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Partial Fertility Restoration as Affected by Night Temperature in a Season-dependent Male-sterile Mutant Tomato, *Lycopersicon esculentum* Mill.

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This study was conducted to investigate the influence of night temperature on the restoration of fertility in a season-dependent male-sterile tomato mutant (T-4). Plants were grown in greenhouses, in which minimum and maximum temperatures were set at 10°C and 28°C by heating and ventilation, respectively. Flowers were hand-pollinated and the fruit-set, seed-set, and number of seeds were examined. The rate of fruit-set was high and did not differ much from October to February; almost all fruits formed in October had self-fertile seeds, but 80% of the fruits from November to February were parthenocarpic. The rate of fruit-set dropped from 70% in March to below 10% in May. During this period, most of the fruits were seeded, though fruit-set was low. The number of seeds per seeded fruit varied with the season, being as high as 50 seeds in October, 1–2 seeds per fruit between November and March, and 1–20 seeds per fruit between April and June. A low night temperature of 12°C did not affect fruit-set but resulted in a better seed-set than a high night temperature of 18°C in the greenhouse. Further, pollination of the plants in phytochambers also resulted in a better fruit- and seed-set at 12°C than 24°C. In all cases, the influence of low temperature was more pronounced in autumn than in spring. Fruit-set was 70% at 12°C and 46% at 24°C. Of these fruits, 50% at 12°C and 10% at 24°C were seeded. It was inferred that partial fertility restoration in T-4 can be achieved by manipulation of night temperatures. The female organ was shown to be normal, functional, and compatible with wild-type pollen. From these results, the potential of the male-sterile T-4 mutant for use in a two line hybrid-seed production system was apparent.

Key Words: male-sterile mutant, night temperature, partial fertility, tomato hybrid-seed.

Introduction

Hybrid-seed production of hermaphrodite or monoecious plants requires the maintenance of separate lines of male and female parents. Emasculation must be performed on the seed parents before pollination with the desired pollen (George, 1985). Whether by hand or using chemicals, emasculation is expensive, time consuming, and labour intensive, thereby contributing significantly to the high cost of hybrid-seeds (Lasa and Bosemark, 1994; Sawhney, 2004). Induction of male-sterility in the seed parent has been a useful way to circumvent the problems of high emasculation costs in F_1 tomato hybrid-seed production (Lasa and Bosemark, 1994).

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A number of male-sterile tomato lines have been developed and are currently being used with some level of success in hybrid-seed production (Dhaliwal et al., 2004). However, most of these lines are genic male-sterile mutants (GMS), and hence face the problem of maintenance. Normally, a GMS line is maintained by backcrossing with a heterozygous maintainer line, but the progeny produced are 50% fertile and 50% sterile. In addition to the need for a third line, the maintainer line, an extra problem is created of roguing the fertile plants. Ideally, the male-sterile parent should be facultative so that it can be induced to self-pollinate when desired, thereby avoiding the maintenance of the male-sterile trait in the heterozygous condition. The ability to manipulate the restoration of fertility in GMS lines by environmental control is a desirable approach for the maintenance of these lines. This can lead to the production of 100% male-

sterile seeds, which can be used as female parents in hybrid-seed production, without the need of a maintainer line or of roguing fertile plants (Sawhney, 1983).

A genic male-sterile (GMS) tomato mutant (T-4) was induced by irradiating seeds of a Japanese commercial tomato cultivar 'First' with gamma-rays (Masuda et al., 1999). The T-4 mutant has shown very promising characteristics of fertility restoration under certain environmental conditions. Pollen viability in the male-sterile T-4 mutant plants (BC_1F_3) was partially restored in the autumn season, leading to a hypothesis that the restoration was triggered by low temperature in autumn (Masuda et al., 2000).

Male-sterility in rice *PGMS* (photoperiod-sensitive genic male-sterility) and *TGMS* (thermo-sensitive genic male-sterility) mutants is influenced by photoperiod and temperature (He et al., 1999; Wu et al., 2003). Viraktamath and Virmani (2001) reported that exposure to high temperature induced complete male-sterility in thermo-sensitive genic male-sterile lines while exposure of the young panicle to low temperature restored fertility in Annong S-1, a thermo-sensitive genic male-sterile line. A similar regulation of male-sterility by temperature has been reported in various male-sterile lines of the tomato. Low temperatures were reported to restore male-fertility in the stamenless-2 (*sl-2*) tomato mutant (Singh and Sawhney, 1998). Gomez et al. (1999) similarly reported that the stamenless tomato mutant *sl* had its fertility restored in more than 15% of flowers that developed under low temperature conditions.

