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Effect of modeled microgravity on UV-C-induced interplant communication of *Arabidopsis thaliana*

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ABSTRACT

Controlled ecological life support systems (CELSS) will be an important feature of long-duration space missions of which higher plants are one of the indispensable components. Because of its pivotal role in enabling plants to cope with environmental stress, interplant communication might have important implications for the ecological stability of such CELSS. However, the manifestations of interplant communication in microgravity conditions have yet to be fully elucidated. To address this, a well-established *Arabidopsis thaliana* co-culture experimental system, in which UV-C-induced airborne interplant communication is evaluated by the alleviation of transcriptional gene silencing (TGS) in bystander plants, was placed in microgravity modeled by a two-dimensional rotating clinostat. Compared with plants under normal gravity, TGS alleviation in bystander plants was inhibited in microgravity. Moreover, TGS alleviation was also prevented when plants of the *pgm-1* line, which are impaired in gravity sensing, were used in either the UV-C-irradiated or bystander group. In addition to the specific TGS-loci, interplant communication-shaped genome-wide DNA methylation in bystander plants was altered under microgravity conditions. These results indicate that interplant communications might be modified in microgravity. Time course analysis showed that microgravity interfered with both the production of communicative signals in UV-C-irradiated plants and the induction of epigenetic responses in bystander plants. This was further confirmed by the experimental finding that microgravity also prevented the response of bystander plants to exogenous methyl jasmonate (JA) and methyl salicylate (SA), two well-known airborne signaling molecules, and down-regulated JA and SA biosynthesis in UV-C-irradiated plants.

1. Introduction

With increasing human activities and scientific research being conducted on long-term space missions, controlled ecological life support systems (CELSS) have attracted growing attention. Higher plants, a key component in CELSS, can supply food, oxygen, and fresh water for human crews during long-term spaceflights and lunar or Mars habitation [1–3]. The current concerns regarding the use of higher plants in CELSS mainly relate to the selection of appropriate species and cultivars and the development of feasible culture conditions. Rather less attention has been focused on the balance and stability of plant populations as a small ecological system. In response to environmental changes or stresses, higher plants can not only initiate intraplant defense responses but also broadcast stress cues through interplant communication to prime stress defenses in neighboring plants [4–8]. It has also been reported that UV-C irradiation of *Arabidopsis thaliana* (*A. thaliana*) and tobacco enhances the genetic instability of neighboring plants via airborne interplant communication [9]. Recently, our work showed that UV-C irradiation of *A. thaliana* leads to an epigenetic alleviation of transcriptional gene silencing (TGS). The TGS-silenced loci of *TGS-GUS*, *TSI,* and *180-bp* repeats in neighboring plants are reactivated through airborne interplant communication, in which jasmonate (JA) and salicylate (SA) pathways play pivotal roles in the production of communicative signals in UV-C-irradiated plants and the responses of bystander plants to these signals [10]. Thus, interplant communication is considered to be important in retaining the stability of plant populations [6] and will have a potential effect on plant ecological systems in CELSS when they are exposed to a variable and heterogeneous space radiation environment.

On a space mission, the CELSS will inevitably be subjected to

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another constant space environmental factor, microgravity (10^{-4}–10^{-6} g). Microgravity can affect plants in terms of physiology, biochemistry, and genetics [11]. Additionally, microgravity is also able to modify the effects of radiation on cells or organisms through additive, synergistic, or antagonistic modalities [12]. This emphasizes the need to determine whether microgravity also affects interplant communication. In previous studies, it has been reported that the JA signaling pathway mediates radiation-induced root-to-shoot signaling within *A. thaliana* [13], and, interestingly, that bystander signaling can be affected by modeled microgravity [14]. The nature of interplant communication is considered to be an eavesdropping of plants on the actively or passively divulged internal signals from neighboring plants [15,16]. It is therefore reasoned that interplant communication should also be affected by microgravity. To test this hypothesis, a well-established co-culture experimental system for interplant communication was placed under modeled microgravity conditions created by a two-dimensional rotating clinostat. The epigenetic alleviation of TGS in bystander plants was used as a biological endpoint to evaluate the manifestations of interplant communication under microgravity conditions.

2. Materials and methods

2.1. A. thaliana lines and plant growth

The *A. thaliana* wild-type (Col-0) and mutant line *pgm-1* used in this study were obtained from the Nottingham *Arabidopsis* Stock Center (NASC, UK). The *pgm-1* plant is unable to synthesize starches due to a lack of the gene encoding plastidic phosphoglucomutase (*PGM*) [17]. Line L5-1, which harbors a single insert of a multicopy of *P35S:GUS* (*TGS-GUS*), was a gift from Dr. Ortrun Mittelsten Scheid (Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Austria) [18,19].

Surface-sterilized seeds of *A. thaliana* were sown on growth medium [1 × Murashige and Skoog (MS) mineral salts, agar at 0.8% (w/v), and sucrose at 1% (w/v)] in Petri dishes. After 48 h of stratification at 4 °C, the Petri dishes were placed in a growth chamber at 22 °C with continuous illumination at approximately 100 μM m^{-2} s^{-1}. In order to avoid root breakage during transplantation, the Petri dishes were placed in a vertical orientation so that the roots grew along the agar surface.

2.2. Protocols for co-culture between UV-C-irradiated and bystander plants

Co-culture experiments of *A. thaliana* seedlings were carried out using an enclosed two-divided Petri dish, in which UV-C-irradiated and bystander groups were physically separated, and can communicate with each other only in an airborne manner [10,20]. In detail, five 10-day-old *A. thaliana* plants grown in Petri dishes were subjected to 1200 J m^{-2} of UV-C irradiation using a 15 W UV-C germicidal lamp (100 μW cm^{-2} s^{-1}). The UV-C-irradiated plants were immediately transferred onto the medium on one side of a two-divided Petri dish (Φ = 9 cm), on the other side of which ten 2-day-old plants (bystander plants) had been grown (Fig. 1). The Petri dish was sealed with parafilm. After 10 days of co-culture, the bystander plants were sampled for the subsequent assays. For treatment with volatiles of MeJA and MeSA, an Eppendorf tube cap was mounted in an agar medium on one side of a two-divided Petri dish, on the other side of which ten 2-day-old plants had been grown. After adding 100 μl of MeSA and MeJA solutions into the cap, the Petri dish was immediately sealed with parafilm. After 10 days of treatment, the bystander plants were sampled for the subsequent assays.

