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ORIGINAL ARTICLE

Adverse effects of heat stress in relation to actin cytoskeleton on pollen performance of *Echinopsis chamaecereus* (Cactaceae)

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Abstract
In this study, the effects of heat stress (30 °C, 35 °C, 40 °C) on pollen performance of *Echinopsis chamaecereus* Friedrich & Glaetzle were analyzed. Heat-treated pollen grains showed a insignificant reduction in germination rate. Pollen tubes treated by temperatures above 30 °C exhibited a decrease in tube length and a negative effect on pollen performance according to cumulative stress response index data. Pollen tube abnormality was enhanced by increased temperature, and bifurcated pollen tubes were very dominant than the other abnormalities. Callose accumulation on tube tips was enhanced by increased temperature. In bifurcated pollen tubes, one of the tubes blocked by callose plugs or dense callose accumulation was observed on tips. Actin organization showed disruption, and anisotropy of actin filaments increased at temperatures above 30 °C. The findings show that high temperatures cause destructive effects on male reproductive biology even in species such as cacti known to be temperature tolerant.

Keywords Callose · Cell wall · High-temperature stress · Pollen tube elongation

1 Introduction

Echinopsis chamaecereus Friedrich & Glaetzle belongs to genus *Echinopsis* of Cactaceae family that comprises more than 100 genera and nearly 2000 species (Nyffeler 2002; Mosti et al. 2011; Schlumpberger and Renner 2012). Members of the family have developed different survival mechanisms to avoid extreme environmental conditions because they grow up in extreme habitats such as under high-temperature conditions. According to their drought and heat resistance, the family members attract more attention concomitant with increasing global warming (Nuzhyna et al. 2018). The excessive rise in environmental temperature is the significant stress factors for plants, and temperature stresses give rise to reverse effects on the vegetative and generative development of plants (Hatfield and Prueger 2015). However, the generative growth is highly responsive to temperature changes; especially, male gametophyte is much more undefended to temperature changes (Liu et al. 2006).

Pollen grains are useful indicators for the detection of temperature stress effects because pollen grains have species-specific responses to various stress conditions (Mesihovic et al. 2016). To detect the response to stress, pollen germination rate, tube length, tube abnormality rate, tube wall or cytoskeleton components are generally used parameters (Jia et al. 2017; Çetinbaş-Genç et al. 2019). Since callose is the most plentiful ingredient of the pollen tube wall, the researchers generally have focused on the changes in callose accumulation on the tube to demonstrate the effect of heat stress (Parrotta et al. 2019). Callose is produced by an enzyme named callose synthase. This enzyme is located in the plasma membrane, and its distribution is regulated by the actin cytoskeleton. Therefore, researchers have committed the abnormal callose accumulation at the apex which is induced via changes in the actin cytoskeleton. Tube shank includes extended, collateral actin filaments which attend the fringe region in sub-apex. But apex just includes operative and active filaments. The aim of actin filaments is concerned with the transfer of organelles and vesicles at the apex and to make strong the cell wall in the back of the apex (Qu et al. 2015). So, convenient dispersion of the actin filaments is quite essential to give rise to effective tubes (Jia et al. 2017), whereas the actin cytoskeleton is the primary focus of circumstances with stress, like temperature

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variations (Parrotta et al. 2016). So, callose accumulation and actin structure are significant marks to state stress tolerance.

Reproductive biology was studied in only 2% of the 2000 species of the Cactaceae family (del Carmen Mandujano et al. 2010). Strittmatter et al. (2002) revealed the details of the breeding system and embryological features, and also details of microsporangium development (Strittmatter et al. 2006) of *Consolea spinosissima* Lem. (Cactaceae). Cisneros et al. (2011) presented the information on ovule morphology, embryogenesis and seed development in three *Hylocereus* species (Cactaceae). Sanchez and Vazquez-Santana (2018) examined the embryological data of *Mammillaria dioica* K. Brandegee (Cactaceae). Although heat effects on pollen performance have been presented in numerous species, there is no report conducted to determine whether heat stress affects pollen performance and reproductive success in any member of the Cactaceae family. Therefore, the Cactaceae family represents a wide research area for reproduction, pollination and stress biology studies. The objective of this study is to determine the effect of heat stress on pollen performance in *E. chamaecereus* and to provide a new perspective on this aspect of sensitivity of reproductive biology under extreme temperature.

