Pollen performance in mango (*Mangifera indica* L., Anacardiaceae):

Andromonoecy and effect of temperature.

V. Pérez^{a, c}, M. Herrero^b and J.I. Hormaza^{a, *}

^aSubtropical Fruit Crops Department, Instituto de Hortofruticultura Subtropical y Mediterránea La Mayora (IHSM La Mayora-CSIC-UMA), 29750 Algarrobo-Costa, Málaga, Spain

^bPomology Department, Estación Experimental de Aula-Dei-CSIC, Avda Montañana 1005, 50059 Zaragoza, Spain

^cUnidad técnica del IPNA-CSIC. Laboratorio de Agrobiología Juan José Bravo Rodríguez (Cabildo Insular de La Palma), 38700 La Palma, Canary Islands, Spain

*Corresponding autor: J.I. Hormaza. Tel:(+34) 952 54 89 90. Fax:(+34) 952 55 26 77. E-mail address ihormaza@eelm.csic.es

Abstract

Previous studies in mango (*Mangifera indica*) had reported how low pollen viability can reduce yields. In this work, an improved in vitro pollen germination method for mango is used to assess germination range at different environmental conditions. Since mango is an andromonoecious species, pollen performance was studied in hermaphrodite and male flowers. The results showed a similar number of pollen grains per anther and pollen viability and germination between the two mango flower morphs. Pollen germination was also studied at different temperature conditions, and results showed that percentage of

pollen germination improved along the flowering season with increasing temperatures. Pollen germination at controlled temperatures slowed down at low temperatures. Differences were found between genotypes in pollen behavior at different temperatures opening the possibility of selecting mango genotypes with pollen more adapted to cold temperatures, especially for subtropical conditions.

Keywords

Pollen germination, mango, *Mangifera indica*, andromonoecy, temperature.

1. Introduction

Mango (*Mangifera indica* L., $2n = 40$), a woody perennial species originating from South Asia (Mukherjee, 1972), belongs to the Anacardiaceae in the Sapindales, an order with mainly a tropical or subtropical distribution, with a few species in temperate regions. The family contains over 800 species classified into 70 genera (Bompard, 2009) and includes other cultivated fruit tree species such as pistachio (*Pistacia vera* L.), cashew (*Anacardium occidentale* L.), ambarella (*Spondias dulcis* Forst.), or yellow and purple mombins (*Spondias mombin* L. and *Spondias purpurea* L.). The genus *Mangifera* contains about 70 species mostly restricted to tropical Asia (Kostermans and Bompard, 1993). Total world mango production has reached about 50 million tons in 2017 (FAOSTAT, 2019) making mango one of the five most important fruit crop worldwide (together with bananas, oranges, grapes and apples). A few countries (India, China, Kenya, Thailand, Indonesia, Mexico and Pakistan) account for over 75% of mango production worldwide (FAOSTAT, 2019).

In addition to the traditional production of mango under tropical conditions, there is an increasing interest to expand the range of cultivation of this crop to regions with subtropical climates (Galán-Saúco, 2009), with clear different environmental conditions than those from the area of origin of this species. The mean optimal temperature for mango ranges between 24-30°C (Galán-Saúco, 2009), and this species has proven to be more sensitive to cold than to warm temperatures. Mango trees can tolerate for short periods temperatures close to 50°C (Mukherjee, 1953), but trees are usually damaged after a few hours below 0°C (Campbell et al., 1977). However, when temperature is lower than 15°C, a number of problems have been reported, such as the inhibition of vegetative development (Davenport, 2003; Galán-Saúco, 2009).

