Sex Plant Reprod (2010) 23:219–224
DOI 10.1007/s00497-009-0132-1

ORIGINAL ARTICLE

Tomato pollen respiration in relation to in vitro germination and pollen tube growth under favourable and stress-inducing temperatures

I. C. Karapanos · K. A. Akoumianakis · C. M. Olympios · Harold Christopher Passam

Received: 16 September 2009 / Accepted: 21 December 2009 / Published online: 10 January 2010

Abstract Tomato pollen germination, pollen tube growth and respiratory activity were recorded during incubation in a liquid medium for 7 h over a temperature range of 15–35°C. Although the initial rate of respiration was highest at 30°C, both at 30°C and 35°C respiration decreased after the first hour of incubation due to high temperature impairment of germination and pollen tube growth. The total per cent germination of pollen over the 7-h period was maximal at 15°C whereas pollen tube length was maximal at 25°C. Although the production of CO_2 measured at hourly intervals throughout the incubation period did not correlate to a statistically significant level with either the per cent pollen germination or the length of the pollen tubes alone, nevertheless from 2 h after the start of incubation, it closely correlated with the values for germination × pollen tube length, indicating that the respiratory activity of tomato pollen at a given time is a function of both the per cent germination and the pollen tube growth. We suggest therefore that the rate of respiration might be preferable to a simple germination test for the assessment of pollen germination ability since it expresses not only the pollen germination potential but also the growth vigour of the pollen tubes. In addition, where in vitro tests are designed to assess pollen germination–temperature interactions, they should employ a long incubation period (e.g. 7 h) to permit differences in sensitivity to temperature to be observed.

Communicated by Mauro Cresti.

I. C. Karapanos · K. A. Akoumianakis · C. M. Olympios · H. C. Passam (✉)
Laboratory of Vegetable Production, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece
e-mail: passam@aua.gr

Keywords CO_2 production · Gametophyte vigour · Heat stress · *Lycopersicon esculentum* · Pollen respiration · Pollen tube metabolism

Introduction

The correlation between temperature and pollen germination is usually represented by an optimum curve. In most plant species studied, pollen germinates satisfactorily in vitro over a temperature range of 20–30°C (Johri and Vasil 1961), but germination and pollen tube growth are reduced at temperatures higher than 30°C (Vasil 1987). For tomato pollen in vitro, maximum germination was recorded at 15°C (Maisonneuve and Den Nijs 1984; Karapanos et al. 2006) but a higher temperature (22°C) was more favourable for pollen tube growth (Maisonneuve and Den Nijs 1984). However, in vivo tomato pollen germination is maximal at 25°C, and the rate of pollen tube growth increases with temperature up to a maximum of 35°C (Dempsey 1970).

Although the effect of temperature on pollen germination and pollen tube growth in vitro and in vivo is well documented, corresponding studies of the respiratory activity during these processes are few. Dickinson (1965) reported that the respiration of germinating pollen of *Lilium longiflorum* L. at 30°C showed three phases: a high rate of respiration during hydration, followed by lower respiratory activity during germination and finally a higher rate during pollen tube growth, whereas Hoekstra and Bruinsma (1975) emphasized differences in respiratory activity between bicellular and tricellular pollen species.

Tomato is one of the most important horticultural crops both for field and greenhouse production. Within the Mediterranean Basin (and elsewhere), plants are often exposed to extreme temperatures, and the frequency of this

problem is likely to increase due to the diverse climatic variations associated with the phenomenon of global warming. Sexual reproduction in plants is more sensitive to high temperatures than vegetative growth, and the adverse effects of extreme temperatures on fruit set are ascribed mainly to their effect on the male gametophyte. It has already been forecast that projected changes in climate, leading to an increase in the Earth's surface temperature of between 1.5° and 11°C by 2,100, will pose serious problems for plant reproduction (Stainforth et al. 2005; Raja-Reddy and Kakani 2007). Consequently, research into the reproductive behaviour of crop plants under such conditions of stress is of immediate concern (Karapanos et al. 2009).