2 Materials and method

Pollen grains were germinated at different temperature conditions (25 °C, 30 °C, 35 °C and 40 °C) for 6 h. Germinated pollen grains at 25 °C were used as control (Elbehi et al. 2013). BK medium with 25% sucrose was used as germination media, according to previous studies with minor revisions (Metz et al. 2000; Elbehi et al. 2013). Germination rates and pollen tube lengths were measured considering approximately 500 pollen grains and 100 germinated pollen, respectively. Cumulative stress response index was calculated to appreciate the stress response of pollen tubes using germination rate (GR) and tube length (TL) of control (c) and treatment (t) groups with the following equation (Dai et al. 1994).

$$\mathrm{CSRI} = \left(\frac{\mathrm{GRt} - \mathrm{GRc}}{\mathrm{GRc}} + \frac{\mathrm{TLt} - \mathrm{TLc}}{\mathrm{TLc}}\right) \times 100$$

Abnormality rates were evaluated considering approximately 100 germinated pollen grains for each group. Callose detection was carried out using 0.1% aniline blue at 455 nm wavelengths (Kenrick and Knox 1985). To analyze the quantitative callose accumulation in the pollen tubes, the fluorescence intensity of callose was measured by ImageJ. The analysis was performed on ten different randomly selected pollen tubes from the apex down to 50 µm for each group. Visualization of actin filaments was carried out according to Lovy-Wheeler et al. (2005). For visualization, 6.6-µM Alexa 488-phalloidin stained tubes were investigated at 488 nm wavelengths. Actin filament anisotropy was measured considering ten various randomly chosen tubes at 150-µm segment with the equivalent length for each group. ImageJ FibrilTool plugin was used for measurement (Boudaoud et al. 2014). All of the preparations were analyzed and photographed with KAMERAM software, assisted by a KAMERAM both light and fluorescent camera and an Olympus BX-51 microscope. Statistical analyses were performed by SPSS 16.0 software, and data were subjected to one-way analysis of variance (ANOVA) with a threshold P value of 0.05.

3 Results

Pollen germination rate was highest at 25 °C by about 26%. Although the germination rate was decreased by about 3% at 30 °C, 1% at 35 °C and 5% at 40 °C, the reductions were not significant statistically. In contrast to germination, the longest tubes were observed at 30 °C. The tube length was increased about 14% at 30 °C, but decreased about 1.5% at 35 °C and 14% at 40 °C in comparison with control. Also, results revealed heat stress-induced abnormalities on pollen tube. Minimum tube abnormality was detected at 25 °C by about 2%. The abnormality rates were about 6% at 30 °C, 7% at 35 °C and 12% at 40 °C. It has been also determined that bifurcated pollen tubes were very dominant than the other abnormalities such as swelling or undulating tubes. To clarify the effects of heat stress, the CSRI value of stressed groups was calculated. According to CSRI data, 30 °C was showed a positive effect, while 35 °C and 40 °C were demonstrated a negative effect on pollen performance. Also, the most destructive effect was determined at 40 °C (Table 1).

According to aniline blue staining, callose accumulation on tube tips was enhanced by temperature increment (Fig. 1a). The enhancement was not much more on the tube tips at 30 °C in comparison with control (Fig. 1b). However, callose accumulation on the tube tips was

Table 1 Effects of heat stress on pollen performance of *E. chamaecereus*

	Control	30 °C	35 °C	40 °C
Germination rate (%)	26.00 ± 0.58^{a}	23.67 ± 3.53^{a}	25.00 ± 2.08^{a}	21.00 ± 2.08^{a}
Tube length (µm)	241.11 ± 80.33^{a}	277.0276 ± 84.3^{b}	237.4884 ± 77.89^{a}	205.6184 ± 78.41^{a}
Abnormality rate (%)	1.33 ± 0.33^{a}	5.67 ± 1.33^{b}	7.33 ± 0.33^{b}	11.00 ± 1.15^{c}
CSRI	–	5.9221	−5.3484	−33.9510