Nevertheless, probably, the most sensitive process to relatively low temperatures in mango seems to be the reproductive process with a decrease in pollen viability (de Wet et al., 1989; Issarakraisila et al., 1992; Issarakraisila and Considine, 1994), pollen germination (Dag et al., 2000; Sukhvibul et al., 2000), fertilization failure (Sukhvibul et al., 2000; Huang et al., 2010), and embryo abortion (Lakshminarayana and Aguilar, 1975). Indeed, in plants in general, the reproductive phase is highly sensitive to temperature stresses (Hedhly et al., 2005b; 2009; Thakur et al., 2010; Zinn et al., 2010; Hedhly, 2011) and temperature is a clear limiting factor for the geographical expansion of most plant species and crops (Saxe et al., 2001; Hall, 2010). Temperature plays an important role in the development of male and female reproductive structures and processes (Young et al., 2004; Hedhly et al., 2005a; 2009; Hedhly, 2011) especially during meiosis, which is the most sensitive phase (Young et al., 2004; Hedhly, 2011). After gamete formation, during the progamic phase, which takes place from pollination to fertilization (Linskens, 1975), temperature affects pollen germination and stigmatic receptivity (Hedhly et al., 2003), pollen tube kinetics (Lewis, 1942; Hedhly et al., 2005a;

2005b) and the effective pollination period (Sanzol and Herrero, 2001; Hedhly, 2011).

Moreover, temperature also influences additional factors involved in reproductive success, such as pollinator activity, which is necessary for an adequate pollination in entomophilous species (Arroyo et al., 1985).

Mango is an andromonoecious species (Maheshwari, 1934), with hermaphrodite and male flowers in the same inflorescence (Bertin, 1982) with a variable proportion of male and hermaphrodite flowers depending on the genotype and temperature (Singh, 1954; Singh et al., 1966; Abourayya et al., 2011). Andromonoecy occurs in a reduced proportion (1.7%) of angiosperms, in about 4000 species, since most angiosperms have hermaphrodite flowers, or present a dioecious reproductive system (Yampolsky and Yampolsky, 1922). The presence of different floral sexual morphs in the same tree has been reported in different tropical fruit trees (Ruiz Zapata and Arroyo, 1978), as cashew, litchi or longan (Wunnachit and Sedgley, 1992; Davenport et al., 2005, Pham et al., 2015), and in other species of agronomic interest such as olive (Cuevas and Polito, 2004; Rosati et al., 2011), caper (Zhang and Tan, 2009), pomegranate (Wetzstein et al., 2011), or melon (Poole and Grimball, 1939; Noguera et al., 2005). In mango, hermaphrodite and male flowers have one single functional anther, sometimes two depending on the genotype, per flower that produce pollen. But little is known on pollen performance in these different flower morphs.

An increasing concern for temperature increase in different areas of the world due to global warming (Hedhly et al., 2009) is prompting research to select temperature tolerant genotypes that can yield crops in areas with different conditions from their area of origin (Hall, 2010). These areas also provide an excellent opportunity to evaluate the limits of the species regarding temperature and bring out genotypic differences between cultivars, reflecting their adaptability to limiting environmental conditions. Indeed, the South of

Spain at latitude 36° N is the farthest region from the equator with a significant commercial mango production in the world (Mukherjee, 1997; Calatrava and Sayadi, 2005). Information from this region situated in the environmental limits of mango cultivation can also help to improve crop establishment in other regions with temperate climates. The aim of this work is to evaluate pollen performance under these limiting conditions. This main goal is developed along three steps: a) to optimize an in vitro mango pollen germination medium, b) to analyze pollen performance in hermaphrodite and male flowers, and c) to understand the effect of temperature in pollen germination.

2. Material and methods

2.1. Plant material

Mango trees of the cultivars ‘Kensington’, ‘Ataulfo’, ‘Osteen’, ‘Kent’ and ‘Keitt’ located in a mango cultivar and rootstock collection at IHSM-La Mayora in Algarrobo-Costa (Málaga, Spain) (36° 45’ N; 4° 2’ W; 35 m above sea level) were used in this work. As a first step, flowers from ‘Kensington’, ‘Osteen’ and ‘Kent’ were used to optimize an in vitro pollen germination medium for mango.

2.2. Optimization of an in vitro pollen germination medium

Fresh flowers collected in the morning in the field were kept in Petri dishes placed on top of wet filter paper in a tray covered with an aluminum foil, as a humid chamber, in order to keep them under a high relative humidity and maintain flower turgidity. Since each

mango flower of these varieties has only one functional stamen, pollen from 25 just dehiscid anthers, from 25 flowers, were placed in a 35 mm diameter Petri dish with 2 mL of liquid germination medium. Pollen was removed from the anthers and distributed in the germination medium using a disposable Pasteur pipette by suction-expulsion movements.