Since respiration is a fundamental process of pollen germination, we examined the respiratory activity of tomato pollen in relation to in vitro germination and pollen tube growth under favourable (15–25°C) and stress-inducing (30–35°C) temperatures. The aims of this study were to examine the effect of high temperature stress on pollen respiration and to determine whether the respiration rate can be used as an indicator of germination ability.

Materials and methods

Pollen collection and handling

Pollen was collected in early December from inflorescences 1–4 of young tomato (*Lycopersicon esculentum* Mill. cv. Supermarmande) plants cultivated in the glasshouses of the Agricultural University of Athens. Flowers were collected during the early morning and pollen was extracted within 1–2 h of collection as described by Karapanos et al. (2006). The pollen was then separated into 7-mg lots, enclosed in airtight glass vials (2.8 cm diameter) containing silica gel and stored for up to one week at 4°C. Tests (not presented) showed that during this time no loss of germination occurred.

Respiration measurements

For respiration measurements, the seals on the vials were broken and 1.7 ml of a liquid substrate containing 10% (w/v) sucrose, 15.1% (w/v) polyethylene glycol (PEG-6000), 50 mg l^{-1} H_3BO_3, 300 mg l^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, 200 mg l^{-1} $MgSO_4{\cdot}7H_2O$ and 100 mg l^{-1} KNO_3 (all purchased from Merck, Darmstadt, Germany), pH 7.0, was added to the pollen. All glassware and media were autoclaved prior to use and antibiotics were not added to the incubation medium since preliminary experiments showed this to be unnecessary. The vials were incubated in an airtight, 650-ml glass container, which was placed in the dark at a predetermined temperature and shaken continually on a Unitwist 300 orbital shaker (UniEquip, Martinsried, Germany) at 115 strokes per min. The incubation temperatures employed were from 15° to 35°C at 5°C intervals (±0.5°C). An airtight inlet and outlet linked the glass container to a closed circulating air flow at a rate of 2 l min^{-1} regulated by a Li-Cor LI-670 Flow Control Unit (LI-COR, Lincoln, NE, USA), connected to a Li-Cor LI-6252 CO_2 Analyzer. Measurements were made at 30-s intervals over a total time period of 7 h, using CO_2-free air (i.e., air passed through soda lime) as a base for the reference cell. At the end of the incubation period, pollen germination was stopped with the addition of 0.5 ml acetocarmine to the medium.

Pollen germination and pollen tube elongation

A pollen grain was considered germinated when the pollen tube was equal or larger than the grain diameter (25–30 μm). This criterion was adopted since it has been used as a standard method by other authors (Stanley and Linskens 1974; Dafni and Firmage 2000; Nepi and Franchi 2000). The initiation of pollen germination during the early stages of incubation was assessed by taking 100 μl aliquots of substrate plus pollen at 10-min intervals and examining them on a microscope slide. Once pollen tube emergence had been detected, for each hour over a 7-h incubation period, 3 aliquots of substrate plus pollen at each incubation temperature were placed on microscope slides and photographs were taken at 4 sites per slide (i.e., 12 photographs per temperature treatment and per hour), each field containing 250–400 pollen grains, with the aid of an optical microscope (Olympus BX 40, Olympus Corp., Tokyo, Japan) fitted with a digital video camera with an analysis of 1024 × 768 pixels and digital compression in jpeg format. Germination was measured optically with the aid of the computer image analysis program Image Pro Plus 2.0 (Media Cybernetics Inc., Bethesda, MD, USA) for pinpointing the pollen grains. Where pollen tube growth was recorded, the length of five pollen tubes with a length greater than 30 μm per photograph was measured by tracing and converting the pixel values to μm. The experiments were repeated three times.