Distinct letters point out the statistically significant differences at the $P < 0.05$ level

obvious at 35 °C and 40 °C (Fig. 1c, d). In bifurcated pollen tubes, callose plugs or dense callose accumulation was seen at only one tube (Fig. 1e, f). To reveal the obvious variations of callose accumulation on tube tips, the FI of callose at segment of 50 µm in ten pollen tubes was determined in each treatment groups using software-based analysis. The fluorescence intensity of callose was increased about 22% at 30 °C, 78% at 35 °C and 156% at 40 °C in comparison with control (Fig. 1g). Shank of pollen tubes showed the parallel organization of actin filaments. On the other hand, actin filaments aligned as curt and fluxional in the apex at 25 °C and 30 °C (Fig. 1h, i). Some disruption on actin cytoskeleton was observed at 35 °C (Fig. 1j). Actin filaments lost their parallel bunch appearance in the shank, and also disorganizations were obvious on the apex at 40 °C (Fig. 1k). To analyze the clear differences of actin organizations, actin filaments anisotropy at 150-µm segment of ten pollen tubes of each treatment group was determined using software-based analysis. The anisotropy of actin filaments was decreased about 14% at 30 °C, increased about 7% at 35 °C and 24% at 40 °C in comparison with control (Fig. 1l).

4 Discussion

In the main study, the effects of heat stress on pollen performance of *E. chamaecereus* were analyzed. Based on the results, the germination was inhibited by temperature increment. Consistently, several researchers have been reported the regression of pollen germination by heat stress in many species such as olive, tobacco and hazelnut (Koubouris et al. 2009; Parrotta et al. 2016; Çetinbaş-Genç et al. 2019). The observations also revealed that pollen tube elongation was stimulated at 30 °C when compared to the control. However, tube length was mightily restricted by temperature above 30 °C. Similar results have been presented in pollen tubes of cotton that exposed to 30 °C, 35 °C, 40 °C and 45 °C. It was revealed that temperatures above 35 °C inhibited tube extension (Song et al. 2015). Besides, Parrotta et al. (2016)

