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# Simulated microgravity affects directional growth of pollen tubes in candidate space crops

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**Background:** Long-term space missions will necessarily require producing viable seeds to be used for plant cultivation over time under altered gravity conditions. Pollen is known to play a key role in determining seed and fruit production over seed-to-seed cycles, but very few studies have evaluated pollen functionality under altered gravity.

**Methods:** We performed ground-based experiments to test how simulated microgravity can affect the directional growth of pollen tubes as a potential bottleneck in seed and fruit sets. The effect of clinorotation was assessed in the pollen of *Solanum lycopersicum* L. cv. 'Micro-Tom' and *Brassica rapa* L. var. *silvestris*, both eligible for cultivation in space. Pollen tube length and tortuosity were compared under 1g and simulated microgravity with a uniaxial clinostat.

**Results:** The main results highlighted that simulated microgravity significantly increased pollen tube length and tortuosity compared to 1g conditions. Further, clinorotation prompted a differential effect on pollen germination between *S. lycopersicum* and *B. rapa*. A more in-depth analysis evaluating the effect of gravity on the directional growth of pollen tubes excluded gravitropic responses as responsible for the tube tip position reached after germination.

**Discussion:** This research provides new insights into how altered gravity can interfere with plant reproduction and, in particular, microgametophyte functionality. Our findings represent a basis for further studies aimed at understanding the effect of real microgravity on plant reproduction and developing countermeasures to ensure seed-to-seed cultivation in long-term space missions and achieve self-sufficiency in food supplies from Earth.

## KEYWORDS

simulated microgravity, pollen tube, pollen germination, clinostat, space crops, Micro-Tom, *Brassica rapa*, seed-to-seed

## 1 Introduction

Future space exploration requires plant-based bioregenerative life support systems (BLSS) to produce edible biomass and oxygen, recycle water, absorb carbon dioxide, and support astronauts' psychological health (Kordyum and Hasenstein, 2021). Indeed, plants are essential components of BLSS, and for decades, many experiments with plants have been performed to test the feasibility of plant cultivation in space (e.g., Zabel et al., 2016; De Pascale et al., 2021; Romano and Aronne, 2021).

Early efforts to cultivate plants for extended periods in microgravity resulted in reduced growth and complications in the transition to the reproductive stage (Kordyum et al., 1983; Kuang et al., 1995). In 1982, *Arabidopsis thaliana* became the first plant species to flower in space and produce seeds (Merkies and Laurinavichyus, 1983). However, only half of the seeds were viable and germinated in developing plants without growth defects (Salisbury, 1999). Environmental constraints such as low ventilation and high ethylene concentrations have been considered the main causes of interrupted or reduced reproductive success in many early experiments in space (De Micco et al., 2013). Recently, the advancement of plant growth systems has led to numerous successful seed sets in space and to a seed-to-seed-to-seed cycle of *A. thaliana* that was achieved entirely on the International Space Station (Link et al., 2014).

Overall, plant biology research in space indicates that real and simulated microgravity do not prevent plant reproduction, but they can affect the quality of embryos and the development of seedlings (Kuang et al., 2000b; Musgrave et al., 2000; Link et al., 2014; Izzo et al., 2022). Various alterations in seeds produced in space include delayed embryonic development, modification of storage reserves, delayed starch use in cotyledons, and decreased cell number in cotyledons (Kuang et al., 2000a; Kuang et al., 2000b; Musgrave et al., 2005). Currently, the identification of mechanisms by which space factors can affect plant reproduction is becoming increasingly critical for plant cultivation in future human settlements on the Moon and Mars (Kordyum et al., 2020). Indeed, the completion of the seed-to-seed cycle in the BLSSs is essential to producing viable seeds to be used for plant cultivation over time independently of Earth's supply.

Within the plant reproductive cycle, viable and germinable pollen is needed to trigger the double fertilization responsible for seed formation. Indeed, both pollen viability and germination are positively correlated with fruit and seed set (Paupière et al., 2017; Herrera et al., 2018). Therefore, possible effects of space factors on successful seed production can be addressed by defects in pollen viability and germinability. Previous experiments showed that most *Arabidopsis* and *Brassica* pollen grains produced under microgravity conditions were viable (Kuang et al., 1996; Kuang et al., 2000b). Nevertheless, this is not enough to fulfill the reproductive cycle. Once formed, viable pollen needs to be transferred to the stigma, posing some challenges in microgravity, especially for self-incompatible species that require cross-pollination to succeed in seed production (Kuang et al., 2000a). Furthermore, once it arrives on the stigma surface, pollen must germinate and guide its pollen tube across the ovary into the ovule

to trigger double fertilization and seed formation. In the alternation between sporophyte (diploid) and gametophyte (haploid) generation in sexual plant reproduction, pollen represents the male gametophyte and is therefore an independent organism with a very short lifespan whose survival is essential to achieve the full completion of the plant reproductive cycle (Pacini and Dolferus, 2019).

Pollen is very sensitive to environmental factors that could affect pollen tube development. The earliest stage of pollen development was revealed to be the most sensitive to temperature changes, with a drastic reduction of pollen germination caused by high temperatures during microsporogenesis (Iovane and Aronne, 2021). Moreover, pollen germination is strongly influenced by relative humidity and its combination with temperature conditions (Güçlü and Koyuncu, 2017; Aronne et al., 2021). Indeed, a drastic loss in pollen viability occurs when pollen is exposed to a combination of high humidity and high temperature, whereas this does not happen when pollen is exposed to the same temperatures but lower humidity levels (Iovane et al., 2022a). Additionally, the possibility of plants growing and reproducing in space will depend on their capability to accomplish both sporophyte and gametophyte generations under altered microgravity conditions. In this scenario, investigations into how simulated microgravity can affect pollen germination and tube growth might provide useful insights into space-related issues in plant reproduction.

Among the instruments used to simulate microgravity, clinostats are meant to reduce the effect of constant gravity acceleration on Earth, and they have been largely used to perform ground-based experiments on the effects of simulated microgravity on plant growth and development (Dedolph and Dipert, 1971; Kiss et al., 2019). Even if clinostats do not reproduce weightlessness, ground-based research in simulated microgravity provides useful understandings to predict plant responses in real microgravity (Kiss et al., 2019). Although plants are known to be extremely sensitive to gravity (Muthert et al., 2020; Sathasivam et al., 2021), gravitropism in pollen tubes has not yet been assessed. To date, the microgametophyte (pollen) tube path towards the ovary is known to be guided exclusively by chemotropic stimuli due to specialized pistil tissues (Kandasamy et al., 1994), whereas the possible co-existence with pollen gravitropism has been generally overlooked.