Thus, the ideal male-sterile seed parents show complete sterility during hybrid-seed production and an appropriate way of maintenance when hybrid-seed is not required. Additionally, female fertility must be normal with no defects in physiological or morphological defects that would compromise cross compatibility or fruit appearance. In this paper, we report on the influence of night temperature on fertility restoration and the fruit- and seed-set of the male-sterile T-4 mutant tomato under controlled conditions in spring and autumn. We also report on the functionality of the female organ and its compatibility with the wild-type pollen.

Materials and Methods

Experiment 1: Effect of year-round greenhouse temperature on partial fertility restoration of male-sterile T-4 mutants

Male-sterile tomato mutant (T-4) seeds, obtained from partially restored fertile plants (BC_2F_3) in spring 2001, were sown in vermiculite on 20 August, 2001. Twenty seedlings were transplanted at the 2nd unfolded leaf stage to 10.5 cm diameter pots filled with a medium composed of bark : sand : peat moss at a ratio of 3 : 2 : 1. The seedlings were fertigated daily with half-strength Enshi-standard solution. Ten uniform seedlings were re-transplanted into 18 cm diameter pots about one week before flowering on 25 September. These were raised in a greenhouse, in which minimum and maximum temperatures were set at 10°C and 30°C by heating and ventilation, respectively, until the end of the experiment in mid-June, 2002. The actual diurnal fluctuation in temperature is shown in Fig. 1. These plants were trained to have the main shoot and one lateral shoot just below the 1st flower truss. At anthesis, 5 flowers in each truss were hand-pollinated and the fruit-set capacity was examined. The total number of flowers pollinated throughout the experiment was 982, of which 571 were pollinated in spring between April 1 and June 15. To investigate the influence of the pistil on pollen-tube germination, the functionality of the female organ, its compatibility with wild-type pollen, and hence its potential for use as a seed parent in hybrid-seed production, the T-4 plants were crossed with wild-type pollen in spring. Mature fruits were harvested and the number of seeds in each fruit counted.

Experiment 2: Effect of autumnal greenhouse night temperatures of 12°C or 18°C on partial fertility restoration of male sterile T-4 mutants

Seeds of male-sterile T-4 mutants (BC_2F_3) were sown on 1st September, 2002. The number of plants, planting method, and crop management were as described in experiment 1. The second transplanting in this case was in 18 cm diameter pots. Plants were grown in 2 separate greenhouses where the night temperature was maintained either above 12°C or above 18°C by heating, while the maximum day temperature ranged between 18–28°C throughout the experiment. Hand-pollination was done on the 1st and 2nd flower trusses between 26th October and 8th November, 2002, after which the night temperature was maintained above 12°C for both greenhouses.

Experiment 3: Effect of growth chamber night temperatures of 12°C or 24°C on partial fertility restoration of male-sterile mutants T-4 in autumn and spring

Plants were raised in the same way as in the experiments above and put in two phytochambers where the day temperature was maintained at 27°C and the night temperature at either 12°C or 24°C for the period between 4th June to 13th June in spring and 22nd October to 31st October in autumn 2003. Fluorescent tubes and high-pressure sodium lamps were used to supply a light

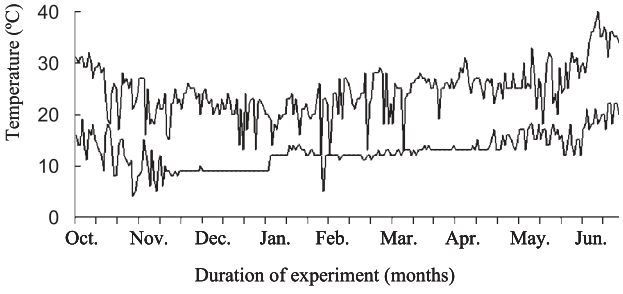

Fig. 1. Diurnal fluctuations between night minimum and day maximum temperatures in the greenhouse during experiment 1.

intensity of 450 μmol·m^{-2}·s^{-1} PPFD for 12 h·d^{-1}. Treatments began when the 1st inflorescence was at anthesis and hand-pollination was done during this period, after which plants were transferred to a greenhouse where the day temperature was maintained below 28°C by ventilation in spring and the night temperature was maintained above 12°C by heating in autumn.

