

Studies on the New Male-Sterility in Japanese Radish, with Special Reference to the Utilization of this Sterility towards the Practical Raising of Hybrid Seeds

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1. Introduction

In Japan it was in the current century that the vegetable breeding has been developed remarkably, with the successful production of a great number of new varieties.

About 75 percent or more of the leading varieties produced in vegetables during recent years belong to F_1 or the similar hybrids.

Moreover, the production of these hybrid varieties in vegetables is increasing more and more, and in some vegetables almost all of the leading varieties have come to be replaced by such hybrid varieties.

Such high development of hybrids in vegetables is based on the practical application of the hybrid vigour including, in general, various useful characteristics such as vigorous growth, uniform quality in the products, hastening of the flowering and of the ripening time, and so on.

The practical application of hybrid vigour in vegetables perhaps goes back to the study of KAKIZAKI (1924) on the F_1 seed production in the egg plant and the first success in the practical raising of F_1 seeds in vegetables was attained in the egg plant by the results of several experiments done by some workers (ANDO et al., 1936; FUJII, 1955; HURUYA, 1943; KAKIZAKI, 1924, 1926, 1928, 1931; KADOTA, 1937, 1940; KUMAZAWA, et al., 1937; NAGAI et al., 1926; NONOMURA, 1939; TAKEUCHI, 1932; TATEISHI, 1927; WATANABE, 1935).

Since then, the utilization of hybrid seeds was executed steadily in various kinds of vegetable having no or little trouble for the economical production of hybrid seeds, such as cucumber, egg plant, tomato, water melon and so on.

In the early days, the practical utilization of hybrid seeds in vegetables was restricted within the sphere of the plants possessing preferable characters for the production of hybrid seeds.

However, there are many vegetables having not such favourable characters in spite of their showing of a considerable hybrid vigour when they were raised under certain suitable cross combinations.

Then, some new crossing methods based on the self-incompatibility were developed by the efforts of many investigators (EAST, 1917; ITO, 1954; KAKIZAKI, 1923, 1925, 1930₁, 1930₂, 1933; MIZUSHIMA et al., 1953; ODLAND et al., 1950; PEARSON, 1929; SHINOHARA, 1924, 1943; STOUT, 1931; TATEBE, 1936, 1942, 1943; TERAOKA, 1923), and these methods are playing now an important rôle in the production of hybrid seeds in some vegetables such as cabbages and Chinese cabbages, in which it is quite difficult to produce hybrid seeds economically if they were crossed with an artificial pollination method.

Self-incompatibility is an efficient character for the production of hybrid seeds in some vegetables, though there prevail a number of important vegetables which are not endowed with such character.

In some cases, the male-sterility becomes a special character which may be converted into a useful means for the production of hybrid seeds.

Several cases of male-sterility have been reported by many workers with various vegetables such as cabbage, carrot, cucurbit, egg plant, melon, onion, pepper, tomato, radish, potato and so on.

Each of these spontaneously caused male-sterilities, excepting some rare cases, was a character which was genetically controlled by a simple recessive gene.

Consequently, it may be difficult to use such recessive gene in the economical production of hybrid seeds.

In a few plants, there were the observations of the differential male-sterilities which were controlled by the cytoplasm or by an interaction between the cytoplasm and the nuclear gene concerned with the sterility.

The male-sterilities which are playing now an important rôle in the production of hybrid seeds or in the breeding projects belong to the type described just above. Moreover it is of the utmost importance that all of the useful male-sterilities reported up to now were originated as the spontaneous mutants occurring in the ordinary populations.

An artificial induction of male-sterility from normal plants has been attempted by some workers with a number of cultivating plants, and the expected results could be obtained only in vegetables such as maize (ERICHSEN, 1963), onion (COHAN et al., 1966), wheat in certain exceptional interspecific recurrent back-crosses (ERICHSEN et al., 1963; FUKASAWA, 1953; KIHARA, 1954) and tobacco (CLAYTON, 1950).

These precedents seem to suggest that one of the important methods to obtain a useful male-sterility may be the general exploration of spontaneous male-sterile mutants in the populations of cultivating plants.

From this point of view, an exploration of male-sterility has been done by the author with the obtaining, from an unidentified variety of Japanese radishes, of a new male-sterility which appears to be of a very convenient and useful characteristic for the production of hybrid seeds or the future breeding.

The works reported here were primarily carried out to make the characteristics of this new male-sterile radish clear and introduce a new method into the field of practical breeding or of the production of hybrid seeds of Japanese radish.

2. Materials and methods

a Varieties used

In addition to the present male-sterile radish strain obtained from an unidentified variety, at Ōnejime, Kagoshima prefecture, several ordinary strains of leading varieties (Kuroba-riso, Kurume-mino and Kurume-okutsumari) which were offered from the Kurume Branch of the Horticultural Research Station and six commercial varieties on the market (Benisuji, Kunitomi, Miyashige, Ōkura, Sakurajima and Tokinashi) were used.

b Methods of cultivation

- i) Each seed was planted in a paper pot of $5\text{cm} \times 5\text{cm} \times 7\text{cm}$ which was filled with the soil prepared well in the early fall. After the fifth true leaf developed fairly well the seedling plants were transplanted into the ridges of 120cm width in the open field, keeping space of $30\text{cm} \times 40\text{cm}$, and they were grown under the ordinary cultural techniques throughout the crossing experiment in the following spring.
- ii) For the purpose of making clear of the influence of environmental condition upon the pollen-formation in the radish, various commercial varieties and the present male-sterile strains were grown under two different conditions.

In one case of them, each two vernalized seeds were planted in a 1/50,000 Wagner's pot in the early fall and grown under glasshouse and field condition from the early fall to the next spring.

In other case, the vernalized seeds were planted one by one in a 9cm clay pot filled with vermiculite, in the late spring, and these pots were placed in an enameled basin containing nutritional solution in the depth of half an inch. These plants were grown under the condition of glasshouse until their growth was damaged seriously by the temperature too high for the sound growth.

c Discrimination of male-sterility

Discrimination of male-sterile plants from normal plants was determined by the careful observation of pollen grains with the aid of the staining with acetic carmine at the three different stages of the flowering such as the begining of flowering, the full bloom and the late flowering.

d Crossings

The pollens collected from the flowers kept free from contamination by covering with a parchment paper bags were used in the crossing test.

The flower-buds used as female were previously emasculated carefully on the day or few day before flowering, and they were pollinated immediately after the emasculation. The paper bags were left for about one week after pollination, then they were removed after the ascertainment of the sign of the development of their ovary.

The individual methods used in each experiment will be dealt with in each section.

3. Observation on the morphological characteristics of the present new male-sterile radish

Up to now, there have been reported at least two instances of male-sterility in Japanese radish, *Raphanus sativus* L. (NISHI, 1958; TOKUMASU, 1951). These male-sterile radishes resemble each other in some characters such as the size and the shape of flowers or flower-buds and the hereditary behaviour of male-sterility.

The present male-sterile radish differed considerably from these two instances of male-sterile radishes mentioned above, showing several abnormalities in the shape or the function of its flowers or flower-buds. In case of the extreme abnormality in flower-buds, complete falling of flower-buds was recognized occasionally, nevertheless the plants showing a slight abnormality continued the development of their buds and opened the flower normally even if their size was reduced to be distinctly smaller than that of normal radish stout in appearance.

At the early stage of development, the buds and the anthers of the present male-sterile radish showed stout appearance but the size of them was smaller than that of normal plants. With the progress of developmental stages the anthers began to shrink and turned out to whitish in their colour and then they became to small and thin matters as if they were made of a piece of paper. Moreover the elongation of filament delayed conspicuously in this male-sterile radish and its anthers commenced to surround the style at relatively low portion when the anthers developed completely (Fig. 6).

On the other hand, the buds of normal radish continued their development regularly and the elongation of style and filaments progressed side by side. Consequently, these anthers were motivated to surround the style at relatively upper portion at the flowering time (Fig. 6).

Morphological abnormalities in the present male-sterile radish may enable us to classify it into the following groups by the extent of the external abnormality.

- Group 1. The size of flower-bud is considerably smaller than that of the normal plant, and the colour of buds begins to fade into yellowish green at the relative early stage of the development, then flower-buds change into yellow and fall finally. During this period the flower-buds continue its development with certain difficulty except elongation of style. Consequently, the style becomes to be crooked in the closed small flower-buds or to be pushed out of the buds (Fig. 8. 9.).
- Group 2. The degree of the falling of flower-buds is slighter than that of the group 1, and considerable numbers of flower-buds continue their development and flowering in the season.
- Group 3. Flowering is considerably difficult because petals are apt to curl and join closely each other.
- Group 4. The size of flower is small, but the flowering proceeds quite normally (Fig. 2.).

The plants belonging to the group 1, 2 or 3 are relatively few and almost all of the new male-sterile plants belong to the group 4.

4. Cytological and histological studies on the pollen-formation in the normal Japanese radish, *Raphanus sativus L.*

The developmental process of pollens of normal fertile radish was observed to be compared with that of the new male-sterile radish

a Materials and methods

The flower-buds of normal plants were cut off at the various stages of development and fixed in acetic alcohol immediately. These buds were embedded in paraffin in the customary manner. The embedded materials were sectioned at a thickness of about 10 μ and stained with Heidenhain's iron alum hematoxylin solution. In some occasion smear method with aceto-carmine was applied, too.

b Results

The young bud usually showed a compact profile of tissues. The archesporium which was constituted of large, stainable and roundish cells began to differentiate at a region adjacent to the dermatogen. With the development of archesporium the tapetum began to differentiate at the outermost portion of archesporium. Then the archesporial cells developed normally and accomplished the formation of pollen-grains through various stages of development.