To date, a few studies have shown that simulated microgravity affects several aspects of pollen tube development, such as alterations in sperm cell migration and callose plug formation (De Micco et al., 2005; De Micco et al., 2006), but gravitropism in pollen tubes has not yet been assessed. Previous studies were performed under *in vitro* conditions where the absence of a Ca<sup>2+</sup> gradient allowed isolation of the effect of simulated microgravity. Thus, to test the hypothesis that chemotropism always coexists with gravitropism in germinating pollen, *in vitro* conditions would be ideal to reveal the effect of altered gravity in orientating pollen tubes. In the framework of future space missions with seed-to-seed crop cycles, it is crucial to study how altered gravity could interfere with pollen germination and tube directional growth to achieve fertilization.

Among candidate space crops, *Solanum lycopersicum* L. cv. 'Micro-Tom' and *Brassica rapa* L. are two promising crops for

cultivation on board spacecrafts, mainly for their compact size and short life cycle (Musgrave et al., 2005; Matsukura et al., 2008; Shikata and Ezura, 2016; Burgner et al., 2020). ‘Micro-Tom’ is a macro-thermal fruity crop that has already achieved a seed-to-seed cycle under simulated microgravity, even though fruit setting was almost halved under clinorotation (Colla et al., 2007). *B. rapa* is a micro-thermal leafy crop that has already been grown from seed to seed under microgravity conditions on the Mir space station in 1997; however, seeds and siliques formed from the second generation of plants on board resulted in compromise (Musgrave et al., 2005). *Brassica* spp. are close relatives of *A. thaliana*, and there is substantial homology between their nuclear genomes (Tatematsu et al., 2004). Particularly, the pollen of *B. rapa* L. var. *sylvestris* has already been investigated for aberrant callose plug deposition during tube development under simulated microgravity (De Micco et al., 2006).

Research on possible gravitropic responses of pollen is needed to have a better understanding of how altered gravity can limit seed and fruit production over repeated seed-to-seed cycles in candidate space crops. In this context, our study is the first to perform *in vitro* pollen germination on a clinostat to isolate the effect of simulated microgravity and unravel the possible gravitropism of the pollen tube. Specifically, our aim was to compare the effect of simulated microgravity and Earth gravity on pollen tube orientation in *S. lycopersicum* L. cv. ‘Micro-Tom’ and *Brassica rapa* L. var. *sylvestris* as potential bottlenecks in regular seed and fruit development for cultivation in space.

## 2 Material and methods

### 2.1 Plant material

The effect of simulated microgravity on pollen germination and tube orientation was assessed in *S. lycopersicum* L. cv. ‘Micro-Tom’ and *B. rapa* L. var. *sylvestris*, both recognized as candidate space crops. ‘Micro-Tom’ and *Brassica* plants were grown from seeds to flower in two different growth chambers. In ‘Micro-Tom’ plants, a photosynthetic photon flux density (PPFD) of  $400 \pm 15 \mu\text{mol m}^{-2} \text{s}^{-1}$  was provided by white LEDs in a  $16 \text{ h d}^{-1}$  photoperiod. Relative humidity was kept at  $70 \pm 5\%$  and the air temperature was set at  $22 \pm 0.5^\circ\text{C}$  both reported as the optimum for tomatoes (Sato et al., 2001; Matsuda et al., 2014). As for *Brassica* plants, the PPFD from white LEDs was  $250 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$  in a  $12 \text{ h d}^{-1}$  photoperiod. Relative humidity and air temperature were kept at  $70 \pm 5\%$  and  $18 \pm 0.5^\circ\text{C}$ , respectively. PPFD was measured during each treatment using a spectroradiometer (SS-110; Apogee Instruments Inc., Logan, UT).

### 2.2 Pollen sampling and viability measurement

Pollen was collected from flowers sampled shortly before anthesis to prevent possible pollen damage once exposed to the external environment. More specifically, undehisced anthers were

detached from 200 flower buds per species, distributed in two different Petri dishes, and left to dehisce in a closed container filled with dry silica gel. This method sped up pollen release from anthers and allowed for the production of one bulk sample of pollen per species within a few hours of flower sampling (Iovane et al., 2022b).

Before clinorotation, the pollen viability of the bulk samples was assessed through the diaminobenzidine (DAB) reaction (Dafni, 1992) to ensure that the pollen used for the experiment was of high quality. We evaluated pollen viability on five pollen samples per species taken from the respective bulk. Each pollen sample was spread into a  $10 \mu\text{l}$  droplet of water previously placed on a slide. One droplet of  $10 \mu\text{l}$  of DAB reagent was added to each pollen sample, and slides were gently warmed on a heating plate and mounted. Pollen grains were scored as viable when stained dark/brown and as not viable when they remained faint/colorless. Each slide mounting a single pollen sample was photographed under a light microscope, and pollen viability was scored through image analyses on six photos per slide. The viability percentage was measured by counting at least 100 pollen grains per photo on a total of 30 photos per species (two species  $\times$  five slides  $\times$  six photos).

### 2.3 Pollen germination and gravity treatments

To test the effect of simulated microgravity on pollen germination, Petri dishes with germinating pollen were split between a control (1g) and a clinostat (cl). In the control treatment, pollen germinated on agar germination medium inside Petri dishes vertically positioned according to the 1g gravity vector. Simulated microgravity treatment was performed on a uniaxial clinostat consisting of two metallic supports and two rotating dishes joined by a rigid shaft. Petri dishes with germinating pollen were fixed on the rotating shaft at a specific distance from the rotating axis to obtain ineffective centrifuge acceleration on pollen, according to a spin velocity of the rigid shaft set at two revolutions per minute. *In vitro* pollen germination was tested using two different agar media optimized for ‘Micro-Tom’ and *B. rapa* (Sato et al., 1998; Karapanos et al., 2010). In more detail, pollen from the bulk samples was placed in 10 Petri dishes per species lined with an agar medium made of 0.9% agar and 15% sucrose (w/v) for ‘Micro-Tom’ pollen and 0.9% agar and 20% sucrose (w/v) for *B. rapa*.

### 2.4 Microscopy and image analysis of germinating pollen

The effect of clinorotation on pollen germination and tube development in agar media was assessed through image analyses comparing pollen germinability, tube length, and tortuosity index between control (1g) and clinostat (simulated microgravity) treatments. Image analysis of germinating pollen was performed with ImageJ v1.53 software on photos taken with a digital camera (EOS 60D, Canon) mounted on a light microscope (Olympus® BX-60). Each photo was taken keeping track of the Petri dish

orientation with respect to the direction of the gravity vector. Germinating pollen was photographed after 24 and 48 h, and germination scoring was performed through image analyses on 400 photos (two species  $\times$  two gravity treatments  $\times$  five Petri dishes  $\times$  two germination times  $\times$  10 photos). Germination percentages were assessed by considering as germinated grains those with a pollen tube longer than the grain diameter. Pollen tube length (L) was assessed on 600 pollen tubes and scored on 200 photos tracing the whole length of the pollen tube from the exine apertures to the pollen tube tip. On the same photos, the pollen tortuosity index was measured to estimate bending/directionality throughout the pollen tube path (Figure 1). The tortuosity index (T) was calculated using the following equation:

$$T = L/C$$

where L (length) is the total length of the pollen tube and C (chord) is the distance between the pollen exine aperture and the pollen tube tip. The reported tortuosity index can vary from 1 ( $L = C$ ) to  $+\infty$  ( $L > C$ ). More specifically, tortuosity values close to 1 indicate that the pollen tube developed straight from the exine to the tube tip, whereas increasing tortuosity values indicate an increase in direction changes during elongation of the pollen tube.