Results

Experiment 1: Effect of year-round greenhouse temperature on partial fertility restoration of male-sterile T-4 mutants

The rate of fruit-set did not differ significantly between October 2001 and February 2002, with nearly 100% of hand-pollinated flowers setting fruit. A slight drop in fruit-set was noticed in March, and a more drastic drop in April, with more than 80% of the flowers pollinated after the second half of April aborting without fruit-set (Fig. 2). However, a transverse section of the fruits revealed that most of the fruits from flowers pollinated between November and March were parthenocarpic. The incidence of parthenocarpy varied seasonally from none in the first half of October to 80–100% between the first half of November and the second half of February. The rate of fruit-set remained lower than 20% and continued to drop between April and June. During this period, most of the fruits were seeded, though fruit-set was low.

The number of seeds per seeded-fruit followed a similar pattern as the frequency of seeded fruits (Fig. 3), with more than 50 seeds per fruit in October, 1–2 seeds per fruit between November and March, and between 1–20 seeds per fruit between April and June. A cross with the normal wild-type pollen yielded 50–200 seeds per fruit, indicating that the poor fruit- and seed-set with hand-pollinated T-4 pollen was not due to a barrier in the pistil, but due to low fertilization capacity of the pollen itself. The female organ was functional and compatible with wild-type pollen.

Experiment 2: Effect of greenhouse autumn night temperatures of 12°C or 18°C on partial fertility restoration of male-sterile T-4 mutants

There was 66.7% and 76.3% fruit-set when the night temperature in the greenhouse during hand-pollination period was maintained at 12°C and 18°C, respectively (Table 1). However, the frequency of seeded fruits was more than double with a night temperature of 12°C (55.1%) than with a night temperature of 18°C (26.3%). The number of seeds per fruit followed a similar pattern as the frequency of seeded fruits, with 7.7 ± 1.3 and 4.4 ± 0.8 (mean ± SE; nearly double) seeds per fruit-set from flowers pollinated when the night temperature was maintained at 12°C and 18°C, respectively.

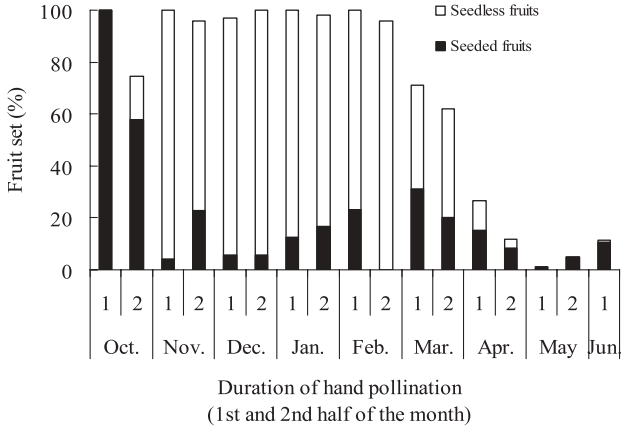

Fig. 2. Rate of fruit-set and proportion of seeded and seedless fruits of T-4 mutant tomatoes hand-pollinated between October 2001 and June 2002.

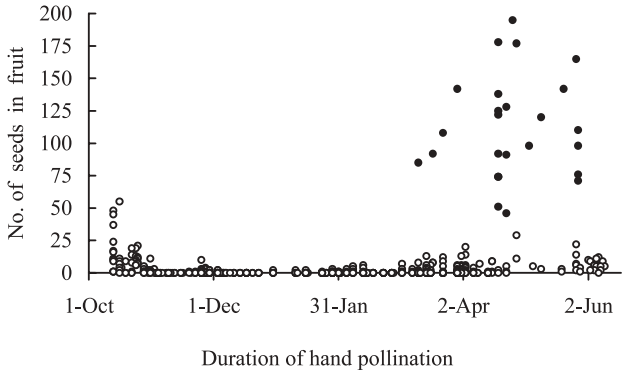

Fig. 3. Number of seeds per fruit from T-4 mutant plants hand-pollinated between October 2001 and June 2002 (open circles), and from those hand-pollined with wild-type fertile pollen between late-March and early-June 2002 (closed circles).