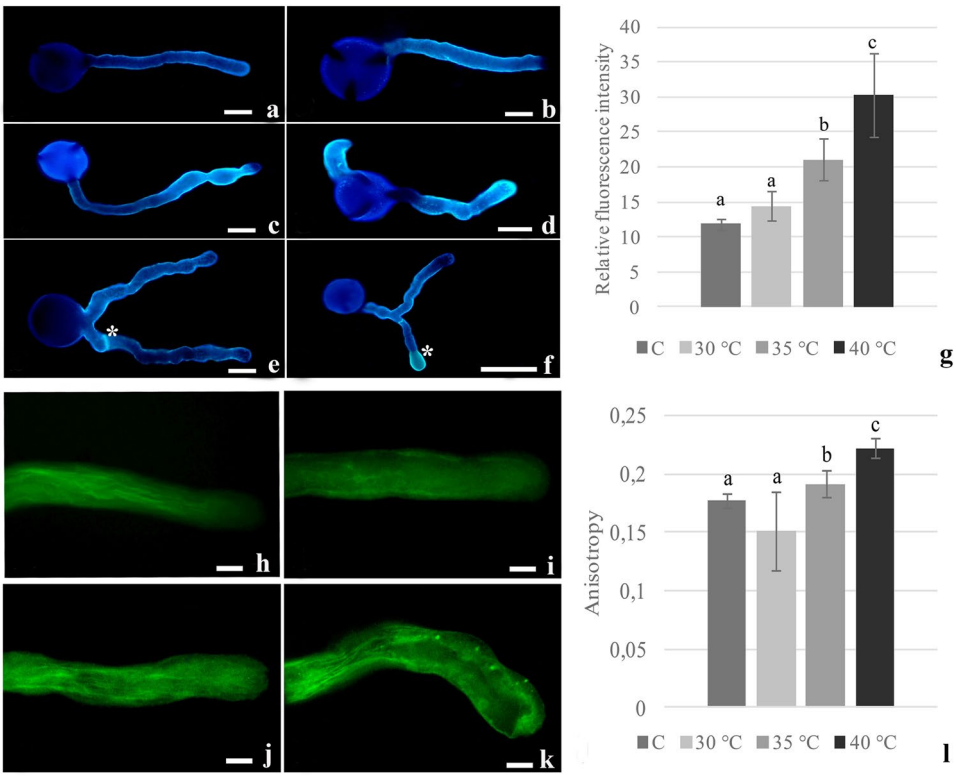

Fig. 1 Representative images of callose accumulation pattern, fluorescence intensity of callose, representative images of actin filament orientation and anisotropy of actin filaments at different temperature conditions. **a** Callose pattern of pollen tube tip at 25 °C, **b** pollen tube tip without callose accumulation at 30 °C, **c** callose accumulation on tube tip at 35 °C, **d** dense callose accumulation on tube tips at 40 °C, **e** bifurcated pollen tube with callose plug at 35 °C (asterisk), **f** bifurcated pollen tube with blocked on tip of a branch by callose at 40 °C (asterisk), **g** graphic representation of the callose fluorescence intensity of pollen tubes from the apex down to 50 µm, **h** parallel actin filaments at 25 °C, **i** parallel actin filaments at 30 °C, **j** actin organizations with some disruption at 35 °C, **k** disorganized actin filaments at 40 °C, **l** actin filaments anisotropy of pollen tubes from the apex down to 150 µm (distinct letters point out the statistically significant differences, and error bars indicate the standard errors). Scale: 20 µm (h–k), 50 µm (a–f)

showed pollen tube lengths of tobacco showed a comparable reduction at heat temperature treatment (35 °C, 37 °C and 40 °C). For allowing to define the temperature that has devastating effects, CSRI values were determined. CSRI data showed that 30 °C has a stimulating effect; however, temperatures above 30 °C have a devastating effect on pollen tube kinetics. Similarly, negative CSRI values have been determined in pollen tubes of almond that exposed to 30 °C and 40 °C (Sorkheh et al. 2018). Although 30 °C is a high-temperature condition with respect to former studies (Song et al. 2015; Sorkheh et al. 2018), positive CSRI value at 30 °C indicates the pollen grains of *E. chamaecereus* which is resistant to this temperature.

Results also revealed that the pollen tube abnormality progressively was enhanced by increased temperature. It was remarkable that bifurcated pollen tubes were very dominant than the other abnormalities such as the poly tube, swelling or irregular diameter. Consistent with the result, researchers noticed various temperatures remarkably which alter the pollen tube morphology (Srinivasan et al. 1999). It has been noticed tumescence on tea pollen tube apex exposed to cold stress (Wang et al. 2016). Also, pollen tubes that exposed the low and high temperatures have exhibited swollen tips in hazelnut (Çetinbaş-Genç et al. 2019). Moreover, Büyükkartal (2002) has been stated that abnormalities such as bifurcation at tube tips arrested the pollen tube elongation in *Trifolium pratense*. Also, Cheung et al. (2003) have been indicated the pollen tube prolongation inhibited after bifurcation of pollen tube tips.

Callose is an important parameter for the assessment of pollen functionality. Callose accumulation on tube tip only seen at blocked tube due to incompatibility or stress (Ünal et al. 2013). According to findings, callose accumulation on tips was enhanced by temperature increment. In bifurcated pollen tubes, one of the tubes was blocked by callose plugs or dense callose accumulation on tips. In the plasma membrane, the placement of callose synthase is conducted by actin filaments. It has been expressed that modification in actin arrange might stimulate unusual callose accumulation in pollen tubes (Cai et al. 2011; Jia et al. 2017).