Almost all of the archesporial cells accomplished their development normally, but a few of them showed abnormality in their shape or stainability at the various stages of development, and failed to develop into the matured pollen-grains. The frequency of such abnormalities was not so high as they were deemed to be responsible for the manifestation of male-sterility.

In normal plants, the tapetal tissue maintained regular cell arrangement until a comparatively late stage of the development of pollen-grains was attained (Fig. 23).

Thereafter, the tapetal tissues began to lose partially their own cell membranes, forming a rudimental layer which was constituted of the fused cells. This layer degenerated and vanished from the anther loculus just before the accomplishment of the pollen formation, and then periclinal tissue of anther developed the formation of pollen-grains thoroughly and completely. (Fig 24. 25).

5. Cytological and histological studies on the pollen-degeneration in the new male-sterile Japanese radish, *Raphanus sativus L.*

Cytological and histological studies on the pollen degeneration in the male-sterile plants which appeared spontaneously in some cultivated plants have been done by several investigators. (ADACHI, 1957, in Hatizyo-Susuki; ARTSHWAGER, 1947, HOSOKAWA et al., 1954, in sugar beet; BOHN et al., 1949, in muskmelon; HIROSE, 1965, in pepper; KATSUMATA, 1965, in carrot; KOBABE, 1958, KOTOTANI et al., 1956, TATEBE, 1952,

in onion; NISHI, 1958, in cabbage, onion, radish and tomato; SINGH et al., 1961, in cucurbit; TOKUMASU, 1957, in radish).

For the purpose of comparing the aspect of pollen degeneration with the results obtained from the several male-sterile plants mentioned above, the cytological and histological studies were carried out with the new male-sterile Japanese radish.

a Materials and methods

The flower-buds of the male-sterile plants which were derived from the original new male-sterile mutants by various cross combinations were cut off at the various stages of their development and fixed immediately, then they were sectioned in the same manner as that of the methods used in the normal plants.

b Results

Some abnormalities in the shape or the stainability of archesporial cells, as described in normal plants, were also observed in the new male-sterile plants. These abnormalities appeared both in the young flower-buds of the male-sterile plant and the normal fertile plant, but the frequency of these abnormalities was very low. This fact may suggest that the abnormalities in archesporial cells play no important rôle for the manifestation of male-sterility. From the other point of view, these phenomena seemed to play an important rôle in the effective development of a greater part of archesporial cells in their encountering with internal unfavourable conditions such as the physiological or the nutritional unbalances in the processes of pollen-formation.

Almost all of the archesporial cells developed normally by the relatively early stage of the formation of microspores, which were followed by the gradual degenerative process and the final death of them. (Fig. 15-18).

Furthermore, certain histological abnormalities, for instance, the early breakdown of tapetal tissue or the formation of plasmodium-like tissue in tapetum, were observed characteristically in this new male-sterile radish. The former appeared rather usually in this male-sterile radish and it seemed to play an important rôle for the manifestation of male-sterility, because the early breakdown of tapetal tissue implies the discontinuance of the supply of nutritive substances into the microspores, which may inevitably induce the degeneration of microspores.

The signs of the early breakdown of tapetal tissue were shown first by the irregularity in cell arrangement, the decrease of cell stainability and the progress of the vacuolization of tapetal cells. Then the breakdown of tapetal tissue was accelerated by the partial vanishing of cell membranes and fusion of tapetal cells. The breakdown of tapetal tissue was likely to happen prior to the visible abnormality in microspores.

With the progress of these abnormalities in tapetal tissue the withering and the discoloration advanced rapidly on the male-sterile anthers. After that, these anthers began to lose their fully round shape and changed gradually into a small and whitish thin matter resembling a piece of paper. When they were observed histologically the degenerated microspores could obviously be recognized as narrow black layers which were sandwiched by the tissues of the anther wall (Fig. 18).

c Discussion

In the process of pollen formation, several abnormalities which are considered to be concerned in the pollen degeneration, such as the cytological, the histological or the phy-

siological abnormality have been observed on the anther of several male-serile plants.

Prior to making discussions on the mechanism of pollen degeneration from the cytological or the histological view point, it may be convenient to classify the male-sterilities into two broad groups by the aspect of tapetal tissue. The one is a group of the male-sterilities which shows a certain abnormality in tapetal tissue, and the other one is a group of the male-sterilities which is induced by the mechanisms other than the abnormality in tapetal tissue.

Male-sterilities which were induced by various mechanisms such as the chromosomal irregularity (BEADLE, 1932, in maize; LESLEY, 1939, in tomato), the abnormal cytokinesis (BEADLE, 1932, in maize), the failure of dextrin production in pollen grains (LESLEY, 1939, in tomato), the unbalance in cell-division-timing (UPCOTT, 1937, in sweet pea) or the pollen degeneration by unknown mechanism (RICK, 1944, in tomato; AOBA et al., 1953, in tomato) may be grouped in the latter group.

Since the successful execution of the histological study of pollen degeneration in *Oenothera* by OEHLKERS (1927) various noticeable abnormalities have been observed in the tapetal tissue of several male-sterile plants.

Other groups of the various male-sterilities classified from the aspects of the degeneration of tapetal tissue are as follows.

1. No abnormality occurs in tapetal tissue but the pollen degeneration takes place at a definite stage of pollen development (BEADLE, 1932, in maize; RICK, 1944, in onion; ADATI, 1957, in Hachizyo-Susuki).
2. Pollen degeneration occurs with the complete failure of tapetum formation (TOKUMASU, 1957, in Japanese radish).
3. Pollen degeneration progresses with a conspicuous abnormality in tapetal tissue.
 - a) Pollen degeneration progresses in intimate relation with the formation of periplasmodium or pseudopodium-like incursions in tapetal tissue. (ARTSCHWAGER, 1947, and HOSOKAWA et al., 1954, in sugar beet; TATEBE, 1954, and NAKAMURA, 1954, in onion; NISHI, 1958, in welsh onion; HIROSE, 1965, in pepper).
 - b) Pollen degeneration progresses in company with the abnormal enlargement and the vacuolization of tapetal cells (JONES et al., 1936, and NISHI, 1958, in onion; ARTSCHWAGER, 1947, in semi-male-sterile sugar beet; KOTOTANI et al., 1960, in a case of male-sterile cabbage; SINGH, 1961, in *Cucurbita maxima*; KATSUMATA, 1956, in carrot).
4. Pollen degeneration is caused by the unbalance in the degeneration-timing of tapetal tissues. (GERD, 1958, in onion; NISHI, 1958, in tomato; KOTOTANI et al., 1960, in cabbage; SINGH et al., 1961, in *Cucurbita maxima*).

Moreover, the male-sterile plants which were induced by the artificial methods showed various abnormalities in their tapetal tissue, pollen mother cells or microspores (NAKAMURA, 1954, in egg plant; NISHI, in *Brassica* CO progeny: CO is a kind of synthetic *Brassica napus* created from the interspecific hybrids between cabbage and Chinese cabbage, OHTA, 1962, in sugar beet), but these abnormalities should be discussed from the point of view other than that of the instances mentioned above.

In Japanese radish, *Raphanus astivus* L., there have been reported some instances of partial or complete pollen degenerating phenomena which were induced by various causes,

such as a simple transmission of chromosome, formation of heteroploid configuration of chromosomes (FUKUSHIMA, 1937, 1949), formation of coenocyte or abnormal cytokinesis (IIZUKA, 1961), formation of heteroploid in the process of the collapse of polyploid form (TOKUMASU, 1960), and a simple recessive gene related with male-sterility (TOKUMASU, 1951).

From the data obtained by the cytological and the histological studies, it may be possible to say that the pollen degeneration of the present new male-sterile radish does not have any relation with the cytological abnormality in pollen-mother cells, and it may be induced by the cause other than the abnormal cell division. Pollen mother cells developed normally until the stage of microspore was reached, then they began to degenerate rapidly with the abnormal breaking of tapetal tissue.

The degenerative aspect of tapetal tissue in the present male-sterile radish resembles somewhat that of the male-sterile radish reported by NISHI (1958). However considerable amount of difference was observed between these two male-sterile forms when they were compared with each other in details.

In case of the present new male-sterile radish, the degeneration of tapetum progressed through the processes of an abnormal enlargement, vacuolization, and partial fusion of tapetal cells by the loss of cell membrane, but the tissue was kept unseparated from anther wall during these degenerative processes.

On the other hand, the tapetal cells of the male-sterile radish reported by NISHI enlarged and began to take rectangular form, then the tapetum after being separated from inner wall of anther proceeded to surround the mass of microspores at the center portion of the loculus of anther.

Consequently it may be possible to conclude that the male-sterile radish reported here is a quite new type of male-sterility in radish as is shown by the name of the new male-sterile radish.

6. On the inheritance of the new male-sterile character in Japanese radish, *Raphanus sativus* L.

In radish, two instances of the male-sterility which appeared spontaneously in commercial varieties growing under a general cultivating condition were reported by TOKUMASU (1951) and NISHI (1958) and as a result of the histological study of their anthers and the aspect of pollen degeneration, it was definitely shown that each of the two instances belongs to the different type of male-sterility.

Also it was confirmed that these sterilities were controlled by a simple recessive gene having relation with male-sterility and showing regular mode of inheritance.