2.3. Protocols for modeled microgravity treatment

A two-dimensional rotating clinostat, constructed by the Center for Space Science and Applied Research of the Chinese Academy of Sciences, was used to produce modeled microgravity, the details of which have been described in a previous study [14]. After UV-C irradiation, the seedlings were transferred onto the surface medium on one side of the two-divided Petri dishes. The Petri dishes were then immediately fixed onto the rotating disk, as shown in Fig. 1. The rotating clinostat rotated continuously at a speed of 2 rpm, and the distance from the *A. thaliana* seedlings to the rotating center was approximately 12 cm. Thus, the seedlings were subjected to a modeled microgravity of about $5 \times 10^{-4} g$. Microgravity experiments were conducted under culture conditions similar to those used in growth chambers. The control plants were fixed statically on the side of the rotating clinostat in a vertical orientation.

2.4. Methylation-sensitive amplification polymorphism (MSAP) analysis

MSAP analysis was performed according to a published protocol [21] with slight modifications, in which the restriction enzymes *Hpa*II and *Msp*I were used to detect methylation polymorphisms. The adapters and primers used for the analysis are listed in Supplemental Table 1. The detailed procedure has been described previously [22].

2.5. Quantitative analysis and histochemical staining of GUS activity

For the analysis of GUS activity in bystander plants, five plants of line L5-1 with uniform size were randomly selected from each experiment. The aerial parts of the plants, not including the hypocotyls, were sampled. A quantitative assay of GUS activity in the aerial plant parts was performed according to a previously described protocol [23]. In the present study, the samples were measured separately. The incubation time in 4-MUG assay solution was 5 h for 12-day-old plants. The fluorescence at 455 nm under excitation at 365 nm was measured using a luminescence spectrophotometer equipped with an ELISA plate reader (Spectra Max M2, Molecular Devices, USA). The final data were the average of 15 plants from three independent experiments. The histochemical staining of L5-1 plants was performed as described previously [24].

2.6. qRT-PCR analysis of RNAs of TGS loci

RNA levels of the *TSI*, *180-bp* repeats, and the JA and SA biosynthesis and signaling genes were measured using qRT-PCR, and the corresponding PCR primers of which are listed in Supplemental Table 2. Five to eight *A. thaliana* plants were collected from each independent experiment. The samples were homogenized in liquid nitrogen, and total RNA was extracted using the Trizol reagent (Invitrogen, USA) according to the manufacturer's protocols. Total RNA was reverse transcribed using the Transcript One-Step gDNA Removal and cDNA Synthesis Supermix kit (TransGen Biotech, China) according to the manufacturer's protocols. qRT-PCR was performed under the following conditions: one cycle of 95 °C for 10 s and 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The *UBQ5* gene was used as an internal control. The final data were the average of three independent experiments, with three technical replicates for each experiment.

2.7. Statistical analysis

All results are presented as means ± SD. The statistical significance of the experiments was determined by performing Student's *t* test. A *P*-value of 0.05 or less was considered to be significant.

3. Results

3.1. The effects of modeled microgravity on interplant communication

In a previously established *A. thaliana* co-culture experimental

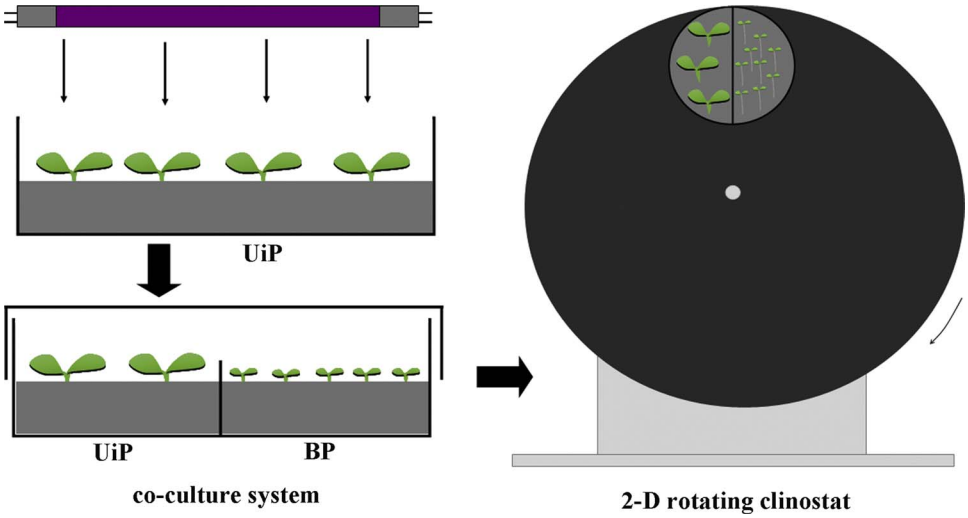

Fig. 1. Schematics of the co-culture experimental system for airborne interplant communication and microgravity treatment. UiP: UV-C irradiated plants, BP: bystander plants.

system, expression of TGS-silenced *TGS-GUS*, *TSI*, and *180-bp* repeats loci in bystander plants were found to be epigenetically activated by 1200 J m^{-2} of UV-C irradiation via airborne interplant communication [10]. Here, we further investigated the effects of microgravity on the airborne interplant communication by placing the co-culture system in a two-dimensional rotating clinostat. It was shown that microgravity significantly inhibited the activation of *TGS-GUS* (GUS activity) in bystander L5-1 plants ($P > 0.05$), as shown in Fig. 2A and B. We also examined the RNA levels of *TSI* and *180-bp* repeats in bystander plants using qRT-PCR after co-culture with UV-C-irradiated plants. As shown in Fig. 2C, microgravity prevented the up-regulation of these TGS-loci (in all cases, $P > 0.05$). These results indicated that airborne interplant communication for alleviation of TGS might be modified in microgravity.

In order to further strengthen this conclusion, *A. thaliana pgm-1* mutant seedlings, which show impaired gravity sensing due to a lack of starch statoliths in the columella cells of the root cap [17,25], were introduced into the co-culture system. When *pgm-1* seedlings were used as the UV-C-irradiated group, activation of *TGS-GUS* in bystander L5-1 plants was inhibited ($P > 0.05$), even under normal gravity, as shown in Fig. 3A. Similar expression levels were observed for the *TSI* and *180-bp* repeats in bystander plants (in both cases, $P > 0.05$), as shown in Fig. 3B. When *pgm-1* mutant plants were used as the bystander group, RNA levels of the *TSI* and *180-bp* repeats in these plants did not significantly increase after co-culture with UV-C-irradiated wild-type plants under normal gravity (in both cases, $P > 0.05$), as shown in Fig. 3C. These results further confirmed an effect of microgravity on airborne interplant communication.