We started the experiments with a germination medium consisting of 20% sucrose, 15% PEG 4000, 0.2 gL⁻¹ MgSO₄, 0.1 gL⁻¹ KNO₃, 0.1 gL⁻¹ H₃BO₃, 0.7 gL⁻¹ Ca(NO₃)₂ (Sukhvibul et al., 1999, excluding N-morpholino ethanesulfonic acid). However, since we obtained a high percentage of burst pollen grains, we tried different concentrations of polyethylene glycol (PEG) and sucrose, both involved in the regulation of osmotic process and prevention of pollen grain bursting. Thus, following Alcaraz et al. (2011) we modified the concentrations of PEG and sucrose evaluating the effect of PEG 8000 at 15%, 20%, 23% and 25% and sucrose at 10%, 15% and 20%.

Following incubation for 24 hours at 20°C, pollen germination was observed using a Leica DML microscope. For each different concentrations of sucrose and PEG, two Petri dishes were analyzed, scoring germinated pollen grains in each one from at least 200 pollen grains. Pollen was considered germinated when the pollen tube length was equal or longer than the diameter of the pollen grain.

2.3. Pollen counting in hermaphrodite and male flowers

Along the flowering season, anthers of hermaphrodite and male flowers from ‘Kensington’, ‘Ataulfo’, ‘Osteen’, ‘Kent’ and ‘Keitt’ (Figure 1) were fixed in ethanol: acetic acid (3:1). Anthers were collected at anthesis, the day of flower opening, and prior to anther dehiscence. After that, 20 fixed anthers were placed in a 1.5mL tube with 100µl

distilled water that contain a small drop of detergent. The detergent was used to avoid the clustering of the pollen grains in the hemocytometre. The anthers were pressed gently to the tube walls with a stick to release the pollen grains. Then, a drop of the solution was placed in a hemocytometre and covered with a coverslip (modified of Niesenbaum, 1992). Pollen grains of the four big squares of the hemocytometre diagonal were counted with between 10 to 17 replicates for each genotype and flower type.

2.4. Pollen viability and germination in hermaphrodite and male flowers.

Pollen viability and germination was analyzed in ‘Kensington’, ‘Ataulfo’, ‘Osteen’, ‘Kent’ and ‘Keitt’ in both flower morphs: hermaphrodite and male flowers. Pollen viability and germination were evaluated using pollen from freshly opened flowers at anthesis, with pollen pre-hydrated in a humid chamber.

To assess pollen viability, fresh pollen from 25 anthers of each flower type were placed in a 35mm diameter Petri dish with the optimized in vitro pollen germination medium and a drop of fluorescein diacetate (FDA) in acetone (2mg/mL) (Heslop-Harrison and Heslop-Harrison, 1970). After 15 minutes in the dark, viable pollen grains were counted in a Leica DN LB2 epifluorescent microscope (Leica, Wetzlar, Germany). Viable pollen fluoresce bright yellow-green and no viable pollen show only exine autofluorescence. Two replicates were performed in each flower morph and genotype, counting a minimum of 200 pollen grains per dish.

To evaluate pollen germination, fresh and pre-hydrated pollen was germinated in the optimized medium. For each floral morph and cultivar, pollen from 25 flowers was used. Small Petri dishes were incubated for 24 hours under each environmental condition. Pollen germination was observed using an optical Leica DML microscope, counting a

minimum of 200 pollen grains per Petri dish. Pollen grains were recorded as germinated when pollen tube length was equal or longer than the diameter of the pollen grain.

2.5. Temperature effect on in vitro pollen germination

The effect of temperature on pollen germination was studied in three cultivars ‘Kensington’, ‘Osteen’ and ‘Kent’. Experiments were performed along the flowering season that, under the environmental conditions of Southern Spain, takes place from April to June.