In a regular pollen tube, actin cytoskeleton is packed into parallel filaments in the shank region (Qu et al. 2015). They are extremely dynamic at the tip and pollen tube growth which depends on the dynamism of apical actin filaments (Liu et al. 2015). Accordingly, convenient organization and dynamics of the actin cytoskeleton are essential to generate well elongating tubes (Parrotta et al. 2016). Nevertheless, actin cytoskeleton has often been considered a focus of stress, like heat or drugs. Gao et al. (2015) have been noticed low-temperature-induced actin cytoskeleton depolymerization in pollen tubes of pear. In the same way, Parrotta et al. (2016) indicated high-temperature-induced alterations of the actin cytoskeleton in tobacco. Similar with these studies, actin organization showed some disruption especially on apex at the temperature above 30 °C in *E. chamaecereus*. To analyze the changes on the dynamism of apical actin filaments, actin filaments anisotropy of pollen tube at 150-µm segment was determined using FibrilTool, Image J software-based analysis. Anisotropy value is a count among 0 and 1 (Boudaoud et al. 2014). And rising of anisotropy refers the reduction in actin dynamics. (Parrotta et al. 2016). Based on the results, the anisotropy of actin filaments was reduced by 14% at 30 °C, increased about 7% at 35 °C and 24% at 40 °C in comparison with control. These results revealed that 30 °C improved the actions of apical actins that leads to speedy growth. In the same way, 35 °C and 40 °C decreased the apical actin actions that lead to slow growth. In a similar fashion, it has been presented that actin filaments exhibited a higher anisotropy and triggered the short pollen tube generation, a consequence of heat treatment in tobacco (Parrotta et al. 2016). Moreover, it has been concluded that actin cytoskeleton disruption causes tip abnormality (Anderhag et al. 2000; Fang et al. 2016). Pollen tubes that exposed the actin-stabilizing chemicals showed distension and dichotomy on tubes (Cardenas et al. 2008; Ketelaar et al. 2012).

In conclusion, temperatures above 30 °C disrupt actin anisotropy and, in connection with this, cause tube abnormalities and excessive callose accumulation on tube tips that may prevent fertilization. The findings show that high temperatures can cause destructive effects on reproductive biology even in species such as cacti known to be temperature tolerant. So, results will provide a new perspective at reproductive biology under stressful condition. Also, results are important for understanding how heat stress affects cell mechanisms.

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Author Contribution AÇG conceived and planned the experiments and wrote the manuscript.

References

Anderhag P, Hepler PK, Lazzaro MD (2000) Microtubules and microfilaments are both responsible for pollen tube elongation in the conifer *Picea abies* (Norway spruce). Protoplasma 214:141–157. https://doi.org/10.1007/BF01279059

Boudaoud A, Burian A, Borowska-Wykret D, Uyttewaal M, Wrzalik R, Kwiatkowska D, Hamant O (2014) FibrilTool, an ImageJ plug-in to quantify fibrillar structures in raw microscopy images. Nat Protoc 9:457–463. https://doi.org/10.1038/nprot.2014.024

Büyükkartal HN (2002) In vitro pollen germination and pollen tube characteristics in tetraploid red clover (*Trifolium pratense* L.). Turk J Bot 27:57–61

Cai G, Faleri C, Del Casino C, Emons AMC, Cresti M (2011) Distribution of callose synthase, cellulose synthase, and sucrose synthase in tobacco pollen tube is controlled in dissimilar ways by actin filaments and microtubules. Plant Physiol 155:1169–1190. https://doi.org/10.1104/pp.110.171371

Cardenas L, Lovy-Wheeler A, Kunkel JG, Hepler PK (2008) Pollen tube growth oscillations and intracellular calcium levels are reversibly modulated by actin polymerization. Plant Physiol 146:1611–1621. https://doi.org/10.1104/pp.107.113035

Çetinbaş-Genç A, Cai G, Vardar F, Ünal M (2019) Differential effects of low and high temperature stress on pollen germination and tube length of hazelnut (*Corylus avellana* L.) genotypes. Sci Hortic 255:61–69. https://doi.org/10.1016/j.scienta.2019.05.024

Cheung AY, Chen CYH, Tao LZ, Andreyeva T, Twell D, Wu HM (2003) Regulation of pollen tube growth by Rac-like GTPases. J Exp Bot 54:73–81. https://doi.org/10.1093/jxb/erg044

