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Restoration of Male Fertility in Seasonally Dependent Male Sterile Mutant Tomato, *Lycopersicon esculentum* cv. First

Masaharu Masuda, Koji Uchida, Kenji Kato and Stephen G. Agong
Faculty of Agriculture, Okayama University, Tsushima Naka, Okayama 700-8530

Summary

The effect of environmental conditions in the spring and autumn on the restoration of male fertility in T-4 male sterile mutant plants which were obtained via gamma (γ)-irradiation of tomato cv. First was investigated. Vegetatively propagated sterile plants gave rise to all male sterile plants in the spring, but the same T-4 plants cultivated under uncontrolled environmental conditions in autumn yielded fertile male. Self-pollination with these fertile males resulted in 50% fruit set and 26 seeds per fruit. The selfed seeds grown in the spring and with exposure to the natural environment developed into sterile males. When these male sterile plants were self-pollinated under natural conditions in autumn, they gave rise to normal male fertile plants with an average of over 35 seeds per fruit. Short or long day treatments had no effects on the male fertility restoration.

The pollen germination percentage on artificial medium was relatively higher in autumn plants compared to that grown in the spring in the T-4 mutant plants, but it remained distinctly lower than the original cv. First in the spring and autumn. Pollen tube growth following germination in the mutant plants was lacked vigor and grew slowly in the spring compared to autumn. No difference in the final pollen tube length between the mutant and normal plants was noted in autumn.

We conclude from these results that pollen and seed viability in the T-4 male sterile mutant plants was restored when exposed to low temperatures in autumn.

Key Words: male sterility, natural male fertility restoration, tomato mutant.

Introduction

The use of male sterile plants eliminates the labor-intensive process of emasculation and, therefore, significantly reduces the cost of hybrid seed production. The hybrid seed produced by the male sterile system also assures high hybrid purity. Genic male sterility (GMS) is not commonly used in hybrid seed production, primarily because of the difficulty in maintaining pure male-sterile lines. In GMS, the sterile lines are maintained through multiplication as progenies of back-cross of F_1 to its parents (F_1BCP1) and/or F_1-selfed (F_2). In the former case, if sterility is inherited as a simple Mendelian factor, the ratio of fertile to sterile plants should be 1:1. Thus, in this breeding system, 50% fertile plants are not used. However, if male sterility can be restored depending on a given growing condition, it would be a very economical means to maintain a pure sterile line by self-pollination.

One possible approach is to select for chemically-or environmentally-sensitive GMS lines in which fertility can be restored by appropriate treatments (Sawhney, 1997). For tomato plants, more than 40 *ms* genes in GMS are known; however, only a few of these *ms*-systems have been applied in hybrid seed production, because they allow cross-pollination without emasculation (Georgiev, 1991).

Recently we obtained a new type of male sterile mutant in tomato cv. First by irradiating dry seeds with gamma rays. This mutant is not distinguishable in size and color of flower from parent cv. First. Also, the mature mutant pollen grains are morphologically identical to the parent ones and are stainable with acetocarmine. However, almost mature mutant pollen stained with iodine turnes black instead of red in the parent pollen. No fruit set was obtained by self-pollination of the mutant in spring, because the pollens were functionally sterile (Masuda et al., 1999).

The present study examined the pollen behavior of this male-sterile mutant (T-4) as affected by the environmental pressures in spring vs autumn with respect to restoring male fertility and for assessing the suitability of the system for maintenance of *ms* tomato plants.

Materials and Methods

Experimental plants were derived from plants that failed to produce fruits via self-pollination in the M2

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progeny. The original parental plants had been raised from seeds exposed to gamma rays (Masuda, et al., 1998b). The non-fruiting plants were recognized as male sterile and were maintained by cuttings or cross-pollination with cv. First. The plants were transplanted individually into 1/2000 Wagner-pots filled with soil and irrigated every other day with a nutrient solution.