Table 1. Effect of night temperature during anthesis and pollination on fruit and seed-set in a greenhouse.

Min. night temperature[z]	No. of flowers pollinated	No. of fruits[y]		No. of seeds per fruit[x]
		seeded	seedless	
12°C	78	43 (55.1)	9 (11.5)	7.7 ± 1.3
18°C	80	21 (26.3)	40 (50.0)	4.4 ± 0.8

[z] This experiment was conducted in 2 separate greenhouses set at a maximum temperature of 28°C and minimum temperatures of 12 and 18°C in autumn.

[y] Values in parenthesis show % of flowers pollinated. The rest of the flowers aborted.

[x] Data are means (of seeded fruits) ± SE.

Table 2. Effect of low and high night temperatures during anthesis and pollination on fruit and seed-set.

Season	Min. night temperature[z]	No. of flowers pollinated	No. of fruits[y]		No. of seeds per fruit[x]
			seeded	seedless	
Spring	12°C	21	0 (0)	2 (9.5)	0
	24°C	27	0 (0)	0 (0)	0
Autumn	12°C	38	12 (31.6)	14 (36.8)	9.3 ± 2.2
	24°C	46	2 (4.3)	19 (41.3)	7.5 ± 1.1

[z] The experiment was conducted in the growth chambers set at a maximum temperature of 28°C and minimum temperatures of 12 and 24°C in autumn and repeated in spring.

[y] Values in parenthesis show % of flowers pollinated. The rest of the flowers aborted.

[x] Data are means (of seeded fruits) ± SE.

Experiment 3: Effect of growth chamber night temperatures of 12°C or 24°C on partial fertility restoration of male-sterile T-4 mutants in autumn and spring

In the phytochamber with spring-grown plants, there was an extremely low fruit-set with only 2 out of 21 flowers pollinated when the night temperature was maintained at 12°C setting (seedless) fruits, with all the flowers pollinated when the night temperature was maintained at 24°C aborting (Table 2). In contrast, in autumn-grown plants, 68.4% and 45.7% of the flowers pollinated fruited when the night temperature was maintained at 12°C and 24°C, respectively. Of these flowers, 31.6% and 4.3% under 12°C and 24°C treatments set seeded fruits, respectively. The mean number of seeds per fruit was 9.3 and 7.5 under 12°C and 24°C treatments, respectively.

Discussion

Restoration of fertility in genic male-sterile mutants by temperature changes has most commonly been reported in rice, where thermo-sensitive genic male-sterile (*TGMS*) lines are sterile at high temperatures (>25°C) and fertile at lower temperatures (He et al., 1999; Wu et al., 2003). The BC_1F_3 progeny of the male-sterile tomato T-4 mutant used in this study had been observed to be fertile in autumn and sterile in spring (Masuda et al., 2000). It was initially hypothesized that its fertility restoration could be under the influence of photoperiod. To test this hypothesis, an experiment was conducted where plants were grown under short and long daylength conditions. However, there were insignificant variations in the performance of male-sterile mutant plants over both photoperiod regimes (Masuda et al., 1999).