These facts seem to suggest that both sterilities are not always favorable characters in the commercial production of hybrid seeds or the improvement of breeding methods.

For the purpose of clarifying the inheritance of this new male-sterile character, and to show how it may be incorporated into normal commercial varieties, various crossing experiments mentioned in the following were carried out.

a Materials and methods

All of the present new male-sterile strains used here originated in the three spontaneous mutants taken from an unknown variety of Japanese radish (not identified) which were growing under an escaped condition at Ōnejime cho, Kagoshima prefecture.

In addition to this new male-sterile strains several commercial varieties were also used as materials for the crossing experiments.

Generally, the crossings were carried out by the bud-pollination technique. The pollens gathered from the flowers which were protected by the covering with paper bag to keep them free from contamination were pollinated on the flowers castrated 1~3 days before the flowering.

In order to reveal the hereditary behavior of this male-sterility, various crossings were attempted using the original as well as newly raised strains and various normal commercial varieties.

The scheme of cross combinations was as follows

1. The crossing among the plants of the original male-sterile strain.
 - a₁ Male-sterile plant × normal fertile plant.
 - b₁ Selfing of fertile plant.
 - c₁ Fertile plant × fertile plant.
2. The crossing between the new male-sterile strain and normal commercial varieties.
 - a₂ Male-sterile plant × normal commercial varieties.
 - b₂ Fertile plant of the original new male-sterile strain × normal commercial varieties.
 - c₂ Normal commercial varieties × fertile plant of the original new male-sterile strain.
 - d₂ Back crossing of normal commercial varieties to the male-sterile plants which were derived from the various cross combinations described above.
3. Another crossings.
 - a₃ The selfing of the fertile plants which were derived from the crossing between the male-sterile plants taken from the cross combination b₂ and the fertile plants of the original male-sterile strain.

b Results

As is shown in table 6 and 7, two different segregation ratios of 1 male-sterile plant to 1 fertile plant and 1 male-sterile plant to 3 fertile plants were taken from the F₁ populations of the cross combinations a₁ and b₁ respectively.

The selfing of the normal plants which were derived from the cross combination c₁ gave a closely resembling ratio of 1 male-sterile plant to 3 fertile plants.

All of the F₁ plants, except three plants, which were observed in the cross combination a₂ were male-sterile.

The F₂ plants which were taken through the back crossing of normal commercial varieties to the F₁ plants of the cross combination a₂ usually manifested male-sterility, too.

On the other hand, when the normal commercial plants were pollinated with pollen-grains of the fertile plants of original new male-sterile strain, all of the progenies showed pollen fertility in the next generation.

When the fertile plants of the original male-sterile strain were pollinated with the pollen grains of normal commercial varieties the phenotype in the F_1 population segregated into a ratio of 1 male-sterile to 1 fertile.

The selfing of the fertile plants which were described in the cross combination a_3 gave a segregation ratio of 1 male-sterile to 3 fertile in the next generation.

c Discussion

As mentioned elsewhere, two different male-sterilities controlled by simple recessive genic gene concerning the pollen degeneration were reported in Japanese radishes by TOKUMASU (1951) and NISHI(1958), respectively.

Although the data taken from the crossing among the plants composing the original new male-sterile strain seemed to suggest that the male-sterility was controlled only by simple recessive genic gene (table 6 and table 7), but completely different results were obtained in the crossing between the new male-sterile plants and normal commercial varieties (table 8).

Almost all of the data taken from the cross combinations mentioned above may be consistently accounted by applying of the JOHNS's formula which was introduced to explain the heredity of a male-sterile onion (1943).

In this hypothesis two different cytoplasm of the normal and sterile cytoplasm, and two genic genes, the dominant and the recessive are presumed. The male-sterility will result from the interaction between the cytoplasm and the gene concerning the pollen degeneration.

All of the plants with the normal cytoplasm (designated N) can produce usually viable pollens without reference to their genic gene constitutions.

On the other hand, the plants possessing the sterile cytoplasm (designated S), which is inherited only through the maternal plants, and the homozygous recessive genes (designated $ms\ ms$) will manifest usually male-sterility.

The relationships between these cytoplasm and the genic gene for male-sterility may be designated as in the following.

Gene constitution		Phenotypic appearance
cytoplasm	genic gene	
N	$Ms\ Ms$	male fertile
N	$Ms\ ms$	male fertile
N	$ms\ ms$	male fertile
S	$Ms\ Ms$	male fertile
S	$Ms\ ms$	male fertile
S	$ms\ ms$	male sterile

As is shown in table 6 and 7, the data taken from the crossing experiments a_1 and b_1 agree well with the theoretical ratios of segregation which are expected in the F_1 between the plants having the gene constitution $S\ msms$ and $S\ Msms$, and in the selfing of the plants having the gene constitution $S\ Msms$.

Moreover almost all of the data taken from the F_1 populations of the crossing experiment a_2, b_2, c_2 and d_2 agree well with the ratios of segregation which are expected theoretically from such genotypical combinations as $S\ msms \times N\ msms$, $S\ Msms \times N\ msms$ and

N msms \times S Msms. However, there were the observation of exceptional three male-sterile plants in only one case of the F_1 of the crossing between a new male-sterile plant and a commercial fertile plant of variety Miyashige (table 8). These three plants should be induced exceptionally by the contamination with the pollen-grains possessing Ms gene which came from some source other than Miyashige, because no data could be obtained showing the fact that Miyashige is possessing Ms gene so far.

From the data taken by various crossing experiments it may be possible to say conclusively that each of the male-sterile and the normal plants composing new male-sterile strain possesses the gene constitution S msms and S Msms respectively. Moreover, all of the commercial varieties used in this experiment seemed to possess the gene constitution N msms giving no positive data to show the presence of the commercial varieties possessing S MsMs or N MsMs gene constitution. This fact may be considered as an important specificity for this new male-sterile radish.

In a certain case of male-sterile onion, three types of hereditary behaviors were observed in the F_1 populations from the crossing between the male-sterile plant (line 13-53) and various normal plants (JONES et al. 1943). It was ascertained that there are some varieties having no ms gene among the commercial varieties in the United States and the Union of South Africa (DAVIS. 1957, LITTLE et al., 1944).

Aspect of the distribution of Ms and ms genes may be of importance for the utilization of male-sterility and that of the present new male-sterile radish seems to be most convenient and effective for the purpose of executing the commercial production of hybrid seeds.

7. On the free amino acids in the anther of the new male-sterile radish

It is well known that male-sterile plants are accompanied often by the morphological or the functional abnormalities in their flowers or the flower-buds.

The histological or the cytological abnormalities in the anthers at a given stage of their development have been observed, too, by some workers as mentioned elsewhere in this paper. The causes and the mechanisms of these abnormal phenomena have turned out to be an important point for discussion of the characteristics of male-sterility.

Generally, it may be possible to presume that the visible abnormality will come in sight as the result of the invisible abnormality prior to the visible abnormality.

When the invisible abnormality occurs severely in the organs or the tissues which have a compact relationship with pollen development, it will cause the inhibition or the interruption of the pollen development.

Recently, some attempts to elucidate the invisible differences between the anther of male-sterile plant and that of normal plant have been done by some workers with the help of paper chromatographic techniques.

FUKASAWA reported first of some abnormal constitution and content of the free amino acids in the anther of male-sterile wheat, which was induced by the interspecific crossing (1954). Then the special constitution or content of the free amino acids has been reported by several workers in the various pollen-aborted plants.

To obtain some informations upon the invisible abnormalities which might occur in the male-sterile anther, the detection and the identification of the free amino acids and the sugars in the anther tissue were attempted here with the help of paper chromatography.

a Materials and methods

1200 anthers, in total, were gathered from the male-sterile and the normal fertile plants at the various developmental stages of anthers.

The anthers were mashed with pestle in glass mortar adding small quantity of ether, and they were centrifugalized after the installation for a while. The supernatant was poured out and the residue was mashed again in the same way as the first and centrifugalized. Then the residue was mashed again adding 90 percent ethyl alcohol in water and centrifugalized after the installation for about 3 hours at room temperature.

This treatment was repeated twice, and then the supernatant was gathered into the same evaporating dish and it was concentrated nearly to dryness on a water bath. This sediment was dissolved in 0.3 ml. of water, and each 0.01 ml. of this solution was spotted on TOYO's filter paper No. 50 and developed by two dimensional method. As the primary solvent butanol-acetic acid-water (4 : 1 : 2) was used and as the secondary one was used phenol saturated water at room temperature (about 20° C.). To detect amino acids, 0.1 percent ninhydrin in butanol saturated water was used at 90°C. and Rf and specific colouring of each spot were recorded for identification.