Furthermore, to detect a possible gravitropic response of pollen tubes, we investigated the direction of tube elongation in the control

and clinostat treatments. For this purpose, we divided the images of each germinated pollen grain into four quadrants with respect to the direction of the gravity vector, scored the direction of 300 pollen tubes inside the agar medium, and calculated the percentage of pollen tube tips reaching each quadrant (Figure 2).

We hypothesized that the expected frequencies of pollen tube tip location would be equally distributed across the Petri dish containing the germination medium (25% in each quadrant). In the case of  $P < 0.05$ , the observed frequencies would differ from those expected, therefore showing a preferential growth direction of pollen tube tips. Specifically, in the control treatment, a positive or negative gravitropic response would result if pollen tube tips were preferentially located in quadrants I or III, respectively.

## 2.5 Data analysis

Data were analyzed with IBM<sup>®</sup> SPSS Statistics. Shapiro–Wilk’s test and Levene’s test were respectively used to assess the normality and homogeneity of the variances of the datasets. Differences in pollen germination, tube length, and tortuosity between ground and clinostat treatments were tested using ANOVA ( $P < 0.05$ ) and the Brown–Forsythe robust test for equality of means. The observed and expected frequencies of pollen tube tip location in the different

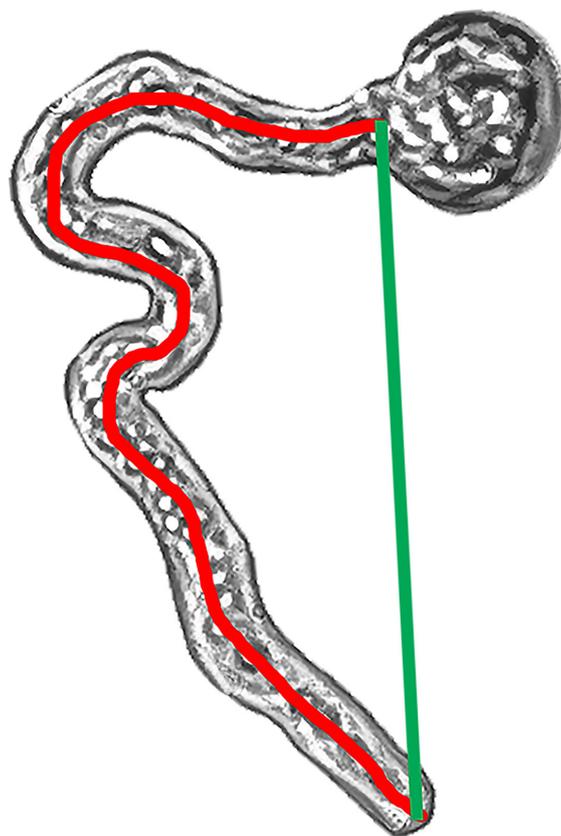
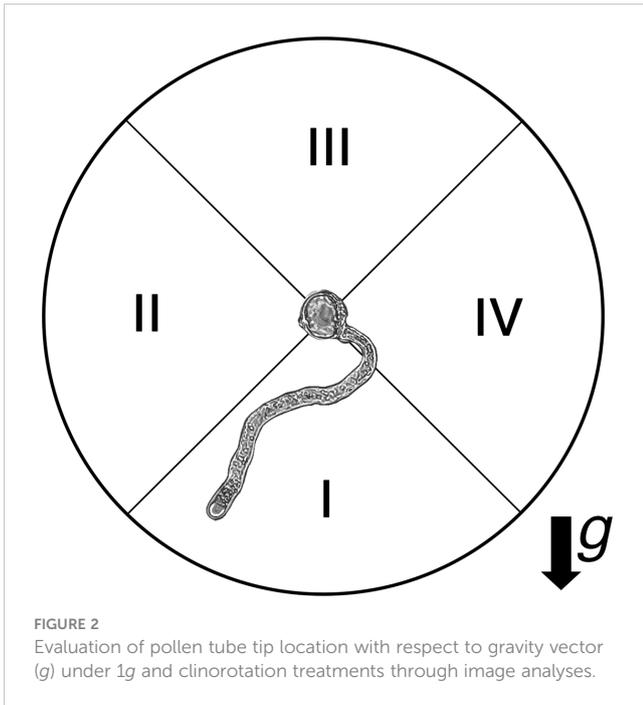


FIGURE 1

Tortuosity index calculated as  $L/C$ —length/chord through image analyses on pollen grains germinated in agar medium. Pollen tube length (L—red line) was traced throughout the whole length of the pollen tube. The pollen tube chord (C—green line) was traced as the straight line between the pollen exine aperture and the tube tip.



quadrants under control and simulated microgravity treatments were compared through a Chi-square test.

### 3 Results

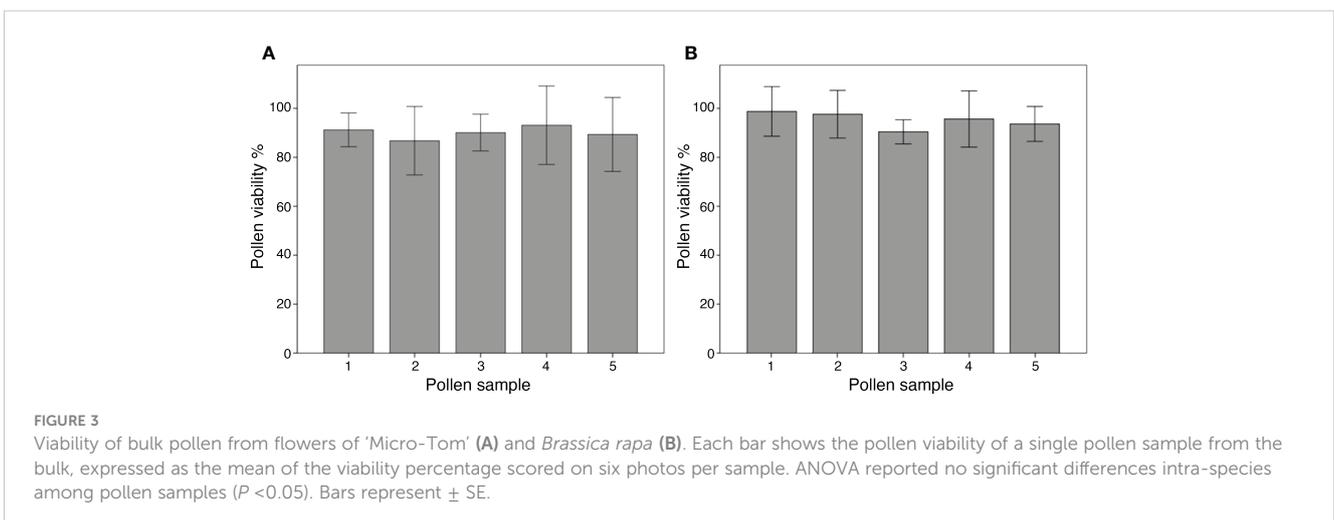
The DAB test for pollen viability showed that pollen was well formed and functional in both species. Moreover, the viability of all pollen samples taken from the bulk of each species was high and uniform, ranging between 87%–91% in ‘Micro-Tom’ and 86%–94% in *B. rapa*, and no significant differences were detected among pollen samples intra-species (Figure 3).