3.2. The interplant communication-shaped status of DNA methylation in microgravity

In addition to the specific TGS-loci, we further wanted to determine whether microgravity also alters the status of other epigenetic marks shaped by interplant communication. For this purpose, genome-wide DNA methylation in bystander plants was examined using MSAP analysis after co-culture with UV-C-irradiated plants. Under conditions of normal gravity, the proportion of methylated CCGG sites increased from 19.93% in control plants to 20.63% in bystander plants, in which the full-methylation ratio (type III and type IV) increased from 17.13% in control plants to 17.48% in bystander plants, and the hemi-methylation ratio from 2.8% to 3.15%, as shown in Table 1. However, when th e co-culture experimental system was subjected to microgravity, the proportion of methylated CCGG sites decreased from 21.68% in control plants to 19.58% in bystander plants (Table 1), in which the full-methylation ratio (type III and type IV) decreased from 18.88% in control plants to 16.43% in bystander plants, whereas the hemi-methylation ratio increased from 2.8% to 3.15%.

The banding patterns of MSAP were further divided into three classes in terms of DNA methylation status. The patterns A–C represented the monomorphic class, in which the methylation pattern remained unchanged; the patterns D–I represented cytosine demethylation patterns; and the patterns J–O represented methylation pattern. As shown in Table 2, under normal gravity conditions, UV-C irradiation resulted in 0.7% demethylation of CCGG sites, and 1.05% methylation of CCGG sites in bystander plants through interplant communication. However, under microgravity conditions, UV-C irradiation caused a 2.46% demethylation of CCGG sites and a 0.35% methylation of CCGG sites in bystander plants. These results suggested that microgravity might modify interplant communications for regulating DNA methylation, and this possibly affects genetic stability of bystander plants due to a correlation between genome-wide demethylation and genetic instability [26].

3.3. The effect of microgravity on the process of interplant communication

Airborne interplant communication involves the production of volatile signals in stressed plants, their airborne propagation between plants, and the responses of bystander plants to the volatile signals. Therefore, it is of interest to determine which biological step(s) in interplant communication is affected by microgravity. In our previous study, we demonstrated that volatile signals are produced and released from UV-C-irradiated plants within 24 h after UV-C irradiation [10]. In order to clarify the effect of microgravity on the production of volatile signals, the co-culture system was immediately placed on a rotating clinostat for 24 h after UV-C irradiation. Then UV-C-irradiated plants were subsequently removed from the co-culture system, and the remaining bystander plants were allowed to grow for 9 days in normal gravity. As shown in Fig. 4A, the activation of *TGS-GUS* in bystander L5-1 plants was significantly inhibited by 24 h of microgravity treatment after UV-C irradiation. In order to determine whether microgravity affects the response of bystander plants to communicative signals, the co-culture system was initially subjected to normal gravity for 24 h after UV-C irradiation. Then the UV-C irradiated seedlings were removed from the co-culture system, and the remaining bystander plants were subjected to microgravity for 9 days. As shown in Fig. 4B, the latter microgravity treatment likewise inhibited the activation of *TGS-GUS* in bystander L5-1 plants. These preliminary results indicated that microgravity might interfere with both the production of volatile signals in UV-C-irradiated plants and the response of bystander plants

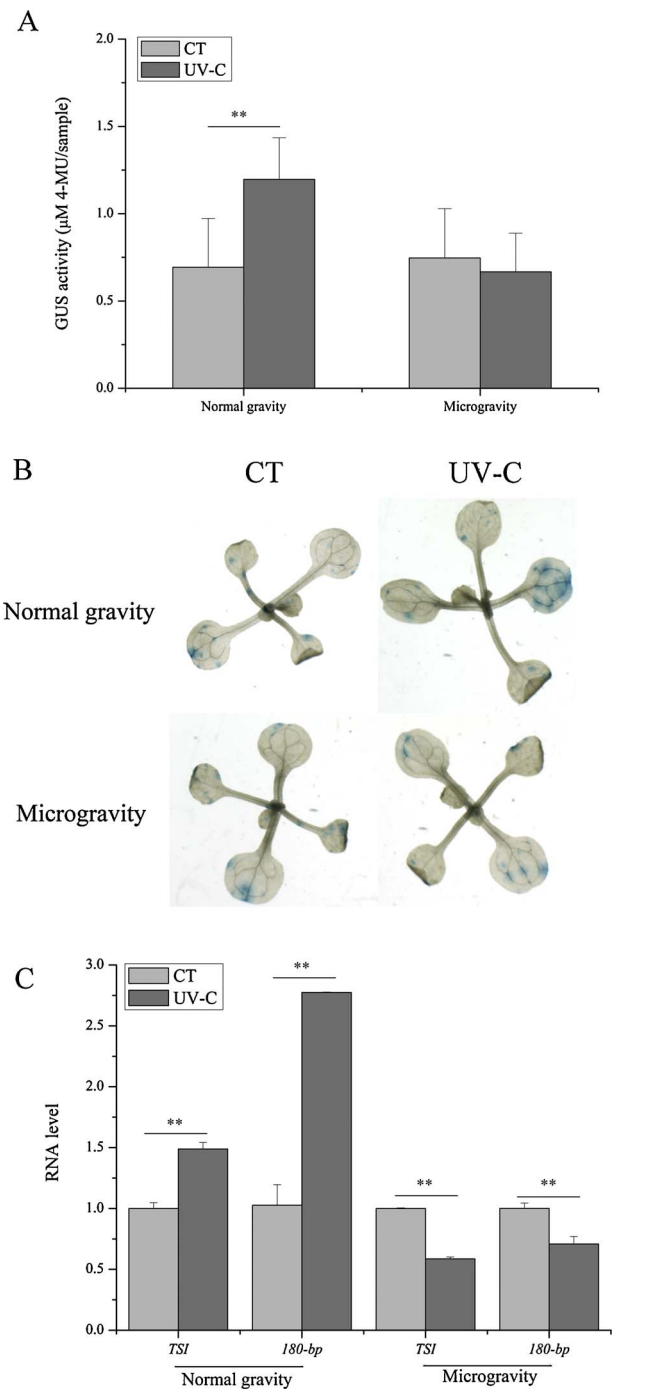

Fig. 2. The repressive effects of modeled microgravity on UV-C-induced interplant communication. A) GUS activity in bystander L5-1 plants co-cultured with wild-type plants exposed to 1200 J m^{-2} of UV-C irradiation under normal gravity or modeled microgravity. B) Histochemical staining of bystander L5-1 plants co-cultured with UV-C-irradiated plants under normal gravity or modeled microgravity. C) RNA levels of the *TSI* and *180-bp* repeats in bystander L5-1 plants after co-culture with UV-C-irradiated plants under normal gravity or modeled microgravity. Results are means ± SD (n = 15 for GUS activity, n = 3 for RNA levels, *t*-test $^{**}P < 0.01$).