Statistical analysis

Pollen germination and pollen tube elongation after 7 h of incubation at each temperature were analyzed using a one way ANOVA procedure, and differences between means were detected using a least significant difference test (LSD) ($P = 0.05$). The initial (first 30 min of incubation) and final (6.5–7.0 h after incubation) rates of respiration were calculated using linear regression of the CO_2 evolved

(μl CO_2 mg^{-1}) in relation to the incubation time. Correlation of the total per cent germination, pollen tube length and the value for germination × pollen tube length with the total volume of CO_2 was recorded at hourly intervals over the 7-h incubation period, as well as with the initial and final rates of respiration (Statgraphics 5.1, Statistical Graphics Corp., Herndon VA, USA). Significance of correlation coefficients was evaluated according the *T* test at the 5% significance level.

Results and Discussion

The onset of germination of tomato pollen during in vitro incubation was observed after 20–30 min at 20–30°C, and after 45–50 min at 15°C. However, despite the delay in initial germination, pollen incubated at 15°C exhibited the highest per cent germination from the second hour of incubation up to the end of the 7-h incubation period. This was due to the relatively greater decrease in the rate of germination of pollen at the higher temperatures (notably 30°C) in comparison with that at 15°C (Fig. 1a). Similarly, pollen tube growth was first observed at 20–30°C, followed later by 15°C. For the first 3 h of incubation, pollen tube growth was highest at 30°C, but by the end of the incubation period, it was highest at 25°C (Fig. 1b). At 35°C, both pollen germination and pollen tube growth were very low. By contrast, in the pollen of *Nicotiana tabacum* L. (like tomato, a member of the Solanaceae) the combination of high humidity and heat (>38°C) resulted in vigour impairment (reduced pollen tube growth rate), but not viability (Shivanna and Cresti 1989; Shivanna et al. 1991).

Tomato pollen germination is susceptible to adverse climatic conditions, especially high and low temperatures (Charles and Harris 1972; Picken 1984; Song et al. 1999). However, it is clear from the data of Fig. 1a and b that if the susceptibility of a tomato cultivar to temperature is to be evaluated by a simple pollen germination test, the result of the test will depend on the time of incubation. A short incubation time (<3 h) may not truly indicate the total germination potential of the pollen lot, or the final length of the pollen tube, and clearly a longer incubation time (7 h) is required.

The respiratory activity of tomato pollen during in vitro germination and pollen tube growth related to the temperature and duration of incubation (Fig. 2). Initially (i.e. up to 30 min.), the respiratory rate was highest in pollen incubated at 25–35°C and lowest at 15°C. After this time, however, the respiratory activity of pollen at 30–35°C decreased in comparison with that at the other temperatures, so that by the end of incubation, the highest amount of CO_2 produced was that by pollen incubated at 25°C followed by that at 20 and 15°C.

The initial respiration rates in the present experiment (Table 1) were 6–10 times higher than those of other species of bicellular pollen, including that of *Lycopersicon*