b Results

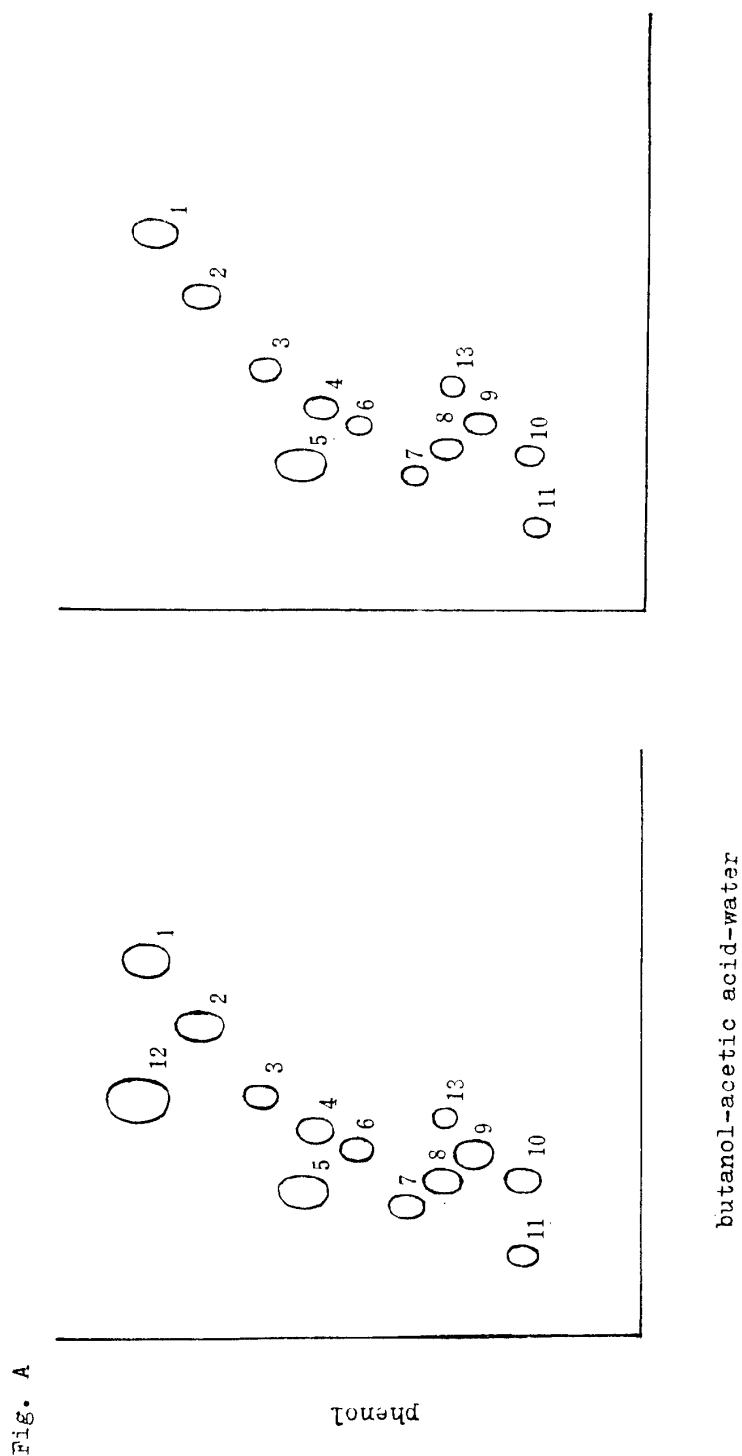
As is shown in Fig. A, 13 spots were clearly detected on the paper chromatograms of the anther of normal plants, at each stage of their development. 10 of these 13 spots were identified to be leucine, valine, proline, alanine, glutamine, threonine, asparagine, serine, aspartic acid and glutamic acid respectively. However the identification of the other three of 13 spots resulted in failure. All of the spots of amino acids identified were larger and more distinct in normal fertile plants than in the male-sterile plants. This fact may suggest that normal anther contains a larger amount of free amino acids as compared with those in the male-sterile anthers.

Out of these amino acids proline showed a peculiar aspect for its chromatogram. Namely, the spot of proline on the paper chromatogram of normal anthers was very large and distinct, though generally the spot of proline was very small and light in colour, and was hard to be detected on the chromatograms of male-sterile anthers.

c Discussion

The report upon the relation between the pollen sterility and the more or less abnormal content or constitution of amino acids in anthers was offered first by FUKASAWA(1954), who observed an abnormality in the amino acid content of the anther of male-sterile maize (WF 9) and wheat which was induced by successive back crossing of *Triticum durum* to *Aegilops ovata*. He found out some differences in the amino acid content between the normal fertile and male-sterile anthers.

It is unknown whether these abnormalities were phenomena brought forth as the result or as the cause of the male-sterilization, but new information may play a part in opening a new sight and new method to make clear the mechanism of inducing the male-sterility.



NAKAZAWA presumed that there might be any differences in the amino acid content or constitution of anthers among the plants which differed in polyploidy, and he found out the facts that the modes of rise and fall of content in some ninhydrin-reacting-substances in the anther of 3 X strain differed from those of 2 X and 4 X individual of *Tradescantia*(1959).

KATAYAMA(1961) studied the relationship between sterility and free amino acid or amide content in the anthers of various rice plants, and detected the fact that only two sterile strains, one of which was derived through haploid form and the other one through chromosome nonparing, lacked proline in their anthers in clear cut contrast to the other sterile strains containing a considerable amount of proline in their anthers. Moreover, he observed the disappearance of proline in the anther of the asynaptic wild senna, on the day prior to flowering even though proline was contained in the anther at the early stage of development (1961). The absence of proline was also reported by HOSOKAWA et al. in the anthers of male-sterile beet (1963).

FUJISHITA (1965) reported the abnormal content of free amino acids in the anther of various fruit vegetables which were grown under low temperature condition. KHOO and STINSON (1957) obtained a result resembling the one observed in the experiment by FUKASAWA. Namely, they failed to detect proline in the anther of a cytoplasmic male-sterile maize.

Without reference to the kind of plant or the type of sterility there had widely been noticed the abnormal content or constitution of free amino acid or amide in the anther of sterile plant. This was considered to be quite attributable to some possible abnormalities in the proline content, excepting some instances in rice plants.

These facts seem to suggest that the abnormality in amino acids or amids content or constitution in the anther will play an important rôle for the manifestation of sterility.

8. On the sugars in the anther of the new male-sterile radish

The purpose of this experiment was to make clear the relationship between the pollen degeneration and the sugar content in anthers.

a Materials and methods

Anthers were gathered at their various developmental stages, 1200 anthers being used in each experiment. The anthers were mashed in a glass mortar with pestle adding a small quantity of ether, then they were filtered and ether fraction was thrown out. This treatment was repeated three times to remove the ether soluble matters. Then methyl alcohol of 90 per cent in water was added to the residue and the mixture was boiled on the water bath for 10 minutes. The supernatant was separated by centrifugalization and the residue was extracted again with methyl alcohol in the same way as the first. Then these two supernatants were poured into an evaporating dish and concentrated nearly to dry on the water bath. The concentrate was resolved in 0.3 ml water and used as the sample for the chromatographical analysis of sugars.

1. Paper chromatography

One way chromatography : The sample prepared as mentioned above was spotted on

the strips of filter paper of 4 *cm* width (TOYO's filter paper No. 50) in the usual manner, and developed by ascending method with butanol-acetic acid-water (4 : 1 : 2) at 20°C. for 18 hours.

Two way chromatography : The sample spotted on the filter paper of 20 × 20 *cm* (TOYO's filter paper No. 50) was developed with the solution of butanol-acetic acid-water (4 : 1 : 2) as the primary solvent and 80 per cent phenol solution in water as the secondary solvent respectively.

2. Paper electrophoresis

The sample spotted on the paper strip of 10 *cm* width was migrated by the constant electric current (1.0 *mA/cm*) of high voltage (800~350) for 2.5 hours in the buffer solution of 1 per cent of borax.

For the detection and the identification of sugars following reagents were sprayed on the paper developed by the methods mentioned above and heated for several minutes in a thermostat.

Reagent 1		Reagent 2	
Resorcin	0.5g	Benzidine	0.5 g
Trichloroacetic acid	5.0g	Acetic acid	20 ml
Ethyl alcohol	100ml	Ethyl alcohol	80 ml
80~90°C for 7~8 minutes		100~110°C for 10 minutes	
Reagent 3			
Benzidine	0.5 g		
Trichloroacetic acid	5.0 g		
Ethyl alcohol	100ml		
80~90°C for 5 minutes			

Reagent 1 gave distinct spots for ketose and for the oligosaccharides which contained fructose, but did not react with aldohexoses.

Reagent 2 gave spots for aldohexose and did not react with other sugars.

Reagent 3 reacted sensitively with both aldohexose and oligosaccharides.

The amount of each sugar was estimated by comparing the size and the colouring reaction of each spot with those of the standard materials.

b Results

In the one way chromatography, both anthers, the anther of the male-sterile and that of normal plants gave the distinct spots (a), and (b), when they were treated with the reagent 3. These spots were identified respectively to be fructose (a) and a mixture of glucose and sucrose (b) from their colouring and *R_f* values.

It was considerably difficult to separate and identify glucose and sucrose clearly, even if the two way development was used, thus electrophoresis was employed to get rid of such difficulty.

The results obtained by these methods were reproduced in table 1.

The size and colouring of each spot for sugars in the normal fertile anthers were larger

Table 1 Developmental stages of anthers and their sugar contents

Sugar	Glucose	Fructose	Sucrose
Young stage			
Normal anther	+++	+++	+++
Sterile anther	+++	+++	+++
Late stage			
Normal anther	+++	++	trace
Sterile anther	+	+	+++

and deeper than those of the sterile-anthers except sucrose, when they were compared with each other at the same developmental stage of anthers.

At the younger stages of the development of anther it was ascertained that the both anthers of male-sterile and normal plant contained three sugars, i. e., glucose, fructose and sucrose. Relative amount of those sugars in the sterile anthers appeared in the decreasing order of sucrose > glucose \doteq fructose, but the quite reverse order of fructose > glucose \doteq sucrose was obtained in the normal fertile anthers.

At the stage of two or three days before the flowering, the aspect of sugar content in the normal anther differed from that of the anther at the young stage of development, namely, relative amount of each sugar occurred in the following order of decreasing, glucose, fructose and a very small amount of sucrose.