Experiment 1. Self-pollination and black pollen appearance in spring and autumn

Six cuttings propagated in summer were kept at temperatures > 14 ℃ in a heated greenhouse during winter. Thirty flowers in total were self-pollinated from April to May 1997 (spring) to confirm their male sterility. Simultaneously, pollens from 10 anthers were stained with acetocarmine and iodine solution, and germinated on artificial medium according to Masuda et al. (1999). Self-pollination and pollen stainability were tested on the same cuttings between late September and late October 1997.

Experiment 2. Segregation of male sterility and black pollen appearance in spring and autumn

The male-sterile segregants arising from the self-pollinated heterozygous plants (*Msms*) were examined for differences in pollen fertility restoration and fruit set between spring and autumn 1997. Four plants with no sign of fruit set were selected randomly in spring and their pollen grains stained with an iodine solution. A pollen germination test was conducted on 20 flowers obtained from the 1st, 2nd and 3rd trusses. Manual shaking of all plants achieved normal fruit set and number of fruit per plant was recorded.

Experiment 3. Restoration of male fertility in autumn

Selfed seeds obtained in autumn 1997 were confirmed as being pure male sterile in spring, 1998. Fifteen plants were selected on the basis of their capability to produce the fruits at the 1st truss after self-pollination by mid-May. In early October to late November 1998, 15 flowers were self-pollinated from each of the five plants that survived the hot summer. Concurrently, stainability with iodine solution and germination ability were examined.

Experiment 4. Restoration of seed fertility under artificial photoperiod control

This experiment was conducted to test whether photoperiodicity played a role in restoration of male fertility. In a two replicate trial, spring (May 6-May 29) and autumn (October 9-October 31) in 1998, the day length was controlled by using a black polythene sheet. The plants were initially raised vegetatively via a cutting and at visible bud stage, three plants were randomly transferred to the two photoperiod regimes consisted of Short Day (SD) with day length of 8-hr (9:00-17:00 and 9:30-17:30, autumn/spring, respectively), and Long Day (LD) of 16hrs (4:00-20:00).

Apart from photoperiod, all the other environmental conditions in the greenhouse were uncontrolled. The supplemental lighting was provided for from 4:00-7:00 and 17:00-20:00 in spring, and from 4:00-9:00 and 16:00-20:00 in autumn using fluorescent lamps with a light intensity of 50 $\mu mol \cdot m^{-2} \cdot sec^{-1}$. At anthesis, thirteen flowers were self-pollinated. Simultaneously, the pollen germination tests were carried out as above.

Experiment 5. Germination and growth rate of pollen

The pollen germination and pollen tube growth rate in both cv. First and T-4 mutant plants were examined by subjecting the pollen grains to artificial germination medium (Masuda et al., 1999). Ten microscopic fields were photographed to assess pollen tube length (μm) at hourly intervals for 7 hr following germination were conducted in mid-May and early November.

Results

Experiment 1. Self-pollination and black pollen appearance in spring and autumn

About 55% of the pollens stained black when the pollens were collected between 10 April to 30 May and 40% of those between 27 September to 27 October (Table 1). On the other hand, 15% of the pollens

Table 1. Seasonally dependency of male fertility restoration in male sterile line of tomato mutant T-4 in 1997.

Pollination period	Range in minimum Temp. (℃)	Change in day length[z]	Black pollen rate (%)[y]	Pollen germination (%)[y]	Fruit set (%)[x]	Seeds no. per fruit
April 10		12hr-53min.				
↓	10-19	↓	54.6 ± 8.0	4.0 ± 1.3	0	–
May 30		14hr-18min.	(1.5 ± 0.2)[w]	(70.4 ± 4.1)	(90)	(122 ± 10)
Sept. 27		11hr-58min.	39.1 ± 7.6	14.9 ± 3.4	53	26 ± 5
↓	6-18	↓	(2.3 ± 0.6)	(73.4 ± 3.4)	(90)	(107 ± 11)
Oct. 27		10hr-54min.				

[z] According to Scientific Chronological Table published in Japan.
[y] Anthers of 10 flowers were examined. Mean ± Standard error (SE).
[x] Thirty flowers from T-4 were self-pollinated in each season.
[w] Data obtained from the original cv. First.

sampled in the autumn germinated, whereas only 4% of those collected in the spring did.