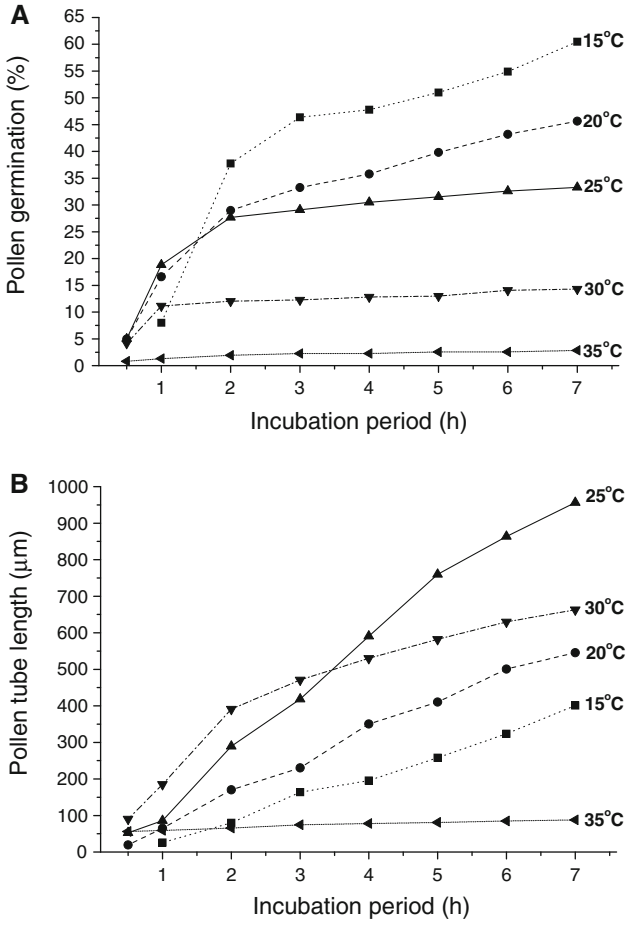

Fig. 1 The course of in vitro tomato pollen germination (**a**) and pollen tube growth (**b**) in relation to temperature during 7 h of incubation

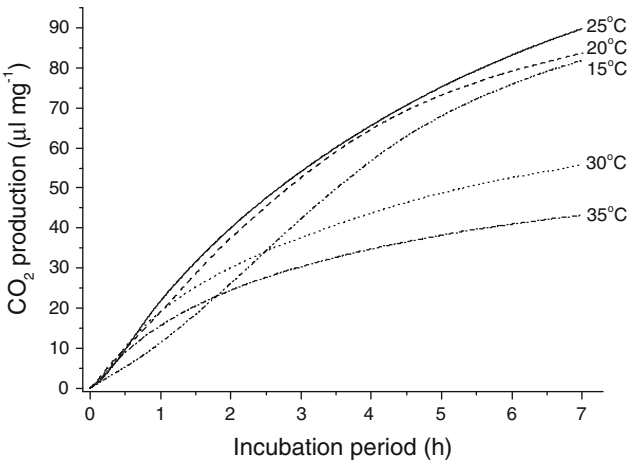

Fig. 2 The respiratory activity of tomato pollen in vitro in relation to temperature during 7 h of incubation. Each *curve* represents the mean of 3 measurements for each incubation temperature

Table 1 The effect of temperature on the initial (0- to 0.5-h incubation) and final (6.5- to 7.0-h incubation) respiration rates, and the total per cent pollen germination, pollen tube length, germination × pollen tube length and total CO_2 production after 7-h incubation

Incubation temperature (°C)	Pollen germination (%)	Mean pollen length (μm)	Germination (%) × pollen tube length	Total CO_2 production (μl mg^{-1})	Initial respiration rate (μl mg^{-1} h^{-1})	Final respiration rate (μl mg^{-1} h^{-1})
15	60.46 ± 6.30 a*	401.2 ± 26.1 d*	24,256	43.18	10.58	5.62
20	45.65 ± 6.92 b	545.4 ± 28.5 c	24,898	55.75	20.23	4.25
25	33.28 ± 7.02 c	956.1 ± 97.0 a	31,819	89.85	19.87	6.30
30	14.30 ± 3.99 d	663.2 ± 69.2 b	9,484	83.66	21.02	3.20
35	2.84 ± 2.61 e	87.8 ± 16.1 e	249	81.85	18.68	1.99

* Means in columns for each pollen lot followed by a different letter are statistically different according to the least significant difference test ($P = 0.05$)

peruvianum Mill. (Hoekstra and Bruinsma 1975). However, in these species, pollen was incubated in an atmosphere of 97% r.h. (Hoekstra and Bruinsma 1975) and not in a liquid medium as in the present experiments. The pattern of the respiratory activity of tomato pollen under moderately high temperatures (30°C) shown in the present study is in agreement with previous results from our laboratory on pollen lots with high, medium and low respiratory activity (Karapanos et al. 2009), whereas Karipidis et al. (2007) found that both fresh tomato pollen and that stored in liquid nitrogen showed a linear consumption of O_2 for 6-h incubation at 16°C. Differences in the susceptibility of pollen respiration to relatively high temperatures have been reported for other species. For example, when the tricellular pollen of *Aster tripolium* L. was incubated in an atmosphere with a high relative humidity at 30 and 35°C, respiratory activity fell off after 10 h, whereas that of the bicellular pollen of *Corylus avellana* L. was not affected under these conditions even after 35 h (Hoekstra and Bruinsma 1975). In the present experiments, tomato pollen showed a greater heat susceptibility than that of *Lilium longiflorum* (Dickinson 1965) and *Pinus mugo* Turra (Nygaard 1969).