On the other hand, the sterile anther at the late stage of development, seemed to contain approximately small and the same amount of glucose and fructose and a considerably large amount of sucrose as compared with that in the normal anthers.

9. On the respiration of anthers of the new male-sterile radish

It may easily be imagined that various complex and exquisite reactions will proceed in anther in the course of pollen formation and these reactions may also have a close relation with the respiration of anther. This study was carried out to know something about the mechanism of pollen degeneration in the new male-sterile radish from physiological view point.

a Materials and methods

From the reasons that the anther of variety Sakurajima is large in the size and easy to be handled, the normal and male-sterile forms of Sakurajima were used. The sterile form was created by the recurrent back-crosses of the normal Sakurajima to a new male-sterile strain.

The anthers were gathered from those plants mentioned above at the two developmental stages, i. e., the early tetrad and a few days before the flowering, and their respirations were measured with Warburg's manometer. Each time of measurement 300 anthers were used in the case of young anthers and 120 anthers were used for the anthers in the later stage of development.

b Results

As is shown in table 2, noticeable differences were observed in the rate of respiration between the male-sterile anther and the normal anther. The respiration of normal anther was more active than that of the male-sterile anther throughout all the stages of development, increasing in amount with the progress of development. On the other hand, the respiration rate of the male-sterile anthers decreased with the progress of their development and such tendency becoming more and more conspicuous in proportion to the advance of the development towards the later stage.

Table 2 Differences in the respiration rate between normal and male-sterile anthers

(1964)

Stage Time	Developing stage of microspores (per 300 anthers)		Stage Time	A few days before flowering (per 120 anthers)	
	O ₂ μl			O ₂ μl	
	sterile	normal		sterile	normal
5'	0.686	0.713	5'	0	2.456
15'	1.544	2.317	15'	0.507	8.770
30'	3.087	5.346	30'	1.352	18.066
45'	4.288	7.841	45'	2.366	28.064
60'	5.317	10.692	60'	3.211	37.711

10. Effect of environmental conditions on the formation of pollen grains in Japanese radish

Aborted pollen grains have often been observed even in the normal fertile plant, and the frequency of these aborted pollen grains differed considerably owing to the characteristics of the respective individuals, varieties or strains, even if the plants were grown under the same condition.

Pollen abortion will be caused by several reasons which are attributable to the internal or the external abnormalities in the process of pollen formation. The cytoplasm or mendelian factors concerning the male-sterility may be considered as an internal abnormality.

In the discussion of the male-sterility it may be the most important thing to distinguish the pollen abortion induced by a hereditary factor from that induced by certain environmental conditions.

In a heritable male sterile onion, BARHAM et al. (1950) ascertained that the male-sterile plants produced viable pollen grains of about 1 per cent, when they were grown under the condition of $70^{\circ}\sim 80^{\circ} F$ during the post meiotic division. In *Capsicum frutescens*, MARTIN (1951) observed some unusual male-sterile plants which showed self fertility and large productivity of seed under field condition, though the plants manifested male-sterility when they were cut back and transferred into greenhouse. Moreover, RUND-FELDT (1958) reported a new type of male-sterile cabbage plants which were made to convert themselves back to the normal fertile plants when they were grown under greenhouse condition.

Stability of the present new male-sterile character was tested under several different environmental conditions.

a Materials and methods

Normal fertile varieties of Miyashige, Sakurajima, Kurume-mino and the new male-sterile strains which were created by recurrent back-crosses of a certain normal varieties to a new male-sterile plant, were grown under several experimental conditions, their pollen productivity being observed.

Experiment 1

Seedling grown in paper pots were transplanted into the ridges prepared previously and cultured under open field condition throughout the growing season extending from early fall to the next late spring.

Experiment 2

Seeds were sown on the filter paper set in the petridish containing water and germinated at 30°C in thermostat. At the high time to germinate they were vernalized for 21 days extending from September 1 to 21 at 5°C, then each two of them were planted in a 1/50,000 Wagner's pot and they were grown under the outdoor condition except the season of midwinter. During nearly about two months of midwinter the plants were protected from the damage by heavy cold in a glasshouse.

Experiment 3

The seeds vernalized for 3 weeks extending from June 1 to 21 in a same manner mentioned above were planted one by one in each unglazed clay pot, about 9 cm in diameter, which was filled with vermiculite. Then they were fed by being placed in the basin containing nutritional solution at a depth of about half an inch.

b Results

The aspect of normal plant :

Growth, bolting and flowering were quite normal under any conditions provided here. But a small number of aborted pollen grains were observed even in the normal plants under the condition of experiment 1.

In experiment 2, the flowering began in late autumn and continued to the next spring except some interruption made during the mid-winter. Considerable numbers of the aborted pollen grains were observed in the flowers opened in the two seasons of late autumn and the early spring of the next year.

On the other hand, the plants, in experiment 3, produced so many aborted pollen grains as amounting to the half or more of the total pollen grains. These facts may suggest that the formation of the aborted pollen grains is highly influenced by the high temperature prevailing during the season of pollen development.

Cytological and histological observations on the degenerative process of these abnormal pollen grains have not been done and the details of the degenerating aspects are left unknown.

Under a high temperature condition the flower-buds showed a tendency to take a

Table 3 Frequency of occurrence of aborted pollen grains and growing condition of plants.

(1963)

Conditions	1. Planted Oct. Flowered in spring		2. Planted Sept. Flowered from autumn through spring		3. Planted June Flowered in early summer	
Fertility	Number of pollens normal sterile		Number of pollens normal sterile		Number of pollens normal sterile	
Sakurajima	510	21	351	40	229	351
Miyashige	515	14	420	20	95	121
Kurume-mino	523	18	436	26	453	375
Normal plants of the new male-sterile strain	524	12	549	58	193	120

In the experiment 2 and 3 the seeds were vernalized for 3 weeks at 5°C before planting.

In the case of experiment 2, the plants were grown under glasshouse during winter and the examination of pollen grains was done in autumn.

distorted or shrivelled form and they began to fall when this condition prevailed over a considerable period.

These abnormalities in flower-buds, however, are out of category of male-sterility even if they play an important rôle in the impediment of fertility in Japanese radishes.

On the other hand, the effect of low temperature condition on the flower-buds or pollen grains seemed to be relatively moderate and the growing or flowering of flower-buds was suspended or delayed gradually in accordance with the drop of temperature.

Aborted pollen grains produced under low temperature condition were definitely small in account as compared with those of the plants grown under high temperature condition.

No viable pollen grains could be observed in the flowers of male-sterile plants even if they were grown under abnormal temperature conditions as mentioned above.

Table 4 Stability of male-sterility in the present male-sterile radishes grown under different conditions.

(1963)

Conditions	1. Planted Oct. Flowered in spring		2. Planted Sept. Flowered from autumn through spring		3. Planted June Flowered in early summer	
Fertility	Number of plants normal sterile		Number of plants normal sterile		Number of plants normal sterile	
Sterile Sakurajima	0	48	0	46	0	52
" Miyashige	0	48	0	47	0	63
" Benisuji	0	52	0	46	0	48
" Kurume-mino	0	42	0	24	0	32

In the experiments 2 and 3 the seeds were vernalized for 3 weeks at 5°C before planting.

These facts seem to show that the male-sterility occurring in the present new male-sterile form is very stable and its manifestation seems to be very hard to be changed by the environmental conditions.

11. Fertility of the female organ in the present new male-sterile radish

When the male-sterile strain is used as the female parent for the artificial raising of hybrid seeds, it is most important that the female organ maintains its normal function for fertility and can produce ample seeds when it is pollinated with normal fertile pollens.

Some instances of the pollen abortion accompanied with a little seed productivity which were due to an abnormality in the function of female organ have been reported in several plants.

In this study the fertility of female organ in the present new male-sterile radish was examined.

a Materials and methods

Bud pollination as well as full-bloom pollination was employed in this study. In the former, flower-buds were emasculated on the day before blooming and pollinated immediately with the pollens collected from the ordinary plant whose flowers were protected beforehand with paraffin-paper bags to preserve the freedom from contamination. After the pollination these flower-buds were covered with paraffin-paper bags and the bags were left for about two weeks and then removed.

In case of the full-bloom pollination the buds were emasculated in the same manner as employed in the bud pollination, then they were pollinated at the date of full blooming.

The number of seeds produced on the treated flowers was counted at the harvest time.

b Results

As is shown in table 5, no differences can be noticed between the normal plant and the new male-sterile plant for the fertility of their female organ.

Table 5 Seed productivity in the male-sterile plants raised through various crosses.

Cross combinations	(1963)				
	Sterile × Normal a	Sterile × Normal b	Sterile × Sakurajima	Sterile × Miyashige	Normal × Normal *
Number of pollinated flowers	37	79	33	52	24
Number of matured pods	32	63	22	37	19
Percentage of matured pods	86	80	67	71	80
Total number of seeds	90	170	57	103	56
Number of seeds per pod	2.8	2.7	2.6	2.8	2.8

a and b are crosses among the original male-sterile strains.

* Cross among the normal commercial plants.

These results will suggest that the pollen degeneration in the new male-sterile radish proceeds without any relation with the function of the female organ and the new male-sterile radish usually maintains the fertility normally in its female organ.

The statement will be endorsed by these facts that the present new male-sterility might be a very convenient character for the production of hybrid seed when it is used as the female character.