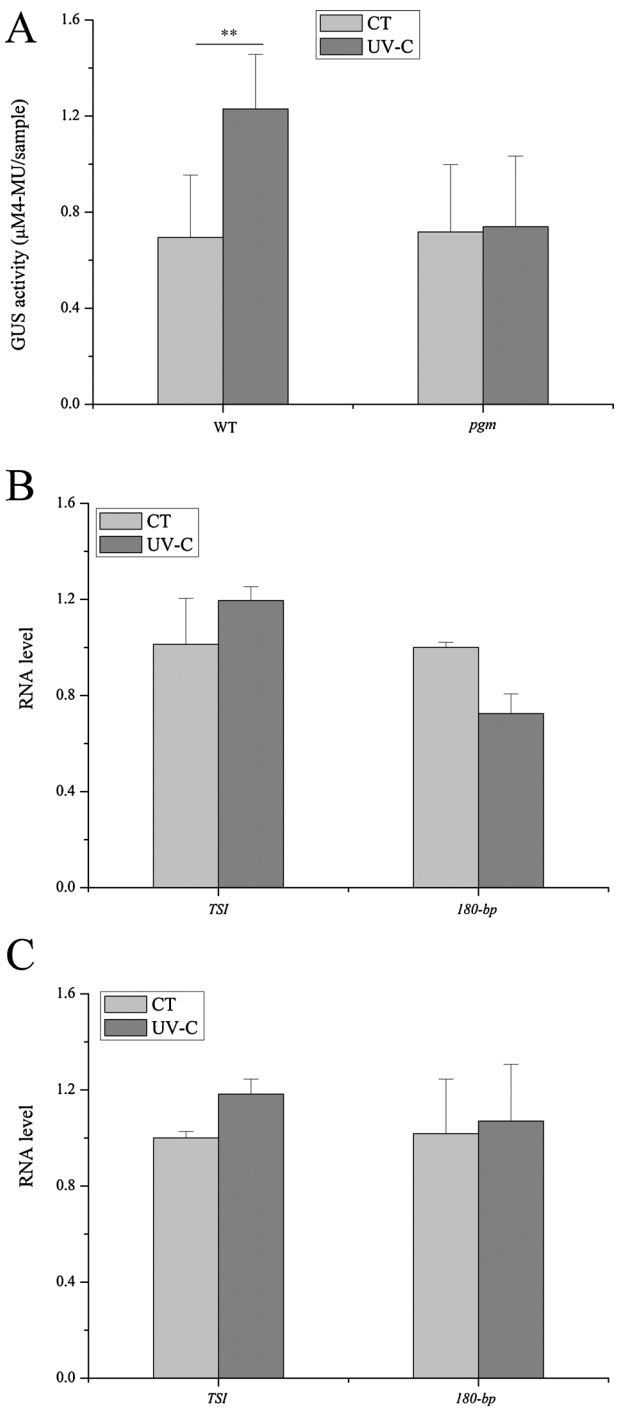

Fig. 3. Interplant communication among plants deficient in gravity sensing. A) GUS activity in bystander L5-1 plants co-cultured with UV-C-irradiated *pgm-1* mutant plants. B) RNA levels of the *TSI* and *180-bp* repeats in the bystander plants co-cultured with UV-C-irradiated *pgm-1* mutant plants. C) RNA levels of the *TSI* and *180-bp* repeats in bystander *pgm-1* mutant plants co-cultured with UV-C-irradiated wild-type plants. Results are means ± SD (n = 15 for GUS activity, n = 3 for RNA levels, *t*-test $^{**}P < 0.01$).

to the volatile communicative signals.

3.4. The responses of plants to exogenous MeJA and MeSA in microgravity

MeJA and MeSA have been identified as volatile signaling molecules in interplant communication, and TGS can be alleviated in *A. thaliana* plants exposed to exogenous MeJA and MeSA [10]. In order to further determine the effect of microgravity on the response of bystander plants to communicative signals, L5-1 seedlings were subjected to these exogenous volatiles, MeJA and MeSA, in microgravity by replacing UV-C-irradiated plants with 100 μl of 0.5 mM MeSA and MeJA solutions in the co-culture system. It was shown that microgravity treatment repressed the activation of *TGS-GUS* by MeJA and MeSA in "bystander" plants compared with that in normal gravity (in both cases, $P < 0.05$), as shown in Fig. 5A. Moreover, the MeJA and MeSA-induced

Table 1
Effect of microgravity on interplant communication-shaped methylation level.

Type	Normal gravity		Microgravity	
	CT	UVC	CT	UVC
I	229	227	224	230
II	8	9	8	9
III	47	49	50	45
IV	2	1	4	2
Total sites	286	286	286	286
Total amplified bands	284	285	282	284
Total methylated bands	57	59	62	56
MSAP(%)[a]	19.93	20.63	21.68	19.58
Full methylated bands	49	50	54	47
Full methylated ratio(%)[b]	17.13	17.48	18.88	16.43
Hemi-methylated ratio(%)[c]	2.8	3.15	2.8	3.15
Non-methylated ratio(%)[d]	80.07	79.37	78.32	80.42

[a] MSAP(%) = [(II + III + IV)/(I + II + III + IV)] × 100.
[b] Fully methylated ratio(%) = [(III + IV)/(I + II + III + IV)] × 100.
[c] Hemi-methylated ratio(%) = [(II)/(I + II + III + IV)] × 100.
[d] Non-methylated ratio(%) = [(I)/(I + II + III + IV)] × 100.

Table 2
Effect of microgravity on interplant communication-shaped methylation pattern.