Flowers were collected in the field at anthesis, but prior to anther dehiscence, from 9:00 to 11:00 a.m., and were brought to the laboratory. The effect of temperature on in vitro pollen germination was evaluated following the optimized germination medium developed in this work.

We performed two experiments: one to evaluate the effect of temperature in the field during the flowering season and another to evaluate pollen germination at controlled temperatures (10°C, 20°C and 30°C) in growth chambers.

For the first experiment, flowers of ‘Kensington’ and ‘Osteen’ were collected daily for four weeks during the flowering season, May to June. To relate pollen germination with temperature during pollen development, a data logger (HOBO pro v2 $\pm$ 0.2°C) was installed in the field and set up to control temperature every 30 minutes. Pollen from freshly opened flowers was incubated for 24 hours.

For the second experiment, the effect of temperature on pollen germination was studied under controlled temperature chambers at 10°C, 20°C and 30°C, and at room temperature (25°C). The temperature treatments were performed in Aralab FitoClima S600 (Rio de

Mouro, Portugal) growth chambers. This was evaluated with pollen from ‘Osteen’, ‘Kensington’ and ‘Kent’. For each cultivar two Petri dishes were incubated for 24 hours at each temperature.

2.6. Statistical analysis

To test significant differences between cultivars (number of pollen grains, pollen viability, pollen germination, pollen germination in the field during the flowering season and pollen germination at controlled temperatures) an ANOVA test was applied and post hoc analyses were done using the Turkey HSD test. Differences between flower types (in the number pollen grains, pollen viability and pollen germination) were compared applying a General Lineal Model (GLM). In all cases, percentages of germinated pollen grains were also arcsine-transformed prior to analysis. All statistical analyses were performed using SPSS 19.00 (IBM Cop., Armonk, NY).

3. Results

3.1. Optimization of an in vitro pollen germination medium for mango

Pollen germination of ‘Kensington’, ‘Osteen’ and ‘Kent’ was assessed in 12 germination media with different concentrations of sucrose and polyethylene glycol (PEG): 10%, 15% and 20% sucrose; and 15%, 20%, 23% and 25% PEG. The results showed differences in pollen germination between the cultivars studied (Figure 2, a). The best pollen germination for the three genotypes was obtained with a concentration of 15% sucrose and 23% PEG. Germination failure was related to pollen bursting. Thus, burst pollen grains were higher in the germination media with lower sucrose and PEG concentrations,

and decreased as PEG and sucrose concentrations increased (Figure 2, b). Consequently, the best pollen germination was obtained with a germination medium consisting of 15% sucrose, 23% PEG 8000, $0.1 \text{ g l}^{-1} \text{ KNO}_3$, $0.2 \text{ g l}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, $0.1 \text{ g l}^{-1} \text{ H}_3\text{BO}_3$, $0.7 \text{ g l}^{-1} \text{ Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$.

3.2. Pollen performance in hermaphrodite and male flowers

To evaluate differences in pollen performance between hermaphrodite and male flowers, the number of pollen grains, pollen viability and pollen germination were compared in these two types of flowers (Figure 1).

The number of pollen grains in the single anther of different flowers was counted. A large variability was found between flowers of each cultivar. ‘Ataulfo’ was the cultivar with the lowest number of pollen grains per anther (464 ± 32 for hermaphrodite and 379 ± 19 for male flowers), and ‘Keitt’ the cultivar with the highest number of pollen grains per anther (1267 ± 109 for hermaphrodite and 981 ± 46 for male flowers). Intermediate values were registered in ‘Kent’ (898 ± 100 for hermaphrodite and 825 ± 45 for male flowers), ‘Kensington’ (585 ± 39 for hermaphrodite and 651 ± 28 for male flowers) and ‘Osteen’ (626 ± 49 for hermaphrodite and 578 ± 41 for male flowers) (Figure 3).

Overall, the number of pollen grains per anther was slightly higher in hermaphrodite than in male flowers, but these differences were only significant in ‘Ataulfo’ ($P < 0.05$) and ‘Keitt’ ($P < 0.01$).