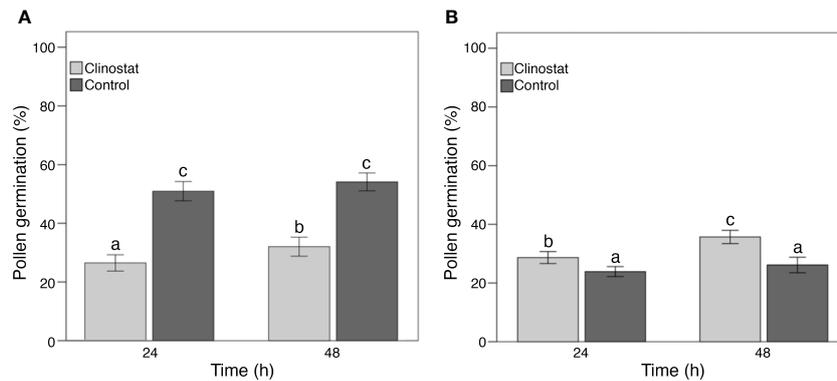
Data on pollen germination showed clear differences between control and clinostat treatments (Figure 4). More specifically, the two-way ANOVA showed a significant ( $P < 0.05$ ) effect of germination time, gravity, and their interaction. The two species

reacted differently to gravity since simulated microgravity reduced pollen germination in ‘Micro-Tom’ but increased pollen germination in *B. rapa* compared to the control condition. However, in both species, simulated microgravity significantly increased pollen germination when prolonging pollen incubation from 24 to 48 h, whereas no differences in pollen germination were found between 24 and 48 h of incubation under the 1g condition.

Thereafter, the pairwise comparison of pollen tube length between control and clinostat treatments highlighted a similar response in ‘Micro-Tom’ and *B. rapa*. Despite the great difference in tube length, pollen of both species developed longer tubes in simulated microgravity than in control conditions (Figure 5). Considering pollen tube length and pollen germination data, results highlighted that simulated microgravity promoted pollen tube length in both species despite reducing the germination rate in *B. rapa*.

As far as pollen tube path in germinated grains, a first visual overview of pollen tubes showed a clear difference between control and clinostat treatments, especially in ‘Micro-Tom’ (Figure 6). More in detail, control pollen developed straighter tubes compared to the simulated microgravity condition, in which tube tips changed their direction more frequently throughout the pollen tube path. The straightness/curviness of pollen tube paths was compared between control and clinostat by measuring the tortuosity index in 300 pollen tubes per species. Results showed that simulated microgravity significantly increased pollen tube tortuosity compared to control. Moreover, ANOVA reported that the difference in tortuosity between control and simulated microgravity was more significant in ‘Micro-Tom’ ( $P < 0.001$ , Figure 7A) than in *B. rapa* ( $P < 0.05$ , Figure 7B), which is also highlighted in the box plots showing the distribution of tortuosity values in the two gravity treatments (Figure 7). Indeed, in ‘Micro-Tom,’ most of the values of the tortuosity index are distributed consistently around the median in the control treatment, whereas a much wider distribution with increasing tortuosity values results in the clinostat treatment. Differently, the tortuosity index of *B. rapa* pollen tubes were similarly distributed around the median both in control and in simulated microgravity, but still had significantly higher values in the clinostat treatment.





**FIGURE 4** Germination of bulk pollen samples from flowers of ‘Micro-Tom’ (A) and *Brassica rapa* (B). Pollen germination was scored after 24 and 48 h both in clinostat (simulated microgravity) and control (1g) conditions. Each bar shows the mean pollen germination percentage scored on 50 photos. Significant differences intra-species are reported with different letters ( $P < 0.05$ ). Bars represent  $\pm$  SE.

Finally, the evaluation of possible gravitropic responses in pollen tubes showed no preferential distribution of pollen tube tips across the Petri dishes, both in control and simulated microgravity treatments (Figure 8). Specifically, we analyzed data assuming no gravitropic response of pollen tubes of ‘Micro-Tom’ and *B. rapa*, therefore hypothesizing an equal distribution (25% in each quadrant) of tube tips across the Petri dish in both control and simulated microgravity. The Chi-square test revealed no significant differences between observed and expected frequencies of pollen tube tips in the four quadrants, independently of the gravity treatments and the species tested (Table S1,  $P < 0.05$ ). Therefore, we confirmed the null hypothesis that the orientation of pollen tubes of ‘Micro-Tom’ and *B. rapa* was not subjected to gravitropic responses.

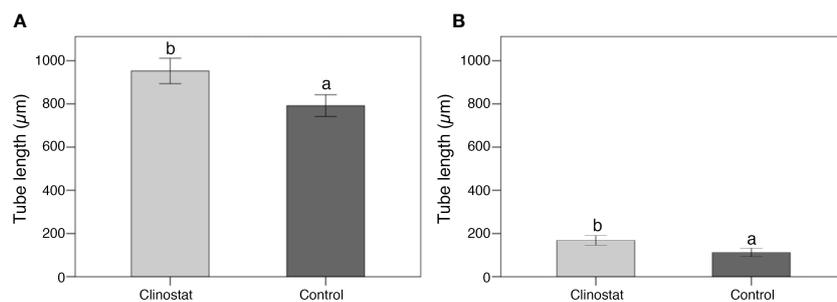
### 4 Discussion

Results on initial pollen viability revealed that growing conditions were optimally set to avoid any interference of abiotic factors with reproductive features. Indeed, pollen samples of both species reported viability over 80%, and no significant differences