Description of Pattern	CLASS	Banding Pattern				Normal Gravity CT (A)-UV-C (B)	Microgravity CT(A)-UV-C(B)
		Sample A		Sample B			
		H	M	H	M		
No change	A	1	1	1	1	226	223
	B	1	0	1	0	46	45
	C	0	1	0	1	8	8
	TOTAL					280	276
	RATIO					98.25%	97.18%
Demethylation	D	1	0	1	1	0	0
	E	0	1	1	1	1	5
	F	0	0	1	1	0	2
	G	0	1	1	0	0	0
	H	0	0	1	0	1	0
	I	0	0	0	1	0	0
	TOTAL					2	7
	RATIO					0.70%	2.46%
Methylation	J	1	1	1	0	0	1
	K	1	1	0	1	3	0
	L	1	0	0	1	0	0
	M	1	1	0	0	0	0
	N	1	0	0	0	0	0
	O	0	1	0	0	0	0
	TOTAL					3	1
	RATIO					1.05%	0.35%

Notes: Column H and Column M represents the pattern after digestion with EcoRI/HpaII and EcoRI/MspI respectively.1 represents the presence of bands, and 0 represents the absence of bands.

expressions of *TSI* and *180-bp* repeats were significantly inhibited in microgravity (in all cases, $P < 0.01$, except for *TSI* in MeSA treatment, $P > 0.05$). We also further examined the response of *pgm-1* plants to MeJA and MeSA, and found that the MeJA and MeSA-induced expression of *TSI* and *180-bp* repeats could also be prevented in a background of impaired gravity sensing (in all cases, $P < 0.01$), as shown in Fig. 5B. These results further indicated that microgravity might interfere with the response of bystander plants to volatile signals in interplant communication.

In the above experiments, MeJA and MeSA were conveyed to "bystander" plants in an airborne manner. This raises the question of whether microgravity can repress the response of bystander plants to volatile MeJA and MeSA by affecting their airborne propagation. To exclude this possibility, *A. thaliana* seedlings were grown in a medium containing 10 μM MeJA and MeSA. The activation of *TGS-GUS* in L5-1 plants was also inhibited in microgravity as compared to those observed in normal gravity (in both cases, $P < 0.05$), as shown in Fig. 5C.

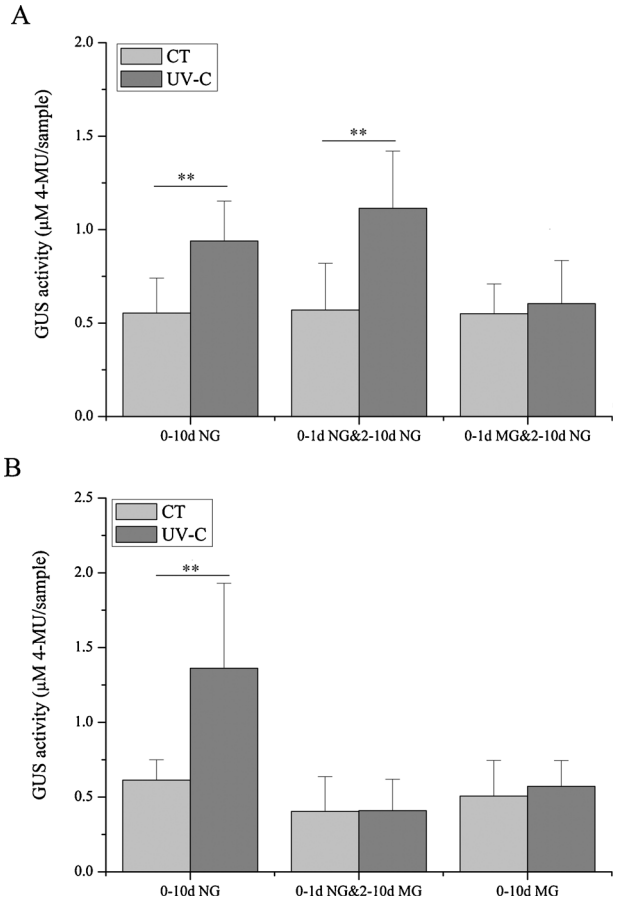

Fig. 4. Effect of microgravity on the process of interplant communication. A) GUS activity in bystander L5-1 plants subjected to 1 day of co-culture with UV-C-irradiated plants in microgravity (marked as 0-1dMG & 2-10dNG). B) GUS activity in bystander L5-1 plants subjected to microgravity within 2–10 days after 1 day of co-culture with UV-C-irradiated plants in normal gravity (marked as 0-1dNG & 2-10dMG). Results are means ± SD (n = 15 for GUS activity, *t*-test $^{**}P < 0.01$). NG: normal gravity, MG: microgravity.

3.5. The effect of microgravity on the JA and SA signal pathways in UV-C-irradiated plants

In order to further determine the effect of microgravity on the production of volatile signals in UV-C-irradiated plants, we measured the mRNA levels of several key genes in JA (*LOX2*, *AOS*, *AOC2*, and *OPR1*) and SA (*PAD4*, *EDS1*, *NDR1*, *EDS5*, and *SID2*) biosynthesis at 6 h and 12 h after UV-C irradiation. At 6 h after UV-C irradiation, with the exception of the *EDS1* gene, the expression of these genes was significantly up-regulated in normal gravity (in all cases, $P < 0.01$), but were repressed in microgravity (in all cases, $P < 0.01$). As marker genes for SA and JA signaling, the expression of *PR2* (SA), but not *PDF1.2* (JA), was down-regulated by microgravity treatment ($P < 0.01$), as shown in Fig. 6A. At 12 h after UV-C irradiation, mRNA levels of the genes in JA and SA biosynthesis with the exception of *EDS1* had almost returned to basal levels in normal gravity (in all cases, $P > 0.05$), and their expressions continued to be reduced under microgravity (in all cases, $P < 0.01$). Interestingly, the expressions of *PDF1.2* and *PR2* were both down-regulated in microgravity (in both cases, $P < 0.01$), as shown in Fig. 6B.

