Characterization and Genetic Analysis of Male Sterile Mutant Induced in Tomato cv.
First, Having Mature Pollen Stainable With Acetocarmine

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Summary

A new male-sterile tomato mutant characterized by morphologically normal flowers and acetocarmine-stainable mature pollens, was induced by irradiating seeds with gamma rays. Mature pollen grains which stain black by iodine solution at anthesis, exhibited a lower germination rate and proved to be self-unfruitful by self-pollination in spring. Thus, the inhibition of starch degradation is associated with the low pollen germination, but whether it causes pollen sterility is unclear.

From the segregation ratio of self-pollinated progenies of heterozygous plant (Mmsms), both inhibition of starch degradation and male sterility were controlled by single recessive genes, respectively, which might be activated at the post-maturation stage of pollen.

Key Words: tomato mutant, male sterility, pollen, iodine-stainable pollen.

Introduction

Genetic male sterility is widely used for hybrid seed production in monococious and hermaphroditic crops to reduce the cost of labor for hand emasculation. Although more than 40 ms genes are known in tomato, most of them are not applicable for a practical hybrid seed production because of the difficulty in maintaining their pureline by seeds (Georgiev, 1991).

Recently three types of male sterile mutants were induced in tomato, cv. First which has been used in Japan as a parent for F1 seed production. These mutants were derived by irradiating dry seeds with gamma rays. In these mutants, pollen degradation becomes evident during pre-meiosis or in the early microsporogenesis (Masuda et al., 1998a). A fourth male sterile mutant, which produced mature pollen stainable with acetocarmine, was identified in the same M2 population as the other three types. In this study, the fourth type was investigated for its pollen sterility and its inheritance pattern.

Materials and Methods

Three clones of male sterile mutant (T-4) have been maintained by cuttings. Ten flowers were self-pollinated for each plant in early May 1996, and pollens collected from each flower at anthesis were stained with 10% acetocarmine. Pollens were collected 2 days before anthesis, at anthesis, and 2 days after anthesis; they were stained with 0.8% iodine solution for 1 hour. Pollen germination rate was examined at 25°C for 2 hours on liquid medium (pH 5.7) containing 50mgH3BO3, 100mgCaCl2 and 100g sucrose per liter.

For genetic analysis of male sterility, the heterozygous fertile F1 hybrids obtained as a backcross between a male sterile mutant and its original, cv. First, were self-pollinated and the seeds sown the following spring.

Results and Discussion

The flowers of T-4 which are indistinguishable in size and color from that of ‘First’, produced morphologically mature pollens stainable normally with acetocarmine (Fig.1). Pollen grains from ‘First’ and T-4 plants, collected 2 days before anthesis stained completely black with iodine solution. At anthesis, pollens of ‘First’ stained red, whereas most of the pollens collected from T-4 stained black. The compound stained black by iodine solution should be starch which indicates that its degradation was inhibited in mature pollens of the mutant. Even 2 days after anthesis most of the T-4 pollens remained black (Fig. 2). Their germination rate was only 9.8%, whereas it was over 90% in ‘First’. The pollen tube growth of T-4 was vigorless. These results indicate that microsporogenesis was completely normal, that mature pollen grains were produced in this mutant, and that sterility is somehow related to the inhibition of starch degradation after maturation. In general, 90% of ms genes express their action at microsporogenesis during anther development, and 10% at microgametogenesis (Horner and Palmer, 1995). Mature male sterile pollen which stains normally with acetocarmine and black with iodine at anthesis have not been reported in tomato. In rice, pollen that stains blue-black with iodine–potassium iodide solution is regarded as being fertile. Not only pollen from normal plants, but also
some from male sterile mutants are stainable, indicating
that such mutants were not distinguishable from normal
plants by the iodine-test in rice (Fujimaki and Hiraiva,
1986). Furthermore, no fruit set in the spring by self-
pollination but seed fertility was partially restored in
autumn so that half of the floweres set seeded fruit
(Masuda et al., 1998b). This suggests that seed fertility
in this mutant is seasonally dependent and T-4 homo-
zygous seeds can be easily mainained by self-pollina-
tion.

In the selfed progenies of a heterozygous plant, the
percentage of pollens stained black with iodine solution
ranged from 2% to 80% (Fig. 3); those with more than
20% black pollens were classified as abnormal type. A
total of 79 progenies segregated into three types; fertile
(65 progenies), sterile with normal stainability (1 prog-
eny), and sterile with abnormal stainability (13 proge-
nies). Segregation ratios of fertile and sterile, and of
normal and abnormal stainability, both fit the ratio of
3:1, indicating that inhibition of starch degradation and
male sterility are controlled by single recessive genes,
respectively (Table 1). Pollen germination rate was less
than 25% in sterile progenies in which more than 20% of
the pollen population stained black (except one),
whereas it was more than 50% in the fertile progenies in
which less than 20% of the pollen population stained

Fig. 1. Flower and pollen grains of a new male sterile mutant (T-4), and its original cv. First. Pollen color
stained with acetocarmine was light red in both lines.

![Image of flower and pollen grains]

Fig. 2. Stage-dependent differences in stainability of pollen grains with iodine solution, between cv.
First and its mutant T-4. At anthesis pollen color was mostly black in mutant and dark red in

![Image of pollen grains at different stages]
Table 1. Segregation of pollen stainability with iodine solution and fertility by vibratory self-pollination in selfed-progenies of a heterozygous tomato plant (Msms).

<table>
<thead>
<tr>
<th>Character</th>
<th>no. of plants</th>
<th>(\chi^2) (3:1)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollen stainability</td>
<td>normal 66</td>
<td>3.076</td>
<td>0.05 ≤ p ≤ 0.10</td>
</tr>
<tr>
<td></td>
<td>abnormal 13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertility</td>
<td>fruting 65</td>
<td>2.232</td>
<td>0.10 ≤ p ≤ 0.25</td>
</tr>
<tr>
<td></td>
<td>non-fruting 14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) More than 20% of pollen grains were stained black.

Fig. 3. Relationship between percentage of pollen stained black with iodine solution and pollen germination rate in fertile and sterile plants. These plants segregated among selfed progenies of a heterozygous tomato plant (Msms).

Ronald A. Hirschi

**Literature Cited**


黒濃。「ファースト」由来の雄性不稔突然変異系統における成熟花粉の染色特性と不稔性の遺伝様式

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**要約**

トマト品種「ファースト」種子へのガンナー線照射によって新タイプの雄性不稔変異系統が見いだされた。本系統は芸が正常に発育し、成熟花粉のアセチルアミン染色において「ファースト」花粉と区別できなかった。しかし、この成熟花粉のほとんどはヨウ素液で黑色に染色され赤褐色に染色される「ファースト」花粉と区別できた。また、本系統の花粉発芽率は極めて低く、春季、自家受粉による着果は認められなかっ

た。花粉のデンプン分解抑制は発芽率の低下に関係していると考えられるが、このことが不稔の原因になっているかどうかは明らかでない。ヘテロ (Msms) 個体の自家受粉による子孫の分離比率からデンプン分解抑制と雄性不稔性はいずれも単一の劣性遺伝子に支配されており、それらの遺伝子は花粉成熟後に働き始めることが明らかとなった。