Pollen viability and germination were also compared in hermaphrodite and male flowers in the five cultivars: ‘Kensington’, ‘Ataulfo’, ‘Osteen’, ‘Kent’ and ‘Keitt’. Pollen viability (Figure 4, a) was higher than 85% for all the cultivars with slight differences between cultivars ($P < 0.001$). Slight differences were also observed in pollen viability

between male and hermaphrodite flowers although they were only significant for ‘Ataulfo’ (85% in male and 93% in hermaphrodite flowers, $P < 0.001$).

Pollen germination (Figure 4, b) showed differences between genotypes ($P < 0.001$).

‘Keitt’ had the highest percentage of germination (81%), followed by ‘Kensington’ (70%), ‘Osteen’ (53%), ‘Kent’ (53%), and ‘Ataulfo’ (48%). No significant differences were observed for the interaction between genotype and flower type.

3.3. *Changes in pollen germination along the flowering season*

Weekly pollen germination was highly variable, ranging from 41% to 68% for ‘Kensington’, and from 38% to 58% for ‘Osteen’. While ‘Kensington’ showed overall higher pollen germination (60%) than ‘Osteen’ (46%), in both cultivars pollen germination and temperature followed a similar pattern (Figure 5). Pollen germination was lower during the first week of the flowering season, with minimum temperatures close to 10°C. In the second week, with higher minimum temperatures, pollen germination increased significantly for ‘Kensington’, but not for ‘Osteen’. In the third week, with maximum temperatures above 25°C, pollen germination increased significantly also for ‘Osteen’. In addition, the highest germination rates were obtained for both cultivars in the fourth and final flowering week, with minimum temperatures over 15°C. Significant differences were found for both genotypes between weeks, ‘Kensington’ ($P < 0.001$) and ‘Osteen’ ($P < 0.001$).

3.4. *The effect of controlled temperatures in pollen germination*

The three cultivars showed differences in pollen germination at room temperature (25°C): ‘Kensington’ (67%), ‘Osteen’ (45%) and ‘Kent’ (58%). Pollen germination at different controlled temperatures showed that the three cultivars performed well at 20°C and 30°C (Figure 6), although at 30°C pollen germination was slightly higher and pollen tubes were longer. Pollen germination was significantly constrained at 10°C for the three cultivars compared to the other temperatures (‘Kensington’, $P < 0.01$; ‘Osteen’, $P < 0.01$; ‘Kent’, $P < 0.01$). However, differences were observed among cultivars. While ‘Osteen’ and ‘Kent’ had a very poor performance at 10°C, ‘Kensington’ was the cultivar with the highest germination percentage at that temperature, although with poor pollen tube growth. This cultivar also showed higher pollen germination at all temperatures compared to ‘Osteen’ and ‘Kent’.

4. Discussion

The optimization of a germination medium for mango allowed the evaluation of pollen germination of different mango genotypes and the effect of temperature on pollen germination.

4.1. Optimization of the in vitro pollen germination medium for mango

Different in vitro pollen germination media were tested in mango after poor results with some of the germination media reported before (Sukhvibul et al., 1999; 2000, Khan and Perveen, 2009, and Huang et al., 2010). Since in most entomophilous species, such as mango, pollen grains are usually bound together to optimize insect transport (Pacini and Franchi, 1993), we used a liquid medium to facilitate the separation of the individual

pollen grains. The medium was optimized by modifying sucrose and polyethylene glycol concentrations to prevent pollen grain bursting. Both sucrose and polyethylene glycol are involved in the osmotic regulation (Visser, 1955; Shivanna and Sawhney, 1995), playing a role in pollen grain bursting (Vasil, 1987); in addition, sucrose also plays an important nutritive role for pollen tube growth. We started as a control with the germination medium previously used in mango (Sukhvibul et al., 1999; 2000) and modified the PEG and sucrose concentrations following the results of Alcaraz et al. (2011) in avocado. The modifications performed resulted in higher pollen germination percentages with reduced pollen bursting, and the germination medium developed was suitable for pollen germination of the three cultivars of mango evaluated.