4. Discussion

Interplant communication could prepare plant populations for more

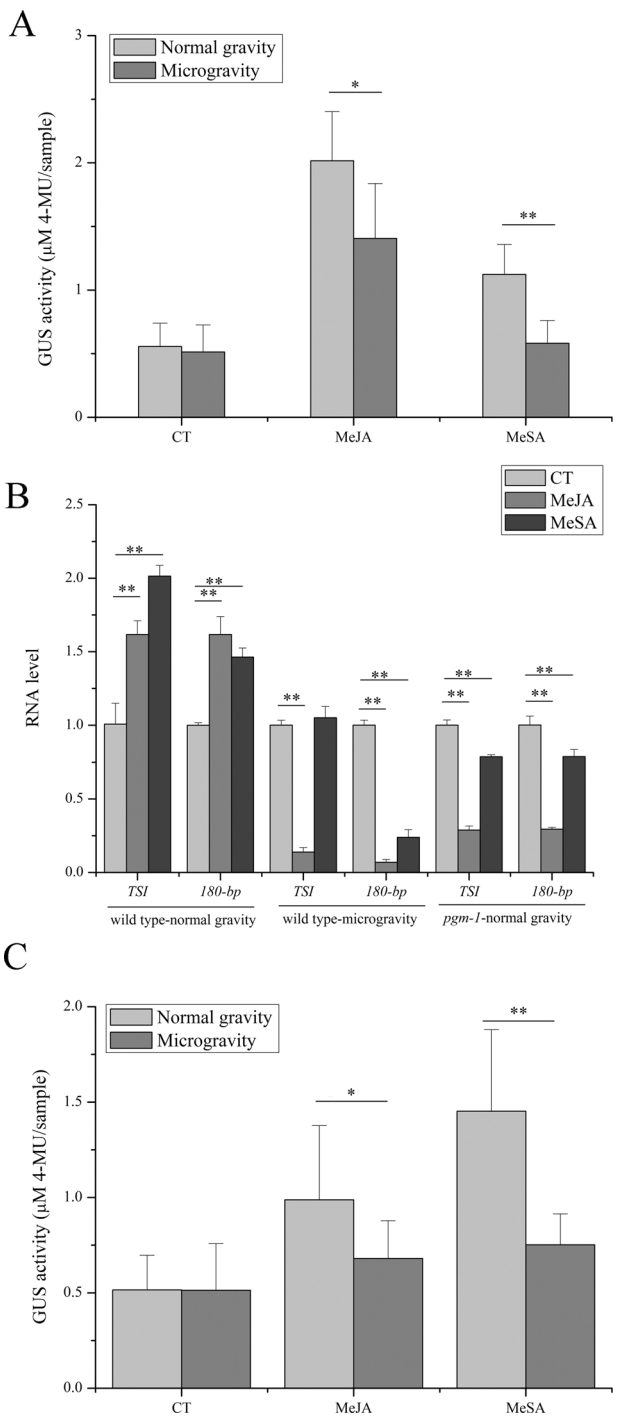

Fig. 5. Responses of plants to exogenous MeJA and MeSA in microgravity. A) GUS activity in "bystander" L5-1 plants exposed to volatiles of MeJA or MeSA. B) RNA levels of *TSI* and *180-bp* repeats in wild-type and *pgm-1* mutant plants exposed to volatiles of MeJA or MeSA. C) GUS activity in L5-1 plants grown in MS medium containing 10 μM MeJA or MeSA. Results are means ± SD (n = 15 for GUS activity, n = 3 for RNA levels, *t*-test $*P < 0.05$, $**P < 0.01$).

robust responses to subsequent environmental stress and is believed to contribute to the stability of plant ecological populations [6]. In this study, we presented the first evidence that interplant communication in response to UV-C-irradiation is modified in microgravity. Considering the important role of interplant communication in the defense response of plant populations [8], this finding indicates that the plant systems in CELSS might be ecologically unstable in long-term space flights. In interplant communications, different biological events in bystander plants are typically induced by distinct signals or signal arrays. For instance, the impairment in JA or SA biosynthesis (*aos* or *sid2*) in UV-C-irradiated plants suppresses the alleviation of TGS [10], but not the induction of homologous recombination in bystander plants [9]. However, in this study, only the status of TGS and DNA methylation were adopted as biological endpoints, and it is therefore difficult to determine whether microgravity modifies interplant communications for other biological events in the same way. Thus, it is desirable to confirm the universality of this phenomenon by investigating more types of biological events, and also other stresses, including the more relevant ionizing irradiation. It has also been reported that under long-term microgravity conditions, plants acclimatize to this stress by modifying their metabolism and oxidative responses and enhancing other tropic responses [27]. However, in the present study, the microgravity treatment was short-term (no more than 10 days), and accordingly, it is not possible to predict whether plants on long-term space missions, which may encompass several plant generations, can develop adaptations to counter the repressive effect of microgravity on interplant communication.

Plants sense gravity via starch statoliths in the columella cells of the root cap, in which physical sedimentation of the statoliths triggers biochemical and physiological signals [28–33] that alter the polar translocation of the plant hormone auxin [34]. In the present study, microgravity treatment and impairment of gravity sensing (*pgm-1*) both prevented the response of bystander plants to volatile signals, including MeJA and MeSA (Fig. 3). It has been reported that the JA and SA signaling pathways play an important role in the response of bystander plants to volatile signals [9,10]. Thus, considering the universal crosstalk among various signaling pathways [32,35–37], it is likely that the microgravity-initiated signaling pathways might antagonize the JA and SA signaling pathways in bystander plants, and subsequently interfere with their responses to volatile signals. However, the initiation of TGS alleviation in bystander plants requires a prolonged time interval (9 days) after perception of the volatile signals, suggesting that TGS alleviation might also be synergistically regulated by plant developmental cues [10]. Auxin is involved in the regulation of plant growth and development [38], and, importantly, its translocation and distribution in plants can also be altered under microgravity conditions or in the *pgm-1* mutant background [37,39–43]. Therefore, it is also conceivable that microgravity might initially modulate the developmental program of bystander plants, and then the altered developmental cues might interfere with the regulatory effects of the volatile signals on TGS status.

In this study, time course analysis showed the repressive effect of microgravity on the production of volatile signals in UV-C-irradiated plants (Fig. 4A), and the expression of genes involved in JA and SA biosynthesis was also significantly down-regulated in microgravity (Fig. 6). Although MeJA and MeSA are two important volatile signaling molecules in interplant communication, plants also produce and emanate other types of volatile organic compounds (VOCs) in response to various environmental stresses [44,45]. Additionally, interplant communication is synergistically implemented by a blend of volatile signals, including MeJA and MeSA [9,10,46,47]. Therefore, the possibility that microgravity might also affect the production of other signaling components cannot be excluded. Impairment of the JA signaling pathway in UV-C-irradiated jasmonic acid receptor (*jar1-1*) mutant plants can also prevent interplant communication, indicating that production of other signaling molecules might be regulated by the JA signaling pathway [10]. In this study, the JA signaling pathway was also down-regulated in microgravity at 12 h after UV-C irradiation, although it is unclear whether this is attributable to direct interaction with microgravity, or down-regulated JA biosynthesis. The results also indicate that microgravity treatment might affect the production of other volatile signals in UV-C-irradiated plants. The SA signaling pathway was also down-regulated in microgravity at 6 h and 12 h after UV-C irradiation (Fig. 6). However, we cannot determine the role of SA signaling in the repressive