Cisneros A, Garcia R, Tel-Zur N (2011) Ovule morphology, embryogenesis and seed development in three *Hylocereus species* (Cactaceae). Flora 206:1076–1084. https://doi.org/10.1016/j.flora.2011.07.013

Dai Q, Shaobing P, Chavez AQ, Vergara BS (1994) Intraspecific responses of 188 rice cultivars to enhanced UVB radiation. Environ Exp Bot 34:433–442. https://doi.org/10.1016/0098-8472(94)90026-4

del Carmen MM, Carrillo-Angeles I, Martinez-Peralta C, Golubov J (2010) Reproductive biology of Cactaceae. In: Ramawat KG (ed) Desert plants. Springer, Berlin, pp 197–230. https://doi.org/10.1007/978-3-642-02550-1_10

ElBehi AW, Orlandi F, Bonofiglio T, Romano B, Fornaciari M, Inglese P, Sortino G, Liguori G (2013) Pollen morphology and reproductive performances in Opuntia ficus-indica (L.) Mill. VIII Int Congr Cactus Pear Cochineal 1067: 217–223. https://doi.org/10.17660/ActaHortic.2015.1067.30

Fang K, Zhang W, Xing Y, Zhang Q, Yang L, Cao Q, Qin L (2016) Boron toxicity causes multiple effects on *Malus domestica* pollen tube growth. Front Plant Sci 7:208. https://doi.org/10.3389/fpls.2016.00208

Gao Y, Zhou H, Chen J, Jiang X, Tao S, Wu J, Zhang S (2015) Mitochondrial dysfunction mediated by cytoplasmic acidification results in pollen tube growth cessation in *Pyrus pyrifolia*. Physiol Plant 153:603–615. https://doi.org/10.1111/ppl.12260

Hatfield JL, Prueger JH (2015) Temperature extremes: effect on plant growth and development. Weather Clim Extrem 10:4–10. https://doi.org/10.1016/j.wace.2015.08.001

Jia MX, Shi Y, Di W, Jiang XR, Xu J, Liu Y (2017) ROS-induced oxidative stress is closely related to pollen deterioration following cryopreservation. Cell Dev Biol Plant 53:433–439. https://doi.org/10.1007/2Fs11627-017-9844-3

Kenrick J, Knox RB (1985) Self-incompatibility in the nitrogen-fixing tree, *Acacia retinodes*: quantitative cytology of pollen tube growth. Theor Appl Genet 69:481–488

Ketelaar T, Meijer HJ, Spiekerman M, Weide R, Govers F (2012) Effects of latrunculin B on the actin cytoskeleton and hyphal growth in *Phytophthora infestans*. Fungal Genet Biol 49:1014–1022. https://doi.org/10.1016/j.fgb.2012.09.008

Koubouris GC, Metzidakis IT, Vasilakakis MD (2009) Impact of temperature on olive (*Olea europaea* L.) pollen performance in relation to relative humidity and genotype. Environ Exp Bot 67:209–214. https://doi.org/10.1016/j.envexpbot.2009.06.002

Liu Z, Yuan YL, Liu SQ, Yu XN, Rao LQ (2006) Screening for high-temperature tolerant cotton genotypes by testing in vitro pollen germination, pollen tube growth and boll retention. J Integr Plant Biol 48:706–714. https://doi.org/10.1111/j.1744-7909.2006.00276.x

Liu X, Qu X, Jiang Y, Chang M, Zhang R, Wu Y, Fu Y, Huang S (2015) Profilin regulates apical actin polymerization to control polarized pollen tube growth. Mol Plant 8:1694–1709. https://doi.org/10.1016/j.molp.2015.09.013

Lovy-Wheeler A, Wilsen KL, Baskin TI, Hepler PK (2005) Enhanced fixation reveals the apical cortical fringe of actin filaments as a consistent feature of the pollen tube. Planta 221:95–104. https://doi.org/10.1007/s00425-004-1423-2

Mesihovic A, Iannacone R, Firon N, Fragkostefanakis S (2016) Heat stress regimes for the investigation of pollen thermotolerance in crop plants. Plant Reprod 29:93–105. https://doi.org/10.1007/s00497-016-0281-y