12. General discussion

Male-sterility is an abnormal phenomenon in which the natural function of male reproductive organ is lost, and it can be induced by various causes, including unfavorable environmental conditions, i. e., special cross combination, morphological abnormality, chromosomal irregularity during the cell division of pollen mother cells, the genic gene or cytoplasm for pollen sterility, chemical or physical treatments and so on.

In the narrower sense, the term of male-sterility generally means the phenomena which fail to produce normal pollen grains, in spite of the fact that all of the organs concerning pollen formation are apparently complete.

In some cases, the category of male-sterility is somewhat more extended than that of the narrow sense, and the failures in fertilization which are to be induced by several other reasons, such as the difficulty of dehiscence of matured anthers, partial deformity or complete lacking of the male reproductive organ and so on, are also included into male-sterility.

Recently, the temporary morphological abnormality or the lacking of normal function of the male reproductive organ, which can be induced artificially by the chemical or physical treatments, is included into male-sterility.

In any case, at first, male-sterility was considered to be an undesirable character. However, as an appreciation for hybrid vigour has been in advanced progress, the methods which can eliminate the tedium of hand pollination are needed. From that point of view, male-sterility is very promising as a very appreciable character which may be made use of as a useful tool by plant breeders.

The report of the pollen abortion in *Suterea* and *Cirsium* by CORRENCE may be the first case of the male-sterility in herbaceous plants (JONES 1965). Thereafter various cases of male-sterility have been reported by many workers with several plants, reaching about 45 species in total (NISHI 1958). They may be classified into several groups by their hereditary modes as is shown in the following.

1. Group, the male-sterility which is controlled only by a certain genic factor (Mendelian factor) concerning male-sterility.

This group may again be divided at least into two different types.

- (a) The one is a male-sterile type which is induced by the homozygous recessive genes concerning male-sterility.
 - (b) The other is a male-sterile type which is controlled by a dominant gene concerning male-sterility.
2. Group, the male-sterile type which is induced by the mutual reaction between the cytoplasm and the genes concerning male-sterility.

3. Group, the male-sterile type which is induced exclusively by the cytoplasm concerning male-sterility.
4. Group, the male-sterility which has no relation to any heritable factors concerning male-sterility, and this group is again divided into two sub-groups.
 - (a) The male-sterility which appears only in the progenies of the special cross combination among the definite species or strains.
 - (b) Temporal male-sterility which is induced by chemical or physical treatments, and not inherited to the progenies.

Hereditary behaviour of male-sterility is highly estimated as an important character for the utilization of sterility in the production of hybrid seeds on the commercial scale.

It happens that when the male-sterilities with various characteristics are classified into some groups as mentioned above, almost all of the male-sterilities which have been reported by many workers with various vegetables, such as cucurbits, cucumber, eggplant, lima bean, muskmelon, radish, tomato and so on, belong to Group 1 (a).

The applications of male-sterility to the production of hybrid seeds have also been attempted by a number of workers, but most of them failed to establish a convenient and useful method, excepting a few cases.

In tomatoes, considerable efforts have been made for the application of male-sterility which was governed by homozygous recessive genic genes, and only a little success was gained, certain weak points being left still to the possible exploitation of some new methods for practical raising of hybrid seeds.

In potatoes, a male-sterility which was governed by a dominant genic gene concerned with male-sterility has been reported (SALAMAN, 1910).

This male-sterile potato plant produced morphologically normal pollen-grains, having no natural function to germinate. This type of male-sterility is considered to be a very convenient and valuable character for the application in the production of hybrid seeds but its original merit can not be fully made use of for the reason that the planting of potatoes is usually carried on through tuber seeds. The existence of such sterility is a significant fact, but it has not yet been observed in plants other than potatoes.

Some instances of male-sterilities reported in beet, carrot, flax, pepper and onion belong to the group 2.

In the cases of flax (BATESON, 1921; CHITTENDEN, 1927; GAIRDNER, 1929), pepper (HIROSE, 1965) and onion (JONES et al., 1943; KOBABE, 1958) the sterility was induced by the mutual reaction between a homozygous recessive gene and cytoplasm concerned with male-sterility. The plants possessing homozygous recessive genes and sterile cytoplasm produced usually aborted pollen grains which have no viability. On the other hand, the plants with gene constitution different from the plants mentioned above produced normal fertile pollens only, and did not produce semi-sterile pollens.

In case of onion, the recessive male-sterile gene is distributed extensively throughout commercial varieties except some strains (DAVIS, 1957). This fact seems to suggest that such male-sterility possesses a high potentiality for the utilization in the production of hybrid seeds, and it was realistically elucidated by JONES et al. (1943).

In a case of beet, the male-sterility was induced by a combination of cytoplasm and two recessive genic genes for male-sterility, however, a big variation was observed in

the viability of pollen grains, extending from normal to complete sterile. Some plants possessing a certain combination of gene and cytoplasm produced incomplete sterile pollen, the so-called partial or semi-male-sterility (OWEN, 1942, 1945).

Several male-sterilities have been observed in carrot, and it may be possible to classify them into two types. The one is a male-sterility which was controlled only by genes such as the digenic genes (one gene Ms_4 is dominant and the other ms is recessive) (HANSHE and GABLEMAN, 1963), or by mutual reaction between digenic genes and modifier genes (HANSHE and GABLEMAN, 1963). The other is a male-sterility which was controlled by mutual reaction among a cytoplasm, dominant genic genes and epistatic gene (THOMPSON, 1961), or among a cytoplasm, genic genes and inhibitor for male-sterility (KATSUMATA et al., 1965).

These male-sterile characters have not yet been practically used in the production of hybrid seeds or the breeding of carrot because of the inconveniences or the complexities in their hereditary mode.

Moreover in maize, several instances of male-sterilities have been reported by many workers. Some of them were shown to be the male-sterility which was controlled by genic gene only (EYSTER, 1921; BEADLE, 1932). The others were the male-sterilities concerned with the cytoplasm for male-sterility.

Cytoplasmic male-sterility in maize was first reported by RHOADES (1933). Since then several additional types have been found. At least, eighty four separate types have been reported (DUVICK, 1965), and they were divided into two strains both by the genic gene constitution required for male-sterility, the degree of fertility in the plants restoring fertility and by the shape of anther of partially sterile plants. (SNYDER, 1954; DUVICK, 1955; EDWARDSON, 1955; JONES, 1956; THOMAS et al., 1956; MOUNDER et al., 1959 and DUVICK, 1965). These two strains were designated by S and T (JONES, 1956) or by the USDA and the Texas (DUVICK, 1965), respectively.

Since 1950s the cytoplasmic male-sterility in maize has been used extensively as an aid in the commercial production of hybrid seeds and in the field of the breeding of maize for the reason that its hereditary feature was simple and convenient enough to be applied on the practical procedure.

The male-sterilities which were induced with tobacco plant (CLAYTON, 1950) and wheat (FUKASAWA, 1953; KIHARA, 1954) may belong to the fourth group.

The F_1 plant of *Nicotiana debneyi* Domin \times *N. tabacum* showed a partial male-sterility and the recurrent back crosses of *N. tabacum* to the F_1 brought about complete male-sterility in the progenies. In case of wheat the recurrent back cross of *Triticum vulgare* to the F_1 plant of *A. obata* \times *T. vulgare* brought about a complete male-sterility in their resultants.

The expectable male-sterility for the breeding or the production of hybrid seeds may belong to the group 2, 3 or 4.

The new male-sterility in Japanese radish reported here belongs to the group 2, and its character was very convenient and useful in the practical production of hybrid seeds or breeding for the reason that the sterility was controlled by mutual reaction between the recessive genic gene and the cytoplasm concerning male-sterility. Moreover, the recessive genic gene was confirmed to be existing homozygously in all commercial varieties used in this experiment. On the other hand no data suggesting the presence of the restorer for

fertility were taken from any crossing experiments conducted here.

The genotype of the present new male-sterile radish was presumed to be S msms, and when the plants possessing S msms gene constitution were crossed with a normal commercial variety possessing N msms gene constitution, all of the progenies would produce habitually no viable pollens.

Already, the similar gene constitution for male-sterility had been reported in onion (JONES, 1943). However considerable differences were noticed in the distribution of the recessive ms gene between onion and radish. Namely, in onion, normal commercial varieties do not always possess the recessive ms genes for male-sterility, and some of them lack the ms genes (LITTLE et al., 1944 ; DAVIS, 1957).

The existence of hybrid vigour has also been confirmed with Japanese radish, and its utilization was contemplated positively (KUMAZAWA, 1935 ; AKIYAMA et al., 1939, 1940). The attempts resulted, however, in failure for the following reasons that the efficiency of crossing was very low and the commercial production of hybrid seeds was not practical in Japanese radish as far as the production of hybrid seeds depends on the artificial castration as usual.

Recently, the seeds named as F_1 appeared in Japanese radishes and they are on sale now commercially. However, the relatively high price of F_1 seeds of Japanese radishes prevailing on the market now seems to suggest that there are considerable difficulties or complexities in the process of the F_1 seed production in Japanese radishes.

As mentioned elsewhere the present male-sterility will give a very convenient and useful clue for the hybrid seed production on the commercial scale. The practical utilization of this sterility may be accomplished successfully if only two steps were taken in earnest.

The first is the introduction of the male-sterile character into the variety or strain which was predetermined to be used as a female parent, i.e., the recurrent back-cross of the predetermined plant to the original new male-sterile strain. Practically, such first step will be accomplished by recurrent back-crosses over 4~5 generations.

When the isolated seed production method is carried out, the back-cross is accomplished very easily by planting the parental strain alternately into ridges, and harvesting the seed on the male-sterile plants.