Pollen fertility was partially restored in the T-4 mutant *ms* plants in autumn with approximately 50% of flowers setting fruit through self-pollination and yielding an average of 26 seeds per fruit in the presumptive male sterile plants. During pollination time in the spring, the minimum temperature (MT) ranged from 10 to 19 ℃, while natural day length (DL) lengthened from 12hr-53min to 14 hr-18min. MT in autumn decreased from 18 to 6 ℃, while DL decreased from 11hr-58min to 10hr-54min. Data obtained from cv. First revealed that seasonal variation had no effect on the fertility parameters in both spring and autumn (Table 1).

Experiment 2. Male sterile segregants and black pollen appearance in spring and autumn

The number of fruits set per plant obtained from the male sterile segregants from the T-4 heterozygous plants during autumn was>10 (Table 2). The environment in autumn restored male fertility, whereas that in the spring had no effect on male sterility. Black pollen rate was not different between spring and autumn, whereas pollen germination percentage was four times higher in autumn than in spring.

Experiment 3. Restoration of male fertility in autumn

Male fertility was significantly restored in autumn with T-4 male sterile homozygous plants as demonstrated by a good yield of seeds per fruit. In spring, no seeds were produced by the same plants (Table 3). Thus, some unknown seasonal variable(s) is significant in the restoration of male fertility which in turn led to the differences in mean pollen germination percentages, fruit set, and seed count per fruit between spring and autumn.

Experiment 4. Restoration of seed fertility under artificial photoperiod control

Photoperiod seemed not to have exerted an influence on the restoration of male fertility in the T-4 tomato male sterile plants (Table 4). There were insignificant variations on the performance of male sterile mutant

Table 2. Seasonal dependency of male fertility restoration in male sterile segregants from T-4 heterozygous (*Msms*) plant in 1997.

Pollination period	Black pollen rate(%)[z]	Pollen germination(%)[z]	Fruit no. per plant[y]
April 8 ↓ June 2	45.7 ± 16.7	3.1 ± 2.7	0
Oct.6 ↓ Oct.20	48.7 ± 12.3	13.3 ± 5.5	>10

[z] Twenty flowers were selected randomly from four plants with no fruit set.
The Pollen was stained with iodine solution and examined for color, and then tested for pollen viability. Mean ± Standard error (SE).
[y] Three trusses per plant were artificially vibrated.

Table 3. Male fertility performance of the male sterile mutants in spring and autumn for seedlings of selfed T-4 homozygous plants in October 1998.

Plant No.	Black pollen rate (%)		Pollen germination (%)		Fruit set (%) and (Seeds no. per fruit)	
	Spring	Autumn[z]	Spring	Autumn[z]	Spring	Autumn
1	19.0	–	4.5	–	0.0	–
2	53.2	–	17.8	–	0.0	–
3	45.0	–	8.7	–	0.0	–
4	66.8	–	2.5	–	0.0	–
5	53.1	–	2.6	–	0.0	–
6	22.7	27.2	9.3	10.9	0.0	50.0 (26.5)
7	36.2	–	8.5	–	0.0	–
8	28.2	–	37.4	–	0.0	–
9	40.0	40.6	3.6	10.1	0.0	64.3 (37.3)
10	18.2	–	7.7	–	0.0	–
11	58.5	–	0.8	–	0.0	–
12	53.8	48.8	5.2	11.3	0.0	35.3 (37.5)
13	63.8	57.5	3.5	11.5	0.0	50.0 (35.3)
14	11.0	47.2	3.5	9.6	0.0	40.0 (42.0)
15	52.5	–	3.1	–	0.0	–
Mean ± SE	41.5 ± 4.5	44.3 ± 5.1	7.9 ± 2.4	10.7 ± 0.4	0.0	47.9 ± 5.0 (35.7 ± 2.5)

[z] Black pollen rate examined in 18 anthers of original cv. First was 2.8 ± 0.7%, and pollen germination rate was 67.9 ± 2.9%.