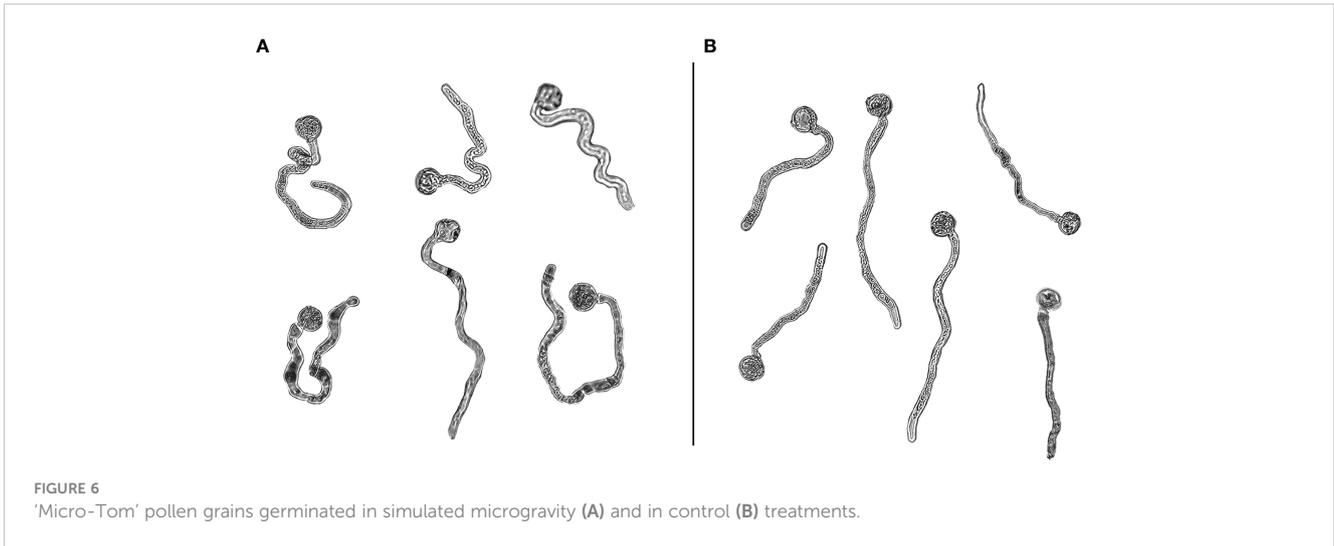
intraspecies between pollen samples proved high uniformity in pollen grains taken from the same bulk (Figure 3). Therefore, the two bulk pollen samples (one bulk per species) represented a proper starting point to perform further analyses and compare results intra- and inter-species concerning further pollen traits under simulated microgravity conditions.

The viability of pollen grains is not sufficient to predict pollen’s capability to elongate a pollen tube and achieve fertilization (Dafni et al., 2005; Pacini and Dolferus, 2019). Our results also demonstrated that pollen with high viability can germinate under simulated microgravity with a significantly different ratio according to the species tested (Figure 4). Considering this, pollen germination rate may be applied as an inclusion criterion in ranking space crops for seed-to-seed cultivation in space (Aronne et al., 2020). Both pollen viability and germination are strongly correlated with fruit and seed set (Paupière et al., 2017; Herrera et al., 2018) and are therefore essential to ensuring repeated seed-to-seed cycles on board a spacecraft.

Since most plant breeding research has been conducted through the sporophyte, the influence of the gametophytic phase, including pollen tube development, has been generally overlooked. However, the possibility of applying gametophytic selection in plant breeding



**FIGURE 5** Pollen tube length in clinostat (simulated microgravity) and control (1g) treatments in ‘Micro-Tom’ (A) and *Brassica rapa* (B) (300 pollen tubes per species). Each bar shows the mean of 150 pollen tube lengths scored on 50 photos. Significant differences intra-species are reported with different letters ( $P < 0.05$ ). Bars represent  $\pm$  SE.

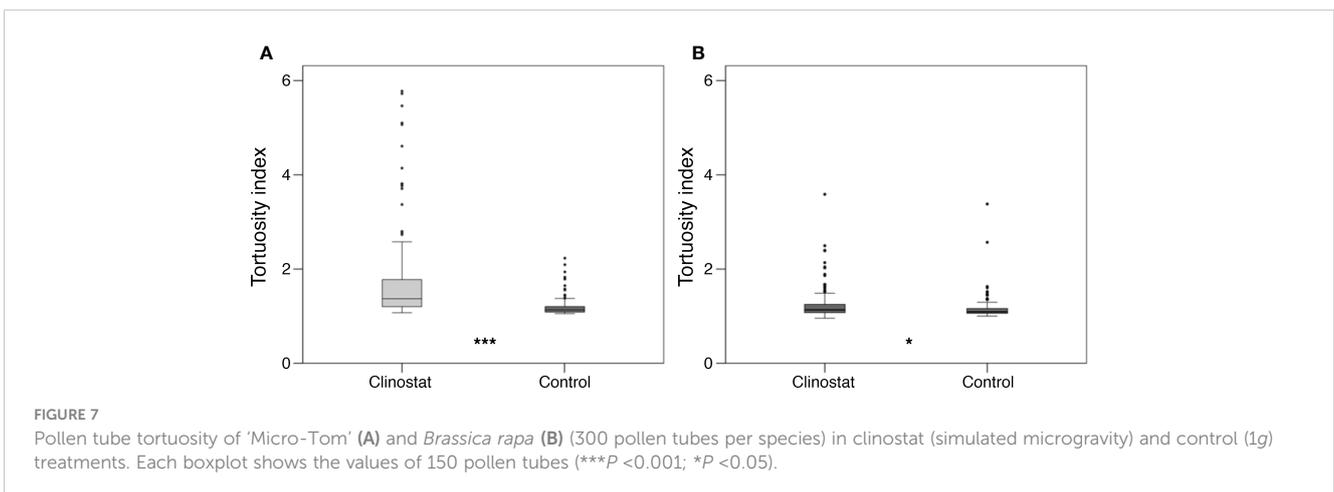


programs on Earth had already been supported by Hormaza and Herrero (1992), who highlighted the overlap in the genetic expression between the sporophyte and the gametophyte in relation to different abiotic stresses (Mulcahy, 1984; Ottaviano and Mulcahy, 1989; Ottaviano et al., 1990). Therefore, the selection of space candidate species and cultivars with the highest germination rate of pollen developed under altered gravity conditions can be considered a potential application of gametophytic selection in a space environment. Since it can be performed in a small environment and over a short period, this breeding procedure could be conducted by astronauts with the aim of screening and selecting species and cultivars that best adapt to real microgravity (De Micco et al., 2013).

To date, research has shown that simulated microgravity affects several aspects of pollen tube development (De Micco et al., 2005; De Micco et al., 2006), but how the gravity vector could affect pollen tube movements is still unknown. Despite this study proving a clear effect of simulated microgravity on the tortuosity of pollen tube movements (Figure 6), our results excluded gravitropism as a pollen tube response affecting the final position of tube tips in the germination medium (Figure 8).