In this study, the influence of night temperature was investigated. Plants were grown within temperature ranges that permit tomato growth and development between mid August and June. Although the fruit-set capacity did not seem to vary much between October and March, most of the fruits set after October were seedless. This may be associated with the low winter night temperatures between November and March. Vardy et al. (1989) reported a similar observation in parthenocarpic tomato 'Severianin', where, under natural low-temperature conditions, only seedless fruits were produced. Low temperature has been reported to inhibit pollen germination and pollen tube growth both *in vitro* and *in vivo* (Maestro and Alvarez, 1988). This could have been the case with the T-4, where most of the pollen applied by hand to the stigma failed to grow to the ovary, but only acted as a stimulant for fruit development. As temperature rose gradually in spring, which is usually the season for hybrid-seed production and fruit-set, the numbers of seeded fruits and seeds per fruit were very minimal. Parthenocarpic fruit-set was almost completely absent. The low winter temperatures that preceded this season could have hampered the development and viability of the T-4 mutant pollen. On the other hand, the extreme day temperatures towards the end of spring (Fig. 1) increased flower abortion but hampered parthenocarpic fruit development. Low temperature has been reported to affect pollen development and viability in chickpea, a crop known to tolerate fairly low temperature conditions (Clerke and Sidique, 2004). Similarly, elevated temperatures were reported to disturb microsporogenesis (Sato et al., 2000), pollen germination, and pollen release (Peet et al., 1998). Based on the characterization of many temperature-sensitive mutants in different organisms, sensitivity to temperature is frequently a property of a mutant protein product, which, at a non-permissive temperature, loses its active conformation or the ability to interact functionally with other proteins (Zachgo et al., 1995). A protein mutant whose conformation is sensitive to night temperatures might have been induced at some stage in the T-4 mutant microsporogenesis.

Fertility restoration was always better enhanced in autumn than in spring even when the night temperature during pollination was similar in both seasons. Greenhouse plants raised from mid August and pollinated in October had the highest number of seeded fruits and seeds per fruit in the greenhouse, while pollination in spring, under similar temperature conditions, resulted in a very low fruit-set, frequency of seeded fruits (Fig. 2), and number of seeds per fruit (Fig. 3). Similarly, there were higher fruit-sets and frequencies of seeded fruits when growth chamber night temperatures were set at both 12°C

and at 24°C in autumn than when the set-up was repeated in spring (Table 2). The growth-chamber plants seem not to have acclimatized as easily to the prevailing greenhouse conditions in spring as in autumn. Further, though the low night temperature of 12°C generally resulted in a higher frequency of seeded fruits and number of seeds per fruit than the higher night temperatures of 18 or 24°C, the effect was more enhanced in autumn than in spring in the growth chamber experiments. In spring, the rate of seeded-fruit-set was, however, higher in the greenhouse (Fig. 3) than in the growth chamber (Table 2), with none at all. These results suggest that restoration of pollen fertility in the T-4 mutant is night-temperature-sensitive and that, for fertility restoration, the temperature condition during plant growth was as important as that during and after pollination. We cannot rule out possibilities of concomitant effects of internal plant factors (hormonal or genetic) working to complement or supplement the effects of night temperature on fertility restoration in the T-4 mutant. However, these need to be examined in future experiments. The T-4 mutant plants seem not to acclimatize easily to changing environmental conditions, and for fertility restoration, optimal conditions should be maintained during growth, anthesis, pollination, and fruit-set. Further, it can be deduced that the optimum night temperature for fertility restoration lies somewhere above 12°C and below 18°C. A high night temperature (24°C) resulted in a high fruit-set but most fruits were parthenocarpic.

Since there was no inhibition of pollen-tube germination in the T-4 pistil when pollinated with the normal pollen, indicating that the female organ was normal, functional, and compatible with wild-type pollen, this mutant has a potential for use as a seed parent in hybrid-seed production. The environmental modulation of fertility provides a further advantage to produce tomato hybrid-seed using the T-4 mutant by a two-line system. In theory, the male-sterile female line can be propagated by growing it under low night temperature conditions (above 12°C and below 18°C) that restore fertility. In practice, however, the temperature controlled male-sterility in the T-4 mutant may pose one potential challenge because of the residual fertility observed in the greenhouse in spring under 'sterility' conditions when hand-pollinated (Fig. 3). A loss of male-sterility and partial seasonal restoration characteristics, coupled with a concomitant regain of pollen fertility with successive backcrosses (data not shown) that is not yet fully understood, is a possible further drawback. It would have been ideal if the hand-pollination in spring resulted in either no fruit-set or parthenocarpic fruits only, and if the F_3 of every mutant progeny gave stable and consistent results. Hand-pollination with T-4 pollen in BC_1F_3 had earlier resulted in no fruit-set in spring (Masuda et al., 2000). In BC_2F_3 used in this study, residual fertility was observed, albeit at low levels. Hand-pollination implies that a very high volume of pollen lands on the stigma, increasing the chance of fertilization. In practice, hybrid-seed impurity is therefore expected to be minimal, but not ultimately absent in spring. Similar problems with residual fertility have been reported in rice TGMS lines and various methods have been proposed to circumvent them (He et al., 1999). Simulation studies on the contamination potential of T-4 pollen in competition with normal pollen during hybrid-seed production are underway.