Table 4. Fruit set in T-4 male sterile plants as affected by short or long day treatments in 1998.

Season (Period)	Day length (hr)	Range in minimum temp. (℃)	Fruit set (%)	Seed no. per fruit
Spring	8	16-23	0.0	–
(May6-29)	16	〃	0.0	–
Autumn	8	13-22	18.2	12.5
(Oct. 9-31)	16	〃	15.4	14.5

plants over both photoperiod regimes during the autumn of 1998. Similarly, in the spring no evidence of a photoperiod effect was traceable to the functioning of the male sterility or fertility systems.

Experiment 5. Germination and growth rate of pollen

Pollen tube growth rate following germination on artificial medium was distinctly higher in pollens collected in the autumn, compared to those harvested in the spring (Fig. 1). Also, pollen tube growth following germination of mutant pollen was weak and grew slowly in spring compared to that in autumn, but no difference in the final pollen tube length between pollen of the mutant and normal plants collected in autumn following the seven hour-period of incubation (Fig. 1).

Discussion

Selfed seeds of male sterile mutant T-4 obtained in autumn were restored to male fertility in the *ms* plants, but reverted to male sterility in the spring, indicating that it is possible to obtain male sterile and fertile plants in the spring and autumn, respectively (Tables, 1, 2 and 3). Sterility or fertility which was assessed by percent pollen germination, percent fruit set, and seed number per fruit in the *ms* plants correlated well with the seasons. That mutant pollen stained black with iodine did not reveal whether fertility was restored in the *ms* plant but the test remains a useful parameter for distinguishing the T-4 mutant plants from original cv. First (Table 3; Masuda et al., 1999).

The male sterility system of T-4 tomato mutant is likely to be controlled by a recessive gene based on the segregation ratio of progenies arising from the selfed heterozygous fertile plants (Masuda et al., 1999). The expression of genic male sterility in tomato has not been studied much but the concept of environmental influence on male fertility was observed in a cabbage mutant by Rundfeldt (1960) who concluded that it was perfectly fertile in winter and absolutely sterile in summer. Recently, Shi and Deng (1986) discovered a photoperiod sensitive rice mutant that was completely sterile in a long day (>14 hrs) but reverts to male fertility in a short day (<13 hrs). Furthermore, Yuan et al. (1993) reveales that, under suitable temperatures, pollen sterility was

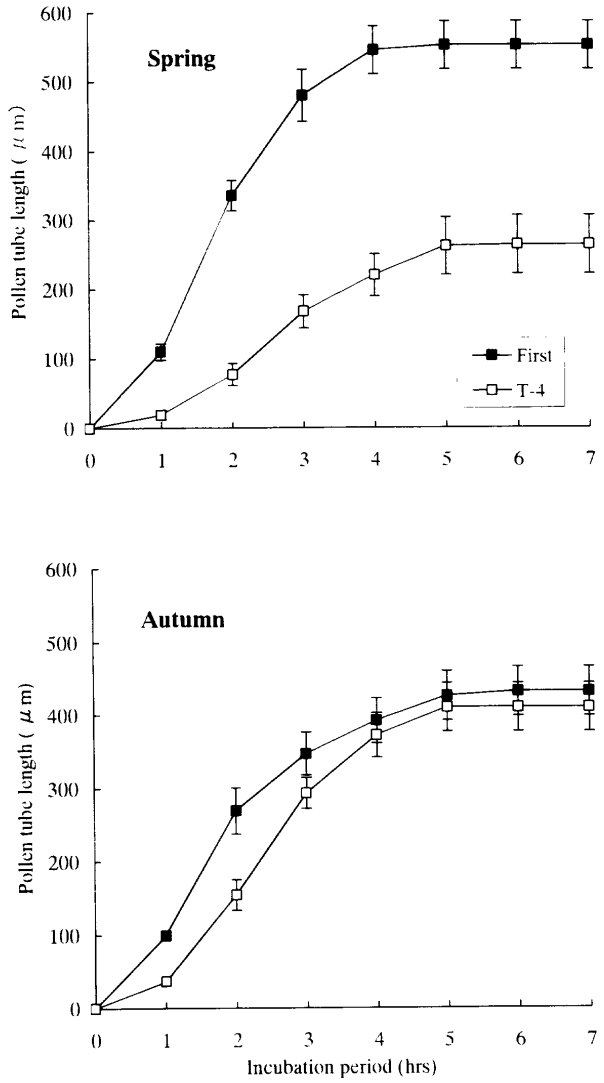

Fig. 1. Comparison of pollen tube growth in cv. First (wild type) and T-4 (male sterile mutant) following germination in spring and autumn. The vertical bars are standard errors (n = 10).