How clinorotation affect the tortuosity of pollen tubes might be addressed by the key role of the cytoskeleton in coordinating vesicle trafficking responsible for pollen tube elongation (Sack, 1991). More specifically, clinorotation may have altered the production of metabolites related to pollen tube development, such as starch and callose, involved in the same ultrastructural changes occurring in the actin cytoskeleton of pollen tubes during the self-incompatibility reactions (De Micco et al., 2006; Cai and Del Duca, 2019). Indeed, the actin filaments located in the sub-apical portion of elongating pollen tubes are reported to be involved in the vesicle trafficking responsible for the formation and maintenance of the tube apex (Vidali et al., 2009; Qin and Yang, 2011; Hepler and Winship, 2014; Fu, 2015). Actin filaments of the cytoskeleton have a crucial role in tube development and growth direction, but how altered gravity can regulate the directionality of pollen tubes remains to be determined (Cheung et al., 2010).

To date, the microgametophyte (pollen) tube path towards the ovary is known to be mainly driven by chemotropic stimuli related to the Ca<sup>2+</sup> gradient throughout the style (Kandasamy et al., 1994; Kim et al., 2019), whereas the possible co-existence with pollen gravitropism has been generally overlooked. Despite *in vitro*



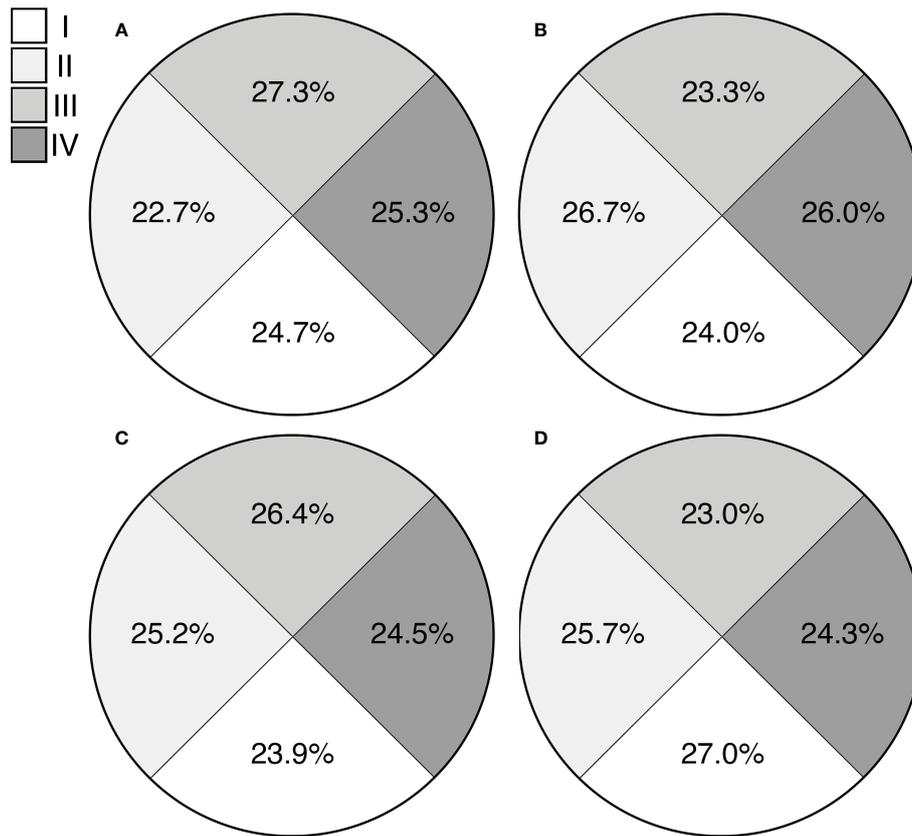


FIGURE 8

Evaluation of tube tip orientation of 'Micro-Tom' in simulated microgravity (A) and control (B) treatments and of *Brassica rapa* in simulated microgravity (C) and control (D) treatments. Percentage values indicate the observed frequencies of pollen tube tips reaching each quadrant.

conditions being assumed to mimic the natural state of pollen during pollen tube elongation, functional differences with *in vivo* or in *semi-vivo* conditions might occur. For instance, pollen tubes elongate faster, tubes live longer, and sperm cells (in bicellular species) form sooner and more reliably *in vivo* than *in vitro* (Kakani et al., 2005; Shi et al., 2018). Pollen tube growth *in vivo* involves interactions with pistil tissues, especially at critical stages such as tube reorientation and the cessation of growth. Thus, considering the role of chemotropism in the directional growth of pollen tubes, we performed the experiment *in vitro* to isolate the effect of the gravity vector on pollen tube directionality and exclude interferences related to chemotropic stimuli occurring *in vivo*.

Overall, our analyses investigating male gametophyte functionality in simulated microgravity suggested that pollen germination and tube directional growth are affected by changes in gravity and highlighted different responses between 'Micro-Tom' and *B. rapa*. To date, research has never considered gravitropism as a driver for pollen tube movement. Our *in vitro* germination tests represented the best first approach to exclude possible chemotropic interferences and isolate the effect of the altered gravity vector on the directional growth of pollen tubes.

Even though our results excluded gravitropism in pollen tubes, further tests are necessary to confirm our results in real

microgravity conditions and in other candidate space crops. In conclusion, a better understanding of how gravity can interfere with plant reproduction is needed to ensure seed and fruit production over repeated seed-to-seed-to-seed cycles, and it is a first step towards self-sufficiency from Earth supplies in long-term space missions.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

MI: Conceptualization, methodology, investigation, visualization, and writing—original draft preparation. LI: Investigation, visualization, and writing—review & editing. LR: Writing—review & editing. GA: Conceptualization, methodology, writing—review & editing, and funding acquisition. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

