggested that the microspores would be disturbed their development during the vacuole enlarging stage, and the critical period of the degeneration of pollens would be the vacuole enlarging stage of microspore.

NAKAMURA⁽¹⁸⁾ pointed out that the occurrence of the large vacuole in microspores was a symptom of the deficiency of nutrient, especially carbohydrates. These vacuous starveling microspores could be recovered sufficient energy to absorb carbohydrates and to accumulate an abundant of starch during the vacuole stage, and then developed into the viable pollens, while, the other ones which failed to recover energy and to absorb carbohydrates became abortive. The similar results were observed in many crops by several workers^(2,5,7,18,24).

During the vacuole disappearing stage of microspore in Satsuma orange, the starch contents under high temperature condition was much greater than that under low temperature and the outdoor control. ONO⁽²²⁾ stated that starch forming activity in guard cells of some plants was not recognized at the comparatively lower temperature (below 10°C) and no starch was found in guard cells. NAKAMURA⁽¹⁸⁾ also reported that the microspores of *Citrus* ceased to accumulate starch owing to the influence of low temperature. Therefore, the high activity of starch synthesizing enzyme system in microspore under high temperature conditions may be one of the causes for the promotion of the microspore development and then the viable pollen would be produced.

The vacuole disappearance and starch accumulation in microspore at the vacuole disappearing stage might be closely related to the various enzymes activities such as amylase, maltase, invertase and hexokinase in anther just at the vacuole enlarging stage. In the present experiment, the activities of these enzymes in anther at the vacuole enlarging stage under high temperatures were greater than that under low temperatures and the outdoor control. The transformation of glucose from starch and maltose which were reserved in anther walls was

promoted by high activities of amylase and maltase. Furthermore, the decomposition of sucrose was mediated by invertase and the formation of glucose and fructose could also be promoted. Then, the increased energy was released by the phosphorylations of glucose and fructose which were mediated by hexokinase. Ultimately, the microspore at the vacuole enlarging stage exposed under high temperature might have utilized quickly that rich energy and absorbed nutrient from periphery of the anther sac and excluded the large vacuole from the cytoplasm. Consequently, a large number of microspores under the high temperature conditions recovered and those recovering microspores could enter into the next growth cycle and became the viable pollen grains resulting in an increase of the pollen fertility. While, enzyme activities in anther and microspore at 25°C were greater than at 20°C temperature treatment. The pollen fertility at 25°C, however, was less than at 20°C temperature treatment. It may be caused by the irregularities of meiotic division in PMCs and mitotic division in pollens and by the disintegration of cytoplasm in pollen grain owing to the high temperature lesion. Furthermore, the nutrient and energy released through the glycolytic pathway at 25°C in anther were utilized by the rapid growth of anther and were not absorbed by the microspore and pollen during their development. On the contrary, the microspore growing under low temperature conditions and the outdoor control could not obtain enough nutrient and energy to exclude the vacuole from cytoplasm in microspore, and then the empty medium sized pollen grains were produced at anthesis.

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温州ミカンの雄性不稔に関する研究
花粉稔性の回復に関係する葯内の炭水化物の代謝
および酵素活性に及ぼす温度の影響

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摘 要

温州ミカンの花粉稔性は、その発達過程における温度条件によって差異があり、温暖条件のもとでは花粉稔性が高められることが知られている。本研究は温州ミカンの鉢植 5~6 年生樹を用い、ガラス室内に設けたビニルハウス内に入れ、その最低温度を 10°C, 15°C, 20°C および 25°C に調節し、花蕾の発育期間中異なる温度条件において、小孢子および花粉の発達と退化の様相を調査するとともに、葯内の炭水化物の代謝および酵素活性について実験を行なった。

温度処理した温州ミカンの花粉稔性を露地に比べると、花粉稔性の上昇率は 15°C 処理区では 14%, 20°C では 23% であった。しかし、25°C では花粉稔性の上昇率はわずか 6% であり、また花蕾は小さく、弱々しい外観を示し、開花後 2~3 週間以内に子房や、幼果が全部離脱した。したがって温州ミカンの稔性を上昇させる温度条件は花粉稔性の上昇率と花蕾の発育の両方を考慮すれば、花蕾から開花までの期間中、最低温度 15~20°C が

適当であると考えられた。

温度処理によつて稔性花粉が増加することは、加温によつて液胞期およびそれ以後の花粉の発達が進行し、デンプンを集積した小孢子の数が増大するためである。高温下で葯内のアミラーゼ、マルターゼ、インペルターゼ、ヘキソキナーゼおよび小孢子内のデンプン合成酵素系の活性を測定した結果それが高まっていたことから、葯壁に貯蔵されていたデンプンが多量に分解され、小孢子に十分な栄養を供給できるようになる。また葯内の解糖経路からも多量のエネルギーが放出されることが考えられる。この結果、液胞をもつた飢餓状態の小孢子はタペート細胞などを含む葯壁から栄養分を吸収して、細胞質内の液胞を排除し、液胞期およびそれ以後の発育過程を円滑に進め、液胞を消失するとともに、栄養物質としてデンプンを十分に集積して稔性花粉となり、花粉稔性を高めたものと考えられる。