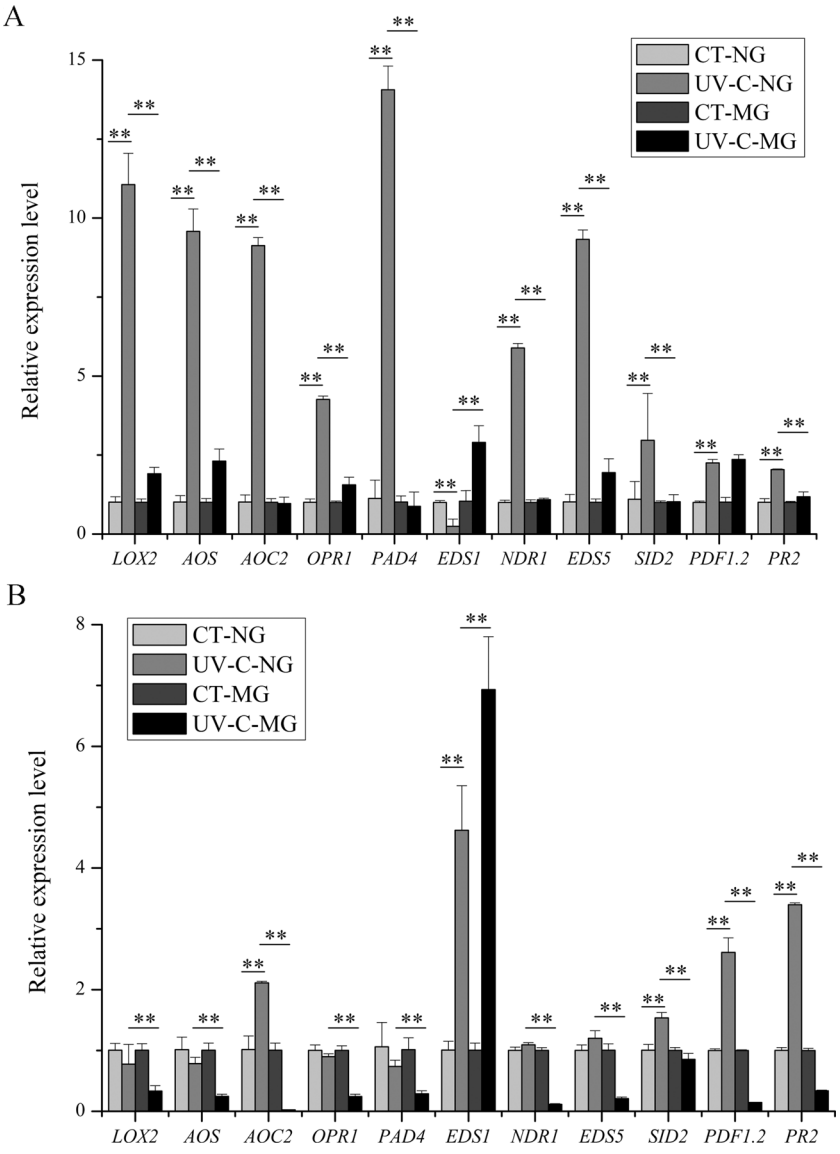

Fig. 6. Effects of microgravity on the JA and SA signaling pathways in UV-C-irradiated plants. The mRNA levels of JA and SA biosynthesis genes and signaling genes in UV-C-irradiated plants were measured at 6 h (A) and 12 h (B) after UV-C irradiation. Results are means ± SD (n = 3, *t*-test $^{**}P < 0.01$). NG: normal gravity, MG: microgravity.

effect of microgravity on the production of volatile signals because the SA signaling pathway is not implicated in the production of interplant-communication volatile signals in conditions of normal gravity [10].

Overall, in the present study, we present robust evidence regarding the effect of microgravity on interplant communication. On the basis of our findings, it is suggested that the modification of interplant communication in microgravity might also be one of the potential factors that affect plant systems in the CELSS of space missions. In future studies, we intend to investigate the universality of the effect of microgravity on interplant communication using more types of biological endpoints, particularly those relating to plant growth and development.

Conflict of interests

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mrfmmm.2017.09.001.

References

[1] D. Chamberland, W.M. Knott, J.C. Sager, R. Wheeler, Controlled ecological life-support system. Use of plants for human life-support in space, J. Fla. Med. Assoc. 79 (1992) 537–544.
[2] D.L. Bubenheim, Plants for water recycling, oxygen regeneration and food production, Waste Manage. Res. 9 (1991) 435–443.
[3] F.B. Salisbury, Growing crops for space explorers on the moon, Mars, or in space, Adv. Space Biol. Med. 7 (1999) 131–162.
[4] K. Chamberlain, Airborne plant–plant communication, Skvortsovia 1 (2014) 112–132.
[5] K. Yoneya, J. Takabayashi, Plant-plant communication mediated by airborne signals: ecological and plant physiological perspectives, Plant Biotechnol. 31 (2014) 409–416.
[6] M. Heil, R. Karban, Explaining evolution of plant communication by airborne signals, Trends Ecol. Evol. 25 (2010) 137–144.
[7] M. Heil, J.C. Silva Bueno, Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 5467–5472.
[8] I.T. Baldwin, R. Halitschke, A. Paschold, C.C. von Dahl, C.A. Preston, Volatile