The second step is accomplished by merely cultivating both a certain fertile strain and the newly obtained male-sterile strain through back-crosses in accordance with the first step. Namely, the growing of both the strains should be done in alternate ridges.

The ratio of the male-sterile plants to the normal pollinator plants may be changed as a rule in proportion to the setting capacity of seeds on the male-sterile plants used as the female parents.

All of the seeds produced on the male-sterile plants may be hybrid seeds between those two strains.

And thus, the economical production of hybrid seeds of Japanese radishes in commercial scale may easily be accomplished by using the new male-sterile strain.

On the other hand, various cases of male-sterility may also be possible to be classified into several groups by the accompanying morphological irregularities or the functional abnormalities in the reproductive organs.

For example the following classification may be allowable :

- Group 1 A group of male-sterilities which are induced by the deformity of reproductive organs.
- Group 2 A group of male-sterilities which are caused by the lacking of anthers.
- Group 3 A group of male-sterilities which are derived from the functional abnormality in the anthers.
- Group 4 A group of male-sterilities which do not show any conspicuous morphological abnormality, except the degeneration of pollens at a given developmental stage of anther.

The male sterilities in tomatoes reported by CURRENCE (1944) and LARSEN et al, (1948) may belong to Group 1. Instances of male-sterilities reported by BEADLE (1932) in maize and LESLIE et al., (1956) in tomatoes may be classified into Group 2. The male-sterile tomatoes by ROVER (1948) and the male-sterile egg plant by JASMIN (1954) belong to Group 3.

Almost all of the male-sterile plants which were reported by many workers with various vegetable crops belong to Group 4.

Although the classification based on the morphological characteristics may be possible, as mentioned above, such way is assumed to be more or less inadequate as compared with that by the hereditary behaviours of male-sterility, because the existence of continuous variations in the morphological abnormality makes it difficult to classify them into definite groups.

In the case of Japanese radishes the size of flower or flower-bud was considerably small in the male-sterile form as compared with that of normal ones (NISHI 1958, TOKUMASU 1951).

In the present male-sterile radish some morphological abnormalities, such as the falling of flower-buds at the relatively early developmental stage, a functional obstruction in the opening of petals and the crooking of style which were assumed to be caused by the functional obstruction were observed. Moreover there prevailed various degrees for the abnormality of flowering, ranging from a slight case in which flowers open normally to a serious case where the falling of flower-buds takes place at the relatively early stage of their development.

In any case, these abnormalities give a great influence on the productivity of hybrid seeds and a careful selection of male-sterile strain is necessary for the effective utilization of this new sterile character.

Mechanism of the pollen degeneration in these male-sterile plants may be elucidated only through the extensive studies which consist of various methods, such as genetical, morphological, physiological, cytological and chemical investigations.

In some cases the pollen degeneration takes place in company with abnormal cell division in pollen mother cells or in the tapetal cells, but in another case it occurs with no relation to the abnormality in tapetum.

As the abnormalities of tapetal tissue accompanied occasionally with male-sterile phenomena the following various aspects, such as, lacking of tapetal tissue, timing unbalance in the collapse of tapetum and abnormal development of tapetal tissue have been reported with several plants.

In Japanese radish, two different pollen degenerations which were considered to have an intimate relation with the timing unbalance or the lacking of tapetum have been observed by TOKUMASU (1957) and NISHI (1958).

In the case of the present new male-sterile radish, pollen degeneration occurred independently of the cell division of pollen mother cells, and the degeneration seemed to proceed with the early breaking of tapetal tissue which appeared usually in the new males-terile radish. Moreover the abnormal development of tapetal tissue was observed, but its frequency was very low and it seemed to have no relation with the manifestation of male-sterility.

On the other hand, it may be rightly presumed that various invisible abnormal phenomena will proceed in the organs or tissues of male-sterile plants.

Recently some workers began to study the correlation between the pollen degeneration and the content or composition of free amino acids in the anther of male-sterile plant.

Already, the abnormalities in the content of proline and various abnormal compositions of free amino acids have been observed with several male-sterile plants.

In case of the present male-sterile radish the content of free amino acids was relatively smaller than that of the normal fertile plants, and this tendency was most conspicuous in the proline content.

Complete explanation for the relationship between the pollen degeneration and the content or composition of amino acids in the anther is left to the future studies.

Moreover, distinct differences were observed in the sugar content in the anther between the present new male-sterile plant and the normal plant. These invisible abnormalities seemed to proceed within the male-sterile plant in advance towards visible abnormalities.

Coexistence of feeble respiration rate and abnormal sugar content, especially the remaining of sucrose until late stage of pollen development, may not be fortuitous but an inevitable result which will be derived from an abnormal enzymatic system in the respiration of the new male-sterile anther.

Fundamental elucidation for the mechanism of pollen degeneration may be given by more detailed and extensive studies and those important portions are all left to the future studies.

13. Summary

1. A search for the useful and convenient character in the production of hybrid seeds or breeding was tried with various varieties of Japanese radish, with special reference to pollen sterility, and a new type of male-sterility was found in an unidentified variety of Japanese radish which was growing under escaped condition at Ōnejime, Kagoshima prefecture.
2. The present new male-sterile radish showed some morphological abnormalities such as the small size in the flower, falling of the flower-bud, crooking of the style in the closed flower-bud or pushing out of the style from the closed flower-bud, difficulty in the flowering and so on, in various degrees in each case.

3. In the present new male-sterile radish the pollen degeneration occurred suddenly at the stage of microspore, and the degeneration seemed to have some relation with the early collapse of tapetal tissue.

On the other hand the plasmodium like incursion of tapetal tissue into the anther loculus was observed rarely, but it seemed to have no direct influence on the manifestation of male-sterility.

4. The sterility of the present new male-sterile radish was induced by the interaction of the homozygous recessive nuclear genes (*ms ms*) and the cytoplasm (*S*) concerning the male-sterility. All of the new male-sterile plants possessed *S ms ms* gene constitution. Moreover it was ascertained that all of the varieties of Japanese radish used here possessed homozygous recessive gene *ms ms*.
5. At least 13 spots of ninhydrin reacting substances were detected on the paper-chromatogram of amino acids of the normal fertile anthers, and 10 of these were identified to be alanine, aspartic acid, asparagine, glutamic acid, glutamine, leucine, proline, serine, threonine and valine respectively.

On the other hand 12 out of 13 spots of ninhydrin reacting substances described above, except the spot of proline, were also detected on the paper chromatogram of amino acids of the new male-sterile anthers. Generally, the size and the colouring of these spots of the new male-sterile anthers were smaller and slighter than those of normal fertile anthers.

6. At least three sugars of fructose, glucose and sucrose were contained in both anther of the new male-sterile plant and of normal fertile plant, and the quantity of these sugars seemed to be larger in normal fertile anther than that of male sterile anther except sucrose contained at the later stage of development of anther.
7. The respiration of normal fertile anther increased with the progress of the stage of development of anther, however, that of the new male-sterile anther began to decrease after early stage of microspore. Moreover, the respiration of normal fertile anther was more active than that of the new male-sterile anther throughout all the stages of development of anther.
8. The sterility of the new male-sterile radish was of a quite stable and hereditary character, and the restoring phenomena of fertility could not be observed in any crossing tests conducted here.
9. All of the male-sterile plants raised from original new male-sterile strain maintained normal fertility in the female organ and showed an excellent productivity of seeds when they were pollinated with normal fertile pollens.

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Segregations of male-sterility in the progenies obtained by the crossings among the present new male-sterile strains.

Table 6

1961				1963			
sterile × normal		ms	N	sterile × normal		ms	N
61. 20-11	× 20- 3	4	: 9	63. 33- 3	× 33-27	13	: 11
" 20-11	× 20-44	15	: 19	" 38-16	× 43-12	11	: 14
" 20-15	× 20- 5	24	: 6	" 39-14	× 43-11	3	: 14
" 20-31	× 20- 5	9	: 11	" 43-14	× 43-12	2	: 1
" 21- 2	× 20- 5	8	: 14	" 43-20	× 43-12	7	: 12
" 21- 8	× 20- 5	7	: 12	" 47- 8	× 47- 6	7	: 12
" 21-35	× 20- 7	9	: 9	" 55-15	× 55-10	12	: 12
" 21-35	× 20-28	11	: 10	" 58- 4	× 58- 6	6	: 7
" 21-37	× 20- 5	13	: 8	" 61-22	× 61-11	11	: 14
" 22-22	× 22-10	10	: 7	" 64- 2	× 64-18	4	: 3
" 26- 5	× 26- 6	9	: 9	" 64- 6	× 64-18	8	: 2
" 26- 9	× 26-12	19	: 12	" 66- 9	× 66-10	14	: 10
" 26-16	× 26-61	0	: 9				
" 26-23	× 26-17	8	: 12				
" 27- 3	× 26-17	8	: 10				
" 27- 5	× 26-17	9	: 11				
" 27-11	× 26-17	12	: 9				
" 29- 9	× 29- 7	5	: 12				
" 27-27	× 29- 7	8	: 10				
N. B.							
ms : male-sterile							
N : normal, fertile							
1862				1964			
sterile × normal		ms	N	sterile × normal		ms	N
62. 35-12	× 35- 4	7	: 9	64. 38-22	× 38-81	6	: 11
" 37- 2	× 37-15	1	: 3	" 44-11	× 44- 4	5	: 17
" 38-18	× 38-16	0	: 2	" 48-11	× 48-11	3	: 10
" 40-13	× 40- 6	9	: 10	" 54- 8	× 54-17	14	: 9
" 42- 6	× 42-11	6	: 4	" 54- 6	× 54-17	11	: 12
" 52-14	× 52- 6	12	: 5	" 78-23	× 78-15	8	: 5
" 55- 1	× 55- 4	13	: 12				
" 58-15	× 58- 2	0	: 2				
" 59-20	× 59- 6	6	: 17				
				1965			
sterile × normal		ms	N	sterile × normal		ms	N
65. 19- 4	× 20- 8	13	: 13	" 21- 6	× 21- 3	10	: 12
" 21- 6	× 21- 3	10	: 12	" 23-13	× 23- 1	12	: 12
" 23-13	× 23- 1	12	: 12				