Literature Cited

Clerke, H. J. and K. H. M. Siddique. 2004. Response of chickpea genotypes to low temperature stress during reproductive development. Field Crops Res. 90: 323–334.

Dhaliwal, M. S., S. Singh, D. S. Cheema and P. Singh. 2004. Genetic analysis of important fruit characters of tomato by involving lines possessing male-sterility genes. Acta Hort. 637: 123–132.

George, R. A. T. 1985. Vegetable seed production. Longman company Ltd, New York.

Gomez, P., M. Jamilena, J. Capel, S. Zurita, T. Angosto and R. Lozano. 1999. Stamenless, a tomato mutant with homeotic conversions in petals and stamens. Panta 209: 172–179.

He, Y. Q., J. Yang, C. G. Xu, Z. G. Zhang and Q. Zhang. 1999. Genetic bases of instability of male-sterility and fertility reversibility in photoperiod-sensitive genic male-sterile rice. Theor. Appl. Gen. 99: 683–693.

Lasa, J. N. and N. O. Bosemark. 1994. Male-sterility. p. 213–228. In: M. D. Hayward, N. O. Bosemark, I. Romagosa and M. Cerezo (eds.). Plant breeding: Principles and prospects. Chapman & Hall, London.

Maestro, M. C. and J. Alvarez. 1988. The effects of temperature on pollination and pollen tube growth in muskmelon (*Cucumis melo* L.). Sci. Hort. 36: 173–181.

Masuda, M., Y. Ma, K. Uchida and K. Kato. 1999. Characterization and genetic analysis of male-sterile mutant induced in tomato cv. First, having mature pollen stainable with acetocarmine. J. Japan. Soc. Hort. Sci. 68: 566–568.

Masuda, M., Y. Ma, K. Uchida, K. Kato and S. G. Agong. 2000. Restoration of male-sterility in seasonally dependent male-sterile mutant tomato, *Lycopersicon esculentum* cv. First. J. Japan. Soc. Hort. Sci. 69: 557–562.

Peet, M. M., S. Sato and R. G. Gardner. 1998. Comparing heat stress effects on male-fertile and male-sterile tomatoes. Plant Cell Environ. 21: 225–231.

Sato, S., M. M. Peet and J. F. Thomas. 2000. Physiological factors limit fruit-set of tomato (*Lycopersicon esculentum* Mill.) under chronic, mild heat stress. Plant Cell Environ. 23: 719–726.

Sawhney, V. K. 1983. Temperature control of male sterility in a tomato mutant. J. Hered. 74: 51–54.

Sawhney, V. K. 2004. Photoperiod-sensitive male-sterile mutant in tomato and its potential use in hybrid seed production. J. Hort. Sci. Biotech. 79: 138–141.

Singh, S. and V. K. Sawhney. 1998. Abscisic acid in a male-sterile tomato mutant and its regulation by low temperature. J. Exp. Bot. 49: 199–203.

Vardy, E., D. Lapushner, A. Genizi and J. Hewitt. 1989. Genetics of parthenocarpy in tomato under a low temperature regime: II Cultivar Severianin. Euphytica 41: 9–15.

Viraktamath, B. C. and S. S. Virmani. 2001. Expression of thermo-sensitive genic male-sterility in rice under varying temperature situations. Euphytica 122: 137–143.

Wu, D., S. Shen, H. Cui, Y. Xia and Q. Shu. 2003. A novel thermo/photoperiod-sensitive genic male-sterile (T/PGMS) rice mu-

tant with green-revertible albino leaf colour marker induced by gamma irradiation. Field Crops Res. 81: 141–147.

Zachgo, S., E. A. Silva, P. Motte, W. Tröbner, H. Saedler and Z. Schwarz-Sommer. 1995. Functional analysis of the *Antirrhinum* floral homeotic *DEFICIENS* gene *in vivo* and *in vitro* by using a temperature-sensitive mutant. Development 121: 2861–2875.