- Aronne, G., Iovane, M., and Strumia, S. (2021). Temperature and humidity affect pollen viability and may trigger distyly disruption in threatened species. *Ann. D. Bot.* 11, 77–82. doi: 10.13133/2239-3129/17157
- Aronne, G., Romano, L. E., and Izzo, L. G. (2020). Subsequent inclusion/exclusion criteria to select the best species for an experiment performed on the ISS in a refurbished hardware. *Life Sci. Space Res.* 27, 19–26. doi: 10.1016/j.lssr.2020.07.002
- Burgner, S. E., Nemali, K., Massa, G. D., Wheeler, R. M., Morrow, R. C., and Mitchell, C. A. (2020). Growth and photosynthetic responses of Chinese cabbage (*Brassica rapa* L. cv. Tokyo bekana) to continuously elevated carbon dioxide in a simulated space station “Veggie” crop-production environment. *Life Sci. Space Res.* 27, 83–88. doi: 10.1016/j.lssr.2020.07.007
- Cai, G., and Del Duca, S. (2019). Pollen tube and plant reproduction. *Int. J. Mol. Sci.* 20 (3), 531. doi: 10.3390/ijms20030531
- Cheung, A. Y., Niroomand, S., Zou, Y., and Wu, H. M. (2010). A transmembrane formin nucleates subapical actin assembly and controls tip-focused growth in pollen tubes. *Proc. Natl. Acad. Sci.* 107 (37), 16390–16395. doi: 10.1073/pnas.1008527107
- Colla, G., Roupael, Y., Cardarelli, M., Mazzucato, A., and Olimpieri, I. (2007). Growth, yield and reproduction of dwarf tomato grown under simulated microgravity conditions. *Plant Biosyst.* 141, 75–81. doi: 10.1080/11263500601153735
- Dafni, A. (1992). *Pollination ecology: a practical approach* (Oxford, UK: Oxford University Press).
- Dafni, A., Kevan, P. G., and Husband, B. C. (2005). *Practical pollination biology*. (Cambridge: Enviroquest Ltd).
- Dedolph, R. R., and Dipert, M. H. (1971). The physical basis of gravity stimulus nullification by clinostat rotation. *Plant Physiol.* 47 (6), 756–764. doi: 10.1104/pp.47.6.756
- De Micco, V., Aronne, G., and De Pascale, S. (2005). Effect of simulated microgravity on seedling development and vascular differentiation of soy. *Acta Astronautica* 58 (3), 139–148. doi: 10.1016/j.actaastro.2005.06.002
- De Micco, V., Pascale, S., Paradiso, R., and Aronne, G. (2013). Microgravity effects on different stages of higher plant life cycle and completion of the seed-to-seed cycle. *Plant Biol. J.* 16, 31–38. doi: 10.1111/plb.12098
- De Micco, V., Scala, M., and Aronne, G. (2006). Effects of simulated microgravity on male gametophyte of *Prunus pyrus*, and *Brassica* species. *Protoplasma* 228, 121–126. doi: 10.1007/s00709-006-0161-7
- De Pascale, S., Arena, C., Aronne, G., De Micco, V., Pannico, A., Paradiso, R., et al. (2021). Biology and crop production in space environments: challenges and opportunities. *Life Sci. Sp. Res.* 29, 30–37. doi: 10.1016/j.lssr.2021.02.005
- Fu, Y. (2015). The cytoskeleton in the pollen tube. *Curr. Opin. Plant Biol.* 28, 111–119. doi: 10.1016/j.pbi.2015.10.004
- Güçlü, F., and Koyuncu, F. (2017). Effects of relative humidity on *in vitro* pollen germination and tube growth in sweet cherries (*Prunus avium* L.). *Sci. Pap. Ser. B Hort.* 61, 15–19.
- Hepler, P. K., and Winship, L. J. (2014). The pollen tube clear zone: clues to the mechanism of polarized growth. *J. Integr. Plant Biol.* 57, 79–92. doi: 10.1111/jipb.12315
- Herrera, S., Lora, J., Hormaza, J. I., Herrero, M., and Rodrigo, J. (2018). Optimizing production in the new generation of apricot cultivars: self-incompatibility, s-RNase allele identification, and incompatibility group assignment. *Front. Plant Sci.* 9, doi: 10.3389/fpls.2018.00527
- Hormaza, J. I., and Herrero, M. Pollen selection. *Theor. Appl. Genet.* (1992) 83 (6-7), 663–672. doi: 10.1007/BF00226682
- Iovane, M., and Aronne, G. (2021). High temperatures during microsporogenesis fatally shorten pollen lifespan. *Plant Reprod.* 35, 9–17. doi: 10.1007/s00497-021-00425-0
- Iovane, M., Cirillo, A., Izzo, L. G., Di Vaio, C., and Aronne, G. (2022a). High temperature and humidity affect pollen viability and longevity in *Olea europaea* L. *Agronomy* 12, 1–12. doi: 10.3390/agronomy12010001
- Iovane, M., Izzo, L. G., Cirillo, A., Romano, L. E., Di Vaio, C., and Aronne, G. (2022b). Flowering and pollen resilience to high temperature of apricot cultivars. *Scientia Hort.* 304, 111261. doi: 10.1016/j.scienta.2022.111261
- Izzo, L. G., Romano, L. E., Muthert, L. W. F., Iovane, M., Capozzi, F., Manzano, A., et al. (2022). Interaction of gravitropism and phototropism in roots of *Brassica oleracea*. *environ. Exp. Bot.* 193, 104700. doi: 10.1016/j.envexpbot.2021.104700
- Kakani, V. G., Reddy, K. R., Koti, S., Wallace, T. P., Prasad, P. V. V., Reddy, V. R., et al. (2005). Differences in *in vitro* pollen germination and pollen tube growth of cotton cultivars in response to high temperature. *Ann. Bot.* 96 (1), 59–67. doi: 10.1093/aob/mci149
- Kandasamy, M. K., Nasrallah, J. B., and Nashrallah, M. E. (1994). Pollen-pistil interactions and developmental regulation of pollen tube growth in *Arabidopsis*. *Development* 120, 3405–3418. doi: 10.1242/dev.120.12.3405
- Karapanos, I. C., Akoumianakis, K. A., Olympios, C. M., and Passam, H. C. (2010). Tomato pollen respiration in relation to *in vitro* germination and pollen tube growth under favourable and stress-inducing temperatures. *Sex Plant Reprod.* 23, 219–224. doi: 10.1007/s00497-009-0132-1
- Kim, Y. J., Zhang, D., and Jung, K. H. (2019). Molecular basis of pollen germination in cereals. *Trends Plant Sci.* 24 (12), 1126–1136. doi: 10.1016/j.tplants.2019.08.005
- Kiss, J. Z., Wolverton, C., Wyatt, S. E., Hasenstein, K. H., and van Loon, J. J. W. A. (2019). Comparison of microgravity analogs to spaceflight in studies of plant growth and development. *Front. Plant Sci.* 10, 1577. doi: 10.3389/fpls.2019.01577
- Kordyum, E., and Hasenstein, K. H. (2021). Plant biology for space exploration – building on the past, preparing for the future. *Life Sci. Sp. Res.* 29, 1–7. doi: 10.1016/j.lssr.2021.01.003
- Kordyum, E., Hedukha, O., Artemeko, O., and Ivanenko, G. (2020). Seed and vegetative propagation of plants in microgravity 7 (5), 500–507. doi: 10.15982/j.issn.2096-9287.2020.20191113001
- Kordyum, E. L., Sytnik, K. M., and Chernyaeva, I. I. (1983). Peculiarities of genital organ formation in *Arabidopsis thaliana* (L.) heynh. under spaceflight conditions. *Adv. Space Res.* 3 (9), 247–250. doi: 10.1016/0273-1177(83)90064-9
- Kuang, A., Musgrave, M. E., and Matthews, S. W. (1996). Modification of reproductive development in *Arabidopsis thaliana* under spaceflight conditions. *Planta* 198, 588–594. doi: 10.1007/BF00262646
- Kuang, A., Musgrave, M. E., Matthews, S. W., Cummins, D. B., and Tucker, S. C. (1995). Pollen and ovule development in *Arabidopsis thaliana* under spaceflight conditions. *Am. J. Bot.* 82 (5), 585–595. doi: 10.1002/j.1537-2197.1995.tb11503.x
- Kuang, A., Popova, A., Xiao, Y., and Musgrave, M. E. (2000a). Pollination and embryo development in *Brassica rapa* L. @ in microgravity. *Int. J. Plant Sci.* 161, 203–211. doi: 10.1086/314254
- Kuang, A., Xiao, Y., McClure, G., and Musgrave, M. E. (2000b). Influence of microgravity on ultrastructure and storage reserves in seeds of *Brassica rapa* L. *Ann. Bot.* 85, 851–859. doi: 10.1006/anbo.2000.1153