induced in rice under long days. Horner and Palmer (1995), likewise, documented widespread utilization of photosensitive genic male sterility for hybrid rice production.

Bishop (1954) recognized that *ms* tomato plant could be completely sterile during the long days in the summer but often produced viable pollen in winter even though the stamens were abnormal. Lower temperatures and Gibberellins (GA_3) also restored fertility in various male sterile tomato mutants (Schmidt and Schmidt, 1981; Sawhney, 1983, 1997). However, it is not evident whether plants carrying these genes are capable of producing enough viable seeds through selfing after restoration of fertility. The achievement of self-fertilization irrespective of day length in the autumn season (Tables 1, 2, 3, 4 and Fig. 1) indicates that fertility restoration system is inducible in the T-4 *ms* plants via an exposure to low temperatures.

The slow germination and growth of mutant pollen grains (Fig.1) strongly correlated the inability of self-

fertilization to proceed in mutant plants in the spring (Tables 1, 2, 3; Fig. 1).

As earlier reported, four types of male sterile mutants (T-1, T-2, T-3, and T-4) in tomato cv. First via irradiation of dry seeds with gamma rays have been isolated (Masuda et al., 1998a; 1998b; Masuda et al., 1999). In T-4 however, unlike the other three forms of *ms* mutants, pollen degradation is not the primary basis for male sterility (Masuda et al., 1999). Although the extent to which seasonal variations affect each of these systems is not yet known, in T-4, temperature is conspicuously an important factor (Tables 1, 2, 3, 4; Fig. 1).

Most *ms* genes in tomato act erratically either during prophase-I or just after meiosis is completed so that microsporogenesis is inhibited or impaired. This behavior can be detected in the early stages of plant development, using the gene marker "aa" (absence of anthocyanin), which facilitates the separation of sterile from fertile plants immediately after their germination (Philouze, 1974). However, the use of this characteristic is not always full proof because about 5% of fertile plants are indistinguishable from the sterile ones (Georgiev, 1991). T-4 *ms* is useful for maintaining the *ms* gene as well as for seed production depending on the season. Hence we conclude that temperature during the season plays an important role in regulating male fertility system within the newly discovered tomato mutant (T-4).

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トマト雄性不稔の突然変異系統における稔性回復の季節依存性

桝田正治・内田浩司・加藤鎌司・S. G. Agong

岡山大学農学部　700-8530　岡山市津島中

摘　要

トマト‘ファースト’にガンマー線を照射して作出したT-4雄性不稔突然変異体の春秋期における稔性回復性について調査した．挿し木によって栄養繁殖したT-4株は春期に全て不稔性を示した．これら同植物体を秋期に自然環境下で栽培したところ，すべての株が稔性回復を示し，自家受粉花の約50%に結実がみられ，果実当たり平均26粒の種子を得た．その自殖種子を翌春に自然環境下で栽培したところ株は全て不稔性を示した．同植物は秋期に稔性回復し果実当たり35粒の種子を得た．短日および長日処理は，不稔性回復に影響しなかった．

T-4株の人工培地上での花粉発芽率は春期より秋期の方が高かったが，その値は原品種‘ファースト’と比べればはるかに低かった．春期のT-4株の花粉は発芽しても花粉管伸長速度が遅く途中で停止したが，秋期には速まり最終の花粉管長においては‘ファースト’のそれと差が無くなった．

これらの結果より，T-4雄性不稔突然変異体の不稔回復は，秋の低温に強く依存しているものと考えられた．