4.2. Pollen and andromonoecy in mango.

In mango, both hermaphrodite and male flowers have anthers with the capacity to produce pollen, although only hermaphrodite flowers can produce fruits. In order to study if differences are present in pollen from the two types of flowers, the number of pollen grains per anther, pollen viability and germination were analyzed in five mango cultivars. Small differences were observed between the pollen from male or hermaphrodite flowers in the cultivars analyzed. Our results showed that the number of pollen grains in hermaphrodite flowers was only slightly higher in all the cultivars analyzed except ‘Kensington’, which was the only genotype with slightly higher number of pollen grains in male flowers. However, significant differences were only obtained for two genotypes of the five analyzed (‘Ataulfo’ and ‘Kent’). A similar study carried out in ‘Ataulfo’ in seven localities in Mexico showed a significantly higher number of pollen grains in hermaphrodite flowers (average 913 pollen grains/anther) than in male flowers (average

643 pollen grains/anther) (Gherke-Vélez et al., 2011). This higher number of pollen grains in hermaphrodite flowers could be related to the possibility that hermaphrodite flowers have more nutritive reserves at anthesis since they will have to set fruits, allow also a better development of the anther. Results in avocado, a species, similarly to mango, with a very low flower to fruit ratio, showed significant differences in nutritive reserves between flowers with the highest reserves being more likely to produce fruits (Alcaraz et al., 2010; 2013).

Pollen viability was high in all the cultivars studied for both flower types. Only in ‘Ataulfo’ lower pollen viability was observed in male flowers, although that was not reflected in differences in pollen germination. Similar results have been reported in pomegranate where similar pollen germination was observed between bisexual and functionally male flowers (Wetzstein et al., 2011). Consequently, our results in mango support that differences in pollen germination are present between cultivars but not between flower morphs. A similar genotype-dependent pollen germination has been previously reported for mango by Duta et al (2013).

4.3. Pollen germination along the flowering season

Although pollen germination was variable along the flowering season for two cultivars studied, ‘Osteen’ and ‘Kensington’, we observed an increase in pollen germination at the end of the flowering season in both cultivars. This increase corresponded with an increase in temperatures. Similar results have been reported for ‘Kensington’, in which pollen viability increased along the flowering season (Issarakraisila et al., 1992). Other studies in regions with subtropical climates have also shown a negative effect of low temperatures on pollen development and viability (Issarakraisila and Considine, 1994; Sukhvibul et al.,

1999; Dag et al., 2000; Sukhvibul et al., 2000; Huang et al., 2010; Gaurang et al., 2015). Recently the effect of low temperatures on pollen development in mango was related to problems in pollen wall development (Lora and Hormaza, 2018).

The results obtained in this work support the hypothesis that temperatures under 15°C restrict pollen germination in mango (Issarakraisila and Considine, 1994; Sukhvibul et al., 2000, Huang et al., 2010) and could be a key parameter to consider when expanding mango cultivation to new regions. This situation is often encountered in the growing area where the experiments were performed. Thus, at the beginning of the flowering season minimum temperatures were close to 10°C. Additional factors, such as the possibility of a better flower quality at the end of the flowering season, should not be discarded.

4.4. Pollen germination under controlled temperature conditions

In order to study the possible differential behavior of pollen from different cultivars, pollen performance was studied at controlled temperatures. The results showed that low temperatures affect pollen germination in the three cultivars studied ('Kent', 'Osteen' y 'Kensington') confirming the results obtained under field conditions. At 10°C pollen germination was reduced and also pollen tubes grew shorter. Within the optimal range of temperature for mango tree development (24-30°C; Galán-Saúco, 2009), we observed an adequate pollen germination. These results were consistent with previous work of pollen germination both in vitro (Sukhvibul et al., 1999; 2000; Huang et al., 2010) and in vivo (de Wet et al., 1989; Dag et al., 2000). Optimal temperatures for mango pollen germination were similar to those reported for some subtropical fruit tree species, such as 25-30°C for avocado (Alcaraz et al., 2011), or 30°C for lychee (Stern and Gazit, 1998) or 23-24°C for longan (Pham et al., 2015).