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2023.1186967/full#supplementary-material>

- Link, B. M., Busse, J. S., and Stankovic, B. (2014). Seed-to-seed-to-seed growth and development of *Arabidopsis* in microgravity. *Astrobiology* 14, 866–875. doi: 10.1089/ast.2014.1184
- Matsuda, R., Ozawa, N., and Fujiwara, K. (2014). Leaf photosynthesis, plant growth, and carbohydrate accumulation of tomato under different photoperiods and diurnal temperature differences. *Sci. Hortic.* 170, 150–158. doi: 10.1016/j.scienta.2014.03.014
- Matsukura, C., Aoki, K., Fukuda, N., Mizoguchi, T., Asamizu, E., Saito, T., et al. (2008). Comprehensive resources for tomato functional genomics based on the miniature model tomato micro-tom. *Curr. Genomics* 9 (7), 436–443. doi: 10.2174/138920208786241225
- Merkies, A. I., and Laurinavichyus, R. S. (1983). Complete cycle of individual development of *Arabidopsis thaliana* haynh plants at salyut orbital station. *Doklady Akademii Nauk SSSR* 271 (2), 509–512.
- Mulcahy, D. L. (1984). “Manipulation of gametophytic populations,” in *Efficiency in plant breeding: proceedings 10th congress on eucarpia*. Eds. W. Lan-gel, A. C. Zeven and N. G. Hogenboom (Wageningen, the Netherlands: Pudoc, Wageningen), p 167–p 175.
- Musgrave, M. E., Kuang, A., Tuominen, L. K., Levine, L. H., and Morrow, R. C. (2005). Seed storage reserves and glucosinolates in *Brassica rapa* l. grown on the international space station. *J. Am. Soc Hortic. Sci.* 130, 848–856. doi: 10.21273/jashs.130.6.848
- Musgrave, M. E., Kuang, A., Xiao, Y., Stout, S. C., Bingham, G. E., Briarty, L. G., et al. (2000). Gravity independence of seed-to-seed cycling in *Brassica rapa*. *Planta* 210 (3), 400–406. doi: 10.1007/PL00008148
- Muthert, L. W. F., Izzo, L. G., van Zanten, M., and Aronne, G. (2020). Root tropisms: investigations on earth and in space to unravel plant growth direction. *Front. Plant Sci.* 10, 1807. doi: 10.3389/fpls.2019.01807
- Ottaviano, E., and Mulcahy, D. L. (1989). Genetics of angiosperm pollen. *Adv. Genet.* 26, 1–64. doi: 10.1016/S0065-2660(08)60222-9
- Ottaviano, E., Sari-Gorla, M., and Mulcahy, D. L. (1990). “Pollen selection: efficiency and monitoring,” in *Isozymes: structure, function, and use in biology and medicine*. Eds. Z. I. Ogita and C. L. Market (New York: Wiley-Liss), p 575–p 588.
- Pacini, E., and Dolferus, R. (2019). Pollen developmental arrest: maintaining pollen fertility in a world with a changing climate. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.00679
- Paupière, M. J., van Haperen, P., Rieu, I., Visser, R. G. F., Tikunov, Y. M., and Bovy, A. G. (2017). Screening for pollen tolerance to high temperatures in tomato. *Euphytica* 213, 230. doi: 10.1007/s10681-017-1927-z
- Qin, Y., and Yang, Z. (2011). Rapid tip growth: insights from pollen tubes. *Seminars Cell & Development. Biol.* 22 (8), 816–824. doi: 10.1016/j.semcd.2011.06.004
- Romano, L. E., and Aronne, G. (2021). The world smallest plants (*Wolffia* sp.) as potential species for bioregenerative life support systems in space. *Plants* 10 (9), 1896. doi: 10.3390/plants10091896
- Sack, F. D. (1991). Plant gravity sensing. *Int. Rev. Cytol* 127, 193–252. doi: 10.1016/S0074-7696(08)60695-6
- Salisbury, F. B. (1999). “Chapter 5 growing crops for space explorers on the moon, Mars, or in space,” in *Advances in space biology and medicine*. Ed. S. L. Bontinc (Stamford, Connecticut: Elsevier), 131–162.
- Sathasivam, M., Hosamani, R., K Swamy, B., and Kumaran G, S. (2021). Plant responses to real and simulated microgravity. *Life Sci. Sp. Res* 28, 74–86. doi: 10.1016/j.lssr.2020.10.001
- Sato, S., Katoh, N., Iwai, S., and Hagimori, M. (1998). Establishment of reliable methods of *in vitro* pollen germination and pollen preservation of *Brassica rapa* (syn. *B. campestris*). *Euphytica* 103, 29–33. doi: 10.1023/A:1018381417657
- Sato, S., Peet, M. M., and Gardner, R. G. (2001). Formation of parthenocarpic fruit, undeveloped flowers and aborted flowers in tomato under moderately elevated temperatures. *Sci. Hortic.* 90, 243–254. doi: 10.1016/S0304-4238(00)00262-4
- Shi, W., Li, X., Schmidt, R. C., Struik, P. C., Yin, X., and Jagadish, S. K. (2018). Pollen germination and *in vivo* fertilization in response to high-temperature during flowering in hybrid and inbred rice. *Plant Cell Environ.* 41 (6), 1287–1297. doi: 10.1111/pce.13146
- Shikata, M., and Ezura, H. (2016). Micro-tom tomato as an alternative plant model system: mutant collection and efficient transformation. *Plant Signal Transduction: Methods Protoc.* 1363, 47–55. doi: 10.1007/978-1-4939-3115-6\_5
- Tatematsu, K., Kumagai, S., Muto, H., Sato, A., Watahiki, M. K., Harper, R. M., et al. (2004). Massugu2 encodes Aux/IAA19, an auxin-regulated protein that functions together with the transcriptional activator NPH4/ARF7 to regulate differential growth responses of hypocotyl and formation of lateral roots in *Arabidopsis thaliana*. *Plant Cell* 16, 379–393. doi: 10.1105/tpc.018630
- Vidali, L., Rounds, C. M., Hepler, P. K., and Bezanilla, M. (2009). Lifeact-mEGFP reveals a dynamic apical f-actin network in tip growing plant cells. *PLoS One* 4 (5), e5744. doi: 10.1371/journal.pone.0005744
- Zabel, P., Bamsey, M., Schubert, D., and Tajmar, M. (2016). Review and analysis of over 40 years of space plant growth systems. *Life Sci. Sp. Res.* 10, 1–16. doi: 10.1016/j.lssr.2016.06.004