Metz C, Nerd A, Mizrahi Y (2000) Viability of pollen of two fruit crop cacti of the genus Hylocereus is affected by temperature and duration of storage. Hort Sci 35:22–24. https://doi.org/10.21273/HORTSCI.35.2.199

Mosti S, Bandara NL, Papini A (2011) Further insights and new combinations in Aylostera (Cactaceae) based on molecular and morphological data. Pak J Bot 43:2769–2785

Nuzhyna N, Baglay K, Golubenko A, Lushchak O (2018) Anatomically distinct representatives of Cactaceae Juss family have different response to acute heat shock stress. Flora 242:137–145. https://doi.org/10.1016/j.flora.2018.03.014

Nyffeler R (2002) Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from trnK/matK and trnL/trnF sequences. Am J Bot 89:312–326. https://doi.org/10.3732/ajb.89.2.312

Parrotta L, Faleri C, Cresti M, Cai G (2016) Heat stress affects the cytoskeleton and the delivery of sucrose synthase in tobacco pollen tubes. Planta 243:43–63. https://doi.org/10.1007/s00425-015-2394-1

Parrotta L, Faleri C, Guerriero G, Cai G (2019) Cold stress affects cell wall deposition and growth pattern in tobacco pollen tubes. Plant Sci 283:329–342. https://doi.org/10.1016/j.plantsci.2019.03.010

Qu X, Jiang Y, Chang M, Liu X, Zhang R, Huang S (2015) Organization and regulation of the actin cytoskeleton in the pollen tube. Front Plant Sci 5:786. https://doi.org/10.3389/fpls.2014.00786

Sanchez D, Vazquez-Santana S (2018) Embryology of *Mammillaria dioica* (Cactaceae) reveals a new male sterility phenotype. Flora 241:16–26. https://doi.org/10.1016/j.flora.2018.02.007

Schlumpberger BO, Renner SS (2012) Molecular phylogenetics of Echinopsis (Cactaceae): Polyphyly at all levels and convergent evolution of pollination modes and growth forms. Am J Bot 99:1335–1349. https://doi.org/10.3732/ajb.1100288

Song G, Wang M, Zeng B, Zhang J, Jiang C, Hu Q, Geng G, Tang C (2015) Anther response to high-temperature stress during development and pollen thermotolerance heterosis as revealed by pollen tube growth and in vitro pollen vigor analysis in upland cotton. Planta 241:1271–1285. https://doi.org/10.1007/s00425-015-2259-7

Sorkheh K, Azimkhani R, Mehri N, Chaleshtori MH, Halasz J, Ercisli S, Koubouris GC (2018) Interactive effects of temperature and genotype on almond (*Prunus dulcis* L) pollen germination and tube length. Sci Hort 227:162–168. https://doi.org/10.1016/j.scienta.2017.09.037

Srinivasan A, Saxena N, Johansen C (1999) Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L): genetic variation in gamete development and function. Field Crops Res 60:209–222. https://doi.org/10.1016/S0378-4290(98)00126-9

Strittmatter LI, Negron-Ortiz V, Hickey RJ (2002) Subdioecy in *Consolea spinosissima* (Cactaceae): breeding system and embryological studies. Am J Bot 89:1373–1387. https://doi.org/10.3732/ajb.89.9.1373

Strittmatter LI, Negron-Ortiz V, Hickey JR (2006) Comparative microsporangium development in male-fertile and male-sterile flowers of Consolea (Cactaceae): when and how does pollen abortion occur. Grana 45:81–100. https://doi.org/10.1080/00173130500537101

Ünal M, Vardar F, Aytürk Ö (2013) Callose in plant sexual reproduction. In: Silva-Opps M (ed) Current progress in biological research. InTech Publ, Rijeka, pp 319–343. https://doi.org/10.5772/53001

Wang W, Sheng X, Shu Z, Li D, Pan J, Ye X, Chang P, Li X, Wang Y (2016) Combined cytological and transcriptomic analysis reveals a nitric oxide signaling pathway involved in cold-inhibited *Camellia sinensis* pollen tube growth. Front Plant Sci 7:456. https://doi.org/10.3389/fpls.2016.00456