Pollen performance was different for the three cultivars studied. While pollen germination was reduced at 10°C for all the cultivars, ‘Kensington’ maintained higher pollen germination at that temperature than the other two cultivars. In a previous report, Sukhvibul *et al.*, (2000) also found an acceptable pollen germination at this temperature for this genotype. This could reflect a different adaptation to cold temperatures at flowering between the different cultivars. A genotype dependent response to temperature has also been reported in other species as pistachio (Acar and Kakani, 2010), avocado (Alcaraz *et al.*, 2011), peach (Hedhly *et al.*, 2005a), longan (Pham *et al.*, 2015) or sweet cherry (Hormaza and Herrero, 1999; Hedhly *et al.*, 2004), suggesting that significant differences in pollen response to low temperatures can be found among different genotypes of the same species. These differences could reflect the adaptation to different flowering times of the different cultivars as it has been shown in pistachio (also a member of the Anacardiaceae), where early flowering cultivars have higher germination rates at low temperatures than late cultivars (Polito *et al.*, 1988). Differences between genotypes in reproductive performance should be taken into account to select the best genotypes for each geographic area (McKee and Richards, 1998). Studies in tomato showed that genes involved in low resistance to low temperature are expressed by the haploid pollen (Zamir *et al.*, 1981; 1982; Clarke *et al.*, 2004) and similar results were obtained for high temperatures in cotton (Rodriguez-Garay and Barrow, 1988), giving support to the ideas that these difference could be used in breeding programs (Hormaza and Herrero, 1992; 1996; Hedhly, 2011).

Taken together the field and control temperature experiments, it appears clear that low temperature has an effect on mango pollen viability and also that there is a differential genotypic response. This opens a way to understand temperature limit conditions at flowering for mango providing a basis for cultivar selection for these suboptimal

conditions. Additional work is underway to analyze the implications of these observations on final fruit set under field conditions.

Competing interests

The authors declare that they have no competing financial interests.

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561 **Figures:**

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Figure 1. Mango flower types: hermaphrodite (♂) and male (♂) of ‘Kensington’, ‘Ataulfo’, ‘Osteen’, ‘Kent’ and ‘Keitt’. Pistil (pt) and stamen (st). Bar = 2mm.

Figure 2. Effect of varying concentration polyethylene glycol (PEG) and sucrose (SUC) in a liquid medium on three mango cultivars (‘Kensington’, ‘Osteen’ and ‘Kent’): a) percentage of pollen germination (+) indicates the relation PEG and SUC with a higher pollen germination b) percentage of burst pollen grains in the 12 germination media. Bars represent mean ± SE.

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579 **Figure 3.** Number of pollen grains per anther, in hermaphrodite and male flowers, in five
580 different mango cultivars: ‘Kensington’, ‘Ataulfo’, ‘Osteen’, ‘Kent’ and ‘Keitt’.
581 Significant differences between cultivars are shown with different letters, and significant
582 differences between type of flowers are indicated with (*), in both cases when $p < 0.05$.
583 Bars represent mean $\pm$ 25% of the values.

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Figure 4. Pollen viability (a) and pollen germination (b), in hermaphrodite and male flowers in five mango cultivars ('Kensington', 'Ataulfo', 'Osteen', 'Kent' and 'Keitt'). Significant differences between genotypes are expressed with different letters, and significant differences between type of flower are indicated with (*), in both cases when $p < 0.05$. Bars represent $\pm$ SE.

Figure 5. Relationship between pollen germination and minimum, maximum and average weekly temperatures recorded for two mango cultivars: a) 'Kensington', and b) 'Osteen'. Differences in pollen germination for each week are expressed with different letters ($p < 0.05$). Error bars $\pm$ SE of germination percentage.

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600 **Figure 6.** Pollen germination at controlled temperatures: 10°C, 20°C and 30°C in three
601 mango cultivars: ‘Kensington’, ‘Osteen’ and ‘Kent’. Differences in germination
602 percentages for each cultivar at the same temperature are shown in lowercase letters; same
603 letters show no significant differences ($p < 0.01$). Capital letters show different percentage
604 of germination in only one temperature for the three cultivars; same letters show no
605 significant differences ($p < 0.05$). Error bars $\pm$ SE of germination percentage.

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