Although the total per cent pollen germination was maximal at 15°C, pollen tube growth was highest at 25°C (Table 1). It appeared that at higher temperatures (30–35°C) there was an initially high rate of pollen metabolism, as reported for pollen of *Lilium longiflorum* (Dickinson 1965) and indicated by the initially high rate of respiration (Table 1), but after about 1 h, this declined due to the progressive cessation of germination and pollen tube growth (Fig. 1a, b). Moreover, at 35°C, pollen tubes remained very short (Fig. 1b; Table 1) and exhibited a tendency to swell and burst.

In other experiments, Karapanos et al. (2009) observed that during incubation at 30°C, tomato pollen showed a high initial rate of respiration, which led to exhaustion of the endogenous respiratory substrates, subsequent ageing and a loss of mitochondrial activity. Moreover, in agreement with the data of the present study, Song et al. (2002) reported that tomato pollen incubated at temperatures higher than 34°C ceased to germinate earlier than those incubated at lower temperatures. Although our experiments and those of Song et al. (2002) show that pollen tube growth stopped within 2 h of incubation at temperatures equal to or higher than 35°C, appreciable respiratory activity may be detected for some time after the cessation of germination, as indicated here by the rates of respiration at 30 and 35°C (Fig. 2) and by the data of Hoekstra and Bruinsma (1975) for tricellular pollen of the Compositae.

In other species (*Antirrhinum*, legumes), high temperatures have been reported to cause pollen tube tip swelling and bursting, apparently due to the enhanced inflexibility of the pollen tube wall (Vasil 1987). By contrast, although tomato pollen germination in vivo is reduced at 30–35°C, the rate of pollen tube growth within the style is apparently enhanced over the same temperature range (Dempsey 1970). Similarly, pollen grains of *Nicotiana tabacum* L. subjected to high relative humidity and heat stress (45°C) failed to germinate in vitro but did germinate in vivo (Shivanna et al. 1991). These observations possibly indicate a temperature-buffering effect of the style tissues during pollen tube extension, or a lack of some factors required for the germination of heat-stressed pollen under in vitro conditions, which are readily available from the stigma (Shivanna et al. 1991).

Although Binder and Ballantyne (1975) found a strong correlation between male fertility and respiration during in vitro germination of pollen of the gymnosperm *Pseudotsuga menziesii* (Mirb.) Franco, there is a paucity of information concerning the respiratory activity of pollen (especially that of angiosperms) in relation to germination and pollen tube growth. From the present experiments, it is shown that neither the initial nor the final rate of respiration correlated with pollen germination or pollen tube length alone. However, the final rate of respiration and the total amount of CO_2 evolved at each hour between 2–7 h of

Table 2 Correlation coefficients (r) for the initial and final rates of respiration and the total production of CO_2 over a 7-h incubation period in relation to pollen germination, pollen tube length and pollen germination × tube length

Correlation	Germination	Pollen tube length	Germination × tube length
Total CO_2 production (hours)			
1	0.705	0.565	0.799
2	0.412	0.495	0.899*
3	0.665	0.403	0.901*
4	0.823	0.489	0.943*
5	0.868	0.537	0.946*
6	0.868	0.601	0.976*
7	0.843	0.658	0.995*
Initial rate of respiration	−0.633	0.344	−0.195
Final rate of respiration	0.774	0.693	0.951*

* Statistically significant correlation at $P = 0.05$

incubation correlated significantly with the value for germination × pollen tube length, which represents a function of both per cent germination and pollen tube elongation vigour (Table 2). This correlation indicates that the measurement of respiratory activity 2 h or more after the start of incubation within the present temperature range (15–35°C) may serve as a reliable and non-destructive method of determining the vigour of tomato pollen during in vitro germination rather than the laborious method of repeatedly measuring the per cent germination and pollen tube growth.