Table 7

1961

normal × normal	ms	N
61.20— 5 × 20— 8	7	: 12
" 22— 3 × 22—10	3	: 13
" 25—10 × 25—13	4	: 11
" 25—25 × 25—31	4	: 14
" 26—17 × 26— 4	2	: 21
" 29—14 × 29— 7	6	: 15

1962

normal × normal	ms	N
62.54— 7 × 54—12	2	: 8
" 55— 9 × 55— 4	2	: 22
" 46—10 × 46— 5	8	: 7
" 52— 3 × 52— 6	3	: 10

1963

normal × normal	ms	N
63.47— 4 × 47— 6	2	: 7
" 55—18 × 55—10	6	: 16
" 58— 8 × 58— 6	0	: 4
" 61—20 × 61—11	4	: 21

1964

normal × normal	ms	N
64.38— 4 × 38— 8	4	: 25
" 42— 2 × 42—22	4	: 5

1965

normal × normal	ms	N
65.25— 8 × 24—10	4	: 17

1961

self	ms	N
61.22—10	5	: 14

1963

self	ms	N
63.33—13	5	: 13
" 47— 6	3	: 7
" 58— 6	0	: 7
" 61—11	1	: 5
" 64—18	2	: 3

Table 8

1961	sterile \times Sakurajima	ms	N	" 40-13 \times B 62-1	9 : 0	
61. 27-3 \times S 61-1	7 : 0	" 42-6 \times B 62-2	14 : 0	" 45-18 \times B 62-3	12 : 0	
" 27-5 \times S 61-2	12 : 0					
" 21-11 \times S 61-1	15 : 0					
" 27-9 \times S 61-3	7 : 0					
" 27-11 \times S 61-1	9 : 0					
" 21-11 \times S 61-2	14 : 0					
" 27-3 \times S 61-2	11 : 0					
" 27-11 \times S 61-2	10 : 0					
sterile \times Miyashige	ms	N				
61. 21-11 \times M 61-1	44 : 0					
" 27-5 \times M 61-2	23 : 0					
Sakurajima \times normal						
61. 1-1 \times 61. 21-11	0 : 18					
1962	sterile \times Sakurajima	ms	N	sterile \times Miyashige \times Miyashige	ms N	
62. 40-12 \times S 92-1	23 : 0			21-11 \times M 61-1 \times M 62-2	24 : 0	
" 37-3 \times S 62-2	18 : 0			sterile \times Sakurajima \times Sakurajima	ms N	
" 40-13 \times S 62-3	23 : 0			21-11 \times S 61-1 \times S 62-2	18 : 0	
" 42-6 \times S 62-1	15 : 0			27-11 \times S 61-2 \times S 62-3	23 : 0	
sterile \times Miyashige	ms	N		sterile \times Sakurajima \times Miyashige	ms N	
62. 35-12 \times M 62-1	16 : 2			27-9 \times S 61-3 \times M 62-2	24 : 0	
" 37-3 \times M 62-2	18 : 0			sterile \times Sakurajima \times Benisuji	ms N	
" 40-13 \times M 62-3	19 : 0			27-3 \times S 61-2 \times B 62-2	22 : 0	
" 42-6 \times M 62-4	10 : 0					
" 45-2 \times M 62-1	20 : 0					
" 54-16 \times M 62-2	22 : 1					
" 55-1 \times M 62-3	21 : 0					
sterile \times Benisuji	ms	N				
62. 37-2 \times B 62-1	3 : 0					
" 37-3 \times B 62-2	8 : 0					
" 38-18 \times B 62-3	14 : 0					
				1962	commercial variety \times normal	ms N
				Sakurajima S 62-2 \times 38-16	0 : 20	
				S 62-3 \times 38-8	0 : 20	
				Miyashige M 62-2 \times 39-11	0 : 18	
				M 62-3 \times 38-16	0 : 22	
				Benisuji B 62-2 \times 39-11	0 : 18	
				B 62-3 \times 38-8	0 : 16	
				1963	sterile \times Miyashige	ms N
				63. 34-1 \times 103-9	24 : 0	
				" 39-5 \times 103-9	22 : 0	
				" 62-8 \times 103-10	23 : 0	
				" 68-22 \times 103-10	26 : 0	
				sterile \times Benisuji	ms N	
				63. 36-2 \times 102-8	24 : 0	
				" 38-9 \times 102-8	26 : 0	
				" 40-8 \times 102-12	27 : 0	

" 53—3 × 102—12	21	:	0	" 66—13 × 87—8 Miyashige	4	:	1
" 69—14 × 102—12	25	:	0	" 38—1 × 84—14			
sterile × Kurobariso	ms		N	Kurume-mino	15	:	11
63.52—2 × 105—26	21	:	0	" 54—17 × 84—3			
" 58—1 × 105—26	21	:	0	Kurume-mino	8	:	7
" 59—16 × 105—28	27	:	0	" 66—13 × 84—3	6	:	5
" 61—18 × 105—28	29	:	0	1964			
sterile × Kurume-mino	ms		N	sterile × Kurobariso × Kurobariso	...	ms	N
63.58—1 × 104—50	26	:	0	64.52—4 × 85—9	...	24	:
" 59—22 × 104—50	22	:	0	64.52—5 × 85—12	...	26	:
" 61—13 × 104—30	7	:	0	64.60—8 × 85—12	...	16	:
" 61—18 × 104—30	9	:	0	64.60—4 × 85—91	...	18	:
" 62—1 × 104—30	26	:	0	64.60—12 × 85—9	...	25	:
sterile × Sakurajima	ms		N	sterile × Kurume-mino × Kurume-mino	ms		N
63.65—7 × 106—12	24	:	0	64.59—9 × 84—3	...	25	:
1964				64.59—2 × 84—3	...	21	:
sterile × Benisuji	ms		N	64.63—7 × 84—3	...	26	:
64.80—1 × 29—14	25	:	0	64.63—3 × 84—14	...	25	:
" 80—19 × 29—42	7	:	0	sterile × Okutsumari × Okutsumari	ms		N
sterile × Kurobariso	N			64.61—1 × 86—13	...	26	:
64.64—11 × 85—9	23	:	0	64.61—2 × 86—9	...	26	:
" 64—8 × 85—9	24	:	0	64.61—8 × 86—9	...	26	:
" 70—1 × 85—12	24	:	0	1965			
" 70—19 × 85—9	27	:	0	sterile × Kurobariso ×			
" 70—5 × 85—12	26	:	0	Kurobariso × Kurobariso	ms		N
sterile × Kurume-mino	ms		N	65.28—10 × 110—17	...	17	:
64.63—4 × 84—3	15	:	0	65.28—12 × 110—9	...	26	:
" 69—2 × 84—3	26	:	0	sterile × Okutsumari ×			
" 69—7 × 84—14	20	:	0	Okutsumari × Okutsumari	ms		N
" 72—1 × 84—14	16	:	0	65.39—3 × 111—4	...	24	:
sterile × Sakurajima	ms		N	65.39—10 × 111—22	...	25	:
64.75—12 × 100—12	4	:	0	sterile × Miyashige ×			
" 75—2 × 100—22	4	:	0	Kurume-mino × Kurume-mino	ms		N
" 78—23 × 100—32	4	:	0	65.42—8 × 109—1	...	17	:
normal × commercial var.	ms		N	65.42—8 × 109—8	...	2	:
64.38—1 × 85—12 Kurobariso	14	:	11	65.42—8 × 109—13	...	21	:
" 38—1 × 87—8 Miyashige	13	:	16	65.42—18 × 109—8	...	18	:

Explanation of figures

- Fig. 1. An aspect of the flowering and the setting of seed pod in normal fertile radish.
- Fig. 2, 3. Two different types of the flowering and of the setting of seed pod in the new male-sterile radish.
- Fig. 4-7. Flowers, buds, anthers and styles of normal (upper) and of the new male-sterile radish (lower).
- Fig. 8, 9. Abnormal types of the style which appeared in some plants of the new male-sterile radish at the stage of flowering.
- Fig. 10-12. Cell division of the pollen mother cells in the new male-sterile radish.
- Fig. 13. Tetrads of the new male-sterile radish.
- Fig. 14. Longitudinal section of a new male-sterile anther at the stage of tetrad.
- Fig. 15, 16. Longitudinal (15) and cross (16) sections of the new male-sterile anther at the early degenerating stage of tapetal tissue.
- Fig. 17. Degenerating microspores in the new male-sterile radish.
- Fig. 18. Longitudinal section of a degenerated anther of the new male-sterile radish.
- Fig. 19. An instance of abnormal development of tapetal tissue into the anther loculus of the new male-sterile radish.
- Fig. 20-25. Aspects of the development of pollen grains and of the degeneration of tapetal tissue in normal fertile radish.