The tomato pollen used in the present experiments was produced under favourable climatic conditions (25–28°C/15°C day/night temperatures) and therefore had a reasonably high per cent germination (>60% at 15°C) and correspondingly high respiratory activity (Table 1; Fig. 2). According to Hoekstra and Bruinsma (1975), however, pollen with a low per cent germination exhibits reduced respiratory activity and therefore produces relatively low amounts of CO_2. In a preliminary experiment with tomato, using pollen formed under conditions of low light intensity and low night temperatures (23–27°C/8–12°C day/night temperatures), in vitro germination was only 16.5 ± 5.5% and pollen tube growth was slow (912.3 ± 56.4 μm over a 7-h incubation period) at 15°C. Although in this case the rate of CO_2 production was also low (28 μl mg^{-1} over the same period), nevertheless the total CO_2 production and the final rate of respiration after 7-h incubation correlated significantly with the value for germination × pollen tube length. Moreover, the effect of high temperature on respiration, germination and pollen tube growth followed a similar pattern as here (Karapanos 2007).

In view of the importance of tomato as a horticultural crop in a world threatened by increased global warming and consequent temperature stress, there is an urgent need to elucidate stress effects on production and ways of screening for stress tolerance, since enough genetic variability is still present within many crops to enable the selection of new cultivars (Hedhly et al. 2009). Although temperature stress primarily affects tomato fruit set by impairing the fertility (Robinson et al. 1965; El Ahmadi and Stevens 1979; Sato et al. 2006) and performance (Dempsey 1970; Charles and Harris 1972; Shelby et al. 1978; Song et al. 1999, 2002; Sato and Peet 2005) of the male gametophyte, there are currently very few data available on tomato pollen metabolism during germination under such unfavourable conditions. The present study demonstrates that the respiratory activity of germinating tomato pollen in vitro under elevated (30°C) and high (35°C) temperatures decreases over time and relates to the negative effect of heat stress on pollen germination and tube growth. In addition, to date, no reliable in vitro method for assessing tomato pollen vigour exists, and histochemical methods based on the reduction in tetrazolium bromide have also not proved successful (Hoekstra and Bruinsma 1975). From our results, we propose first that in vitro pollen germination tests must be carried out over a relatively long (7 h) incubation period so as to permit adverse temperature effects over more of the progamic phase to be seen. Secondly, we propose that the final respiration rate (after a 7-h incubation period) or the amount of CO_2 released during incubation (between 2 and 7 h) may provide a better indication of pollen performance than the measurement of per cent germination alone since it correlates with germination × pollen tube length, which is an indicator not only of germination potential but also pollen vigour.

References

Binder WD, Ballantyne DJ (1975) The respiration and fertility of *Pseudotsuga menziesii* (Douglas fir) pollen. Can J Bot 53:819–823

Charles WB, Harris RE (1972) Tomato fruit-set at high and low temperatures. Can J Plant Sci 52:497–506

Dafni A, Firmage D (2000) Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Syst Evol 222:113–132

Dempsey WH (1970) Effects of temperature on pollen germination and tube growth. Tomato Gen Coop Rep 20:15–16

Dickinson DB (1965) Germination of lily pollen: Respiration and tube growth. Science 150:1818–1819

El Ahmadi AB, Stevens M (1979) Reproductive responses of heat-tolerant tomatoes to high temperatures. J Amer Soc Hort Sci 104:686–691

Hedhly A, Hormaza JI, Herrero M (2009) Global warming and sexual plant reproduction. Trends Pl Sci 14:30–36

Hoekstra FA, Bruinsma J (1975) Respiration and vitality of binucleate and trinucleate pollen. Physiol Plant 34:221–225

Johri BM, Vasil IK (1961) Physiology of pollen. Bot Rev 27:325–381

Karapanos IC (2007) Study on factors that influence microsporogenesis of greenhouse tomato (*Lycopersicon esculentum* Mill.) Ph D Thesis, Agricultural University of Athens, pp 221

Karapanos IC, Fasseas K, Olympios C, Passam HC (2006) Factors affecting the efficacy of agar-based substrates for the study of tomato pollen germination. J Hort Sci Biotech 81:631–638

Karapanos IC, Akoumianakis KA, Olympios CM, Passam HC (2009) The effect of substrate, ADP and uncoupler on the respiration of tomato pollen during incubation in vitro at moderately high temperature. Sex Plant Reprod 22:133–140

Karipidis C, Olympios C, Passam HC, Savvas D (2007) Effect of moisture content of tomato pollen stored cryogenically on in vitro germination, fecundity and respiration during pollen tube growth. J Hort Sci Biotech 82:29–34

Maisonneuve B, Den Nijs APM (1984) In vitro pollen germination and tube growth of tomato (*Lycopersicon esculentum* Mill.) and its relation with plant growth. Euphytica 33:833–840

Nepi M, Franchi GG (2000) Cytochemistry of mature angiosperm pollen. Plant Syst Evol 222:45–62

Nygaard P (1969) Studies on the germination of pine pollen (*Pinus mugo*) in vitro. I. Growth conditions and effects of pH and temperature on germination. Physiol Plant 22:338–346

Picken AJF (1984) A review of pollination and fruit set in the tomato (*Lycopersicon esculentum* Mill). J Hort Sci 59:1–13

Raja-Reddy K, Kakani VG (2007) Screening *Capsicum* species of different origins for high temperature tolerance by in vitro pollen germination and pollen tube length. Sci Hort 112:130–135

Robinson RW, Shannon S, Mishanec W (1965) Low temperature influences pollen production and fruit set of tomatoes. Farm Res 31:13–15

Sato S, Peet MM (2005) Effects of moderately elevated temperature stress on the timing of pollen release and its germination in tomato (*Lycopersicon esculentum* Mill.). J Hort Sci Biotech 80:23–28

Sato S, Kamiyama M, Iwata T, Makita N, Furukawa H, Ikeda H (2006) Moderate increase of mean daily temperature adversely affects fruit set of *Lycopersicon esculentum* by disrupting specific physiological processes in male reproductive development. Ann Bot 97:731–738

Shelby RA, Greenleaf WH, Peterson CM (1978) Comparative floral fertility in heat tolerant and heat sensitive tomatoes. J Amer Soc Hort Sci 103:778–780

Shivanna KR, Cresti M (1989) Effects of high humidity and temperature stress on pollen membrane integrity and pollen vigour in *Nicotiana tabacum*. Sex Plant Reprod 2:137–141

Shivanna KR, Linskens HF, Cresti M (1991) Responses of tobacco pollen to high humidity and heat stress: viability and germinability in vitro and in vivo. Sex Plant Reprod 4:104–109

Song J, Nada K, Tachibana S (1999) Ameliorative effect of polyamines on the high temperature inhibition of in vitro pollen germination in tomato (*Lycopersicon esculentum* Mill). Sci Hort 80:203–212

Song J, Nada K, Tachibana S (2002) Suppression of S-adenosylmethionine decarboxylase activity is a major cause for high temperature inhibition of pollen germination and tube growth in tomato (*Lycopersicon esculentum* Mill.). Plant Cell Physiol 43:619–627

Stainforth D, Aina T, Christensen C, Collins M, Faull N, Frame D, Kettleborough J, Knight S, Martin A, Murphy JM, Piani C, Sexton D, Smith LA, Spicer S, Thorpe A, Allen M (2005) Uncertainty in predictions of the climate response to rising levels of greenhouse gases. Nature 433:403–406

Stanley RG, Linskens HF (1974) Pollen: biology, biochemistry, management. Springer, Berlin

Vasil IK (1987) Physiology and culture of pollen. Int Rev Cytol 107:127–174