signaling in plant–plant interactions: “Talking Trees” in the genomics era, Science 311 (2006) 812–815.
[9] Y. Yao, C.H. Danna, F.J. Zemp, V. Titov, O.N. Ciftci, R. Przybylski, F.M. Ausubel, I. Kovalchuk, UV-C-irradiated Arabidopsis and tobacco emit volatiles that trigger genomic instability in neighboring plants, Plant Cell 23 (2011) 3842–3852.
[10] W. Xu, T. Wang, S. Xu, F. Li, C. Deng, L. Wu, Y. Wu, P. Bian, UV-C-induced alleviation of transcriptional gene silencing through plant–plant communication: key roles of jasmonic acid and salicylic acid pathways, Mutat. Res. 790 (2016) 56–67.
[11] G. Perbal, Plant development in microgravity, in: G. Clement, K. Slenzka (Eds.), Fundamentals of Space Biology, The Space Technology Library, 2006, pp. 227–290.
[12] J. Kiefer, H.D. Pross, Space radiation effects and microgravity, Mutat. Res. 430 (1999) 299–305.
[13] T. Wang, W. Xu, C. Deng, S. Xu, F. Li, Y. Wu, L. Wu, P. Bian, A pivotal role of the jasmonic acid signal pathway in mediating radiation-induced bystander effects in *Arabidopsis thaliana*, Mutat. Res. 791 (2016) 1–9.
[14] T. Wang, Q. Sun, W. Xu, F. Li, H. Li, J. Lu, L. Wu, Y. Wu, M. Liu, P. Bian, Modulation of modeled microgravity on radiation-induced bystander effects in *Arabidopsis thaliana*, Mutat. Res. 773 (2015) 27–36.
[15] R. Karban, J. Maron, The fitness consequences of interspecific eavesdropping between plants, Ecology 83 (2002) 1209–1213.
[16] R. Karban, J. Maron, G.W. Felton, G. Ervin, H. Eichenseer, Herbivore damage to sagebrush induces resistance in wild tobacco: evidence for eavesdropping between plants, Oikos 100 (2003) 325–333.
[17] S. Streb, B. Egli, S. Eicke, S.C. Zeeman, The debate on the pathway of starch synthesis: a closer look at low-starch mutants lacking plastidial phosphoglucomutase supports the chloroplast-localized pathway, Plant Physiol. 151 (2009) 1769–1772.
[18] A. Pecinka, H.Q. Dinh, T. Baubec, M. Rosa, N. Lettner, O. Mittelsten Scheid, Epigenetic regulation of repetitive elements is attenuated by prolonged heat stress in *Arabidopsis*, Plant Cell 22 (2010) 3118–3129.
[19] J.B. Morel, P. Mourrain, C. Beclin, H. Vaucheret, DNA methylation and chromatin structure affect transcriptional and post-transcriptional transgene silencing in *Arabidopsis*, Curr. Biol. 10 (2000) 1591–1594.
[20] F.J. Zemp, C. Sidler, I. Kovalchuk, Increase in recombination rate in Arabidopsis thaliana plants sharing gaseous environment with X-ray and UVC-irradiated plants depends on production of radicals, Plant Signal. Behav. 7 (2012) 782–787.
[21] L.Z. Xiong, C.G. Xu, M.A. Saghai Maroof, Q. Zhang, Patterns of cytosine methylation in an elite rice hybrid and its parental lines, detected by a methylation-sensitive amplification polymorphism technique, Mol. Gen. Genet. 261 (1999) 439–446.
[22] W. Xu, T. Wang, S.Y. Xu, S.X. Xu, L.J. Wu, Y.J. Wu, P. Bian, Radiation-induced bystander epigenetic effects demonstrated in *Arabidopsis thaliana*, Radiat. Res. 183 (2015) 511–524.
[23] K. Bomblies, Quantitative GUS activity assay, in: D. Weigel, J. Glazebrook (Eds.), Arabidopsis–A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 2002, pp. 249–252.
[24] L.L. Li, S. Santerre-Ayotte, E.B. Boivin, M. Jean, F. Belzile, A novel reporter for intrachromosomal homoeologous recombination in *Arabidopsis thaliana*, Plant J. 40 (2004) 1007–1015.
[25] T. Caspar, S.C. Huber, C. Somerville, Alterations in growth, photosynthesis, and respiration in a starchless mutant of *Arabidopsis thaliana* (L.) deficient in chloroplast phosphoglucomutase activity, Plant Physiol. 79 (1985) 11–17.
[26] A. Boyko, I. Kovalchuk, Epigenetic regulation of genome stability in plants in response to stress, in: G. Grafi, N. Ohad (Eds.), Epigenetic Memory and Control in Plants, Springer, 2013, pp. 41–46.
[27] H. Zheng, F. Han, J. Le, Higher plants in space: microgravity perception, response, and adaptation, Microgravity Sci. Technol. 27 (2015) 377–386.
[28] E.B. Blancaflor, Regulation of plant gravity sensing and signaling by the actin cytoskeleton, Am. J. Bot. 100 (2013) 143–152.
[29] J.Z. Kiss, Mechanisms of the early phases of plant gravitropism, CRC Crit. Rev. Plant Sci. 19 (2000) 551–573.
[30] J. Zou, Z. Zheng, S. Xue, H. Li, Y. Wang, J. Le, The role of *Arabidopsis* actin-related protein 3 in amyloplast sedimentation and polar auxin transport in root gravitropism, J. Exp. Bot. 67 (2016) 5325–5337.
[31] G. Leitz, B.H. Kang, M.E. Schoenwaelder, L.A. Staehelin, Statolith sedimentation kinetics and force transduction to the cortical endoplasmic reticulum in gravity-sensing *Arabidopsis* columella cells, Plant Cell 21 (2009) 843–860.
[32] Y. Hashiguchi, M. Tasaka, M.T. Morita, Mechanism of higher plant gravity sensing, Am. J. Bot. 100 (2013) 91–100.
[33] M. Toyota, N. Ikeda, S. Sawai-Toyota, T. Kato, S. Gilroy, M. Tasaka, M.T. Morita, Amyloplast displacement is necessary for gravisensing in *Arabidopsis* shoots as revealed by a centrifuge microscope, Plant J. 76 (2013) 648–660.
[34] M. Toyota, T. Furuichi, H. Tatsumi, M. Sokabe, Critical consideration on the relationship between auxin transport and calcium transients in gravity perception of *Arabidopsis* seedlings, Plant Signal. Behav. 3 (2008) 521–524.
[35] A. Robert-Seilaniantz, M. Grant, J.D. Jones, Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism, Annu. Rev. Phytopathol. 49 (2011) 317–343.
[36] M.T. Morita, M. Tasaka, Gravity sensing and signaling, Curr. Opin. Plant Biol. 7 (2004) 712–718.
[37] C. Wolverton, A.M. Paya, J. Toska, Root cap angle and gravitropic response rate are uncoupled in the *Arabidopsis pgm-1* mutant, Physiol. Plant 141 (2011) 373–382.
[38] P.J. Davies, The plant hormones: their nature, occurrence, and functions. in: P.J. Davies, Plant hormones: biosynthesis, signal transduction, action! Springer, 2010, pp,1–15.
[39] J. Ueda, K. Miyamoto, T. Yuda, T. Hoshino, S. Fujii, C. Mukai, S. Kamigaich, S. Aizawa, I. Yoshizaki, T. Shimazu, K. Fukui, Growth and development, and auxin polar transport in higher plants under microgravity conditions in space: BRIC-AUX on STS-95 space experiment, J. Plant Res. 112 (1999) 487–492.
[40] R.J. Ferl, A. Paul, The effect of spaceflight on the gravity-sensing auxin gradient of roots: GFP reporter gene microscopy on orbit, npj Microgravity 2 (2016) 15023.
[41] K. Miyamoto, M. Oka, R. Yamamoto, Y. Masuda, T. Hoson, S. Kamisaka, J. Ueda, Auxin polar transport in *Arabidopsis* under simulated microgravity conditions—relevance to growth and development, Adv. Space Res. 23 (1999) 2033–2036.
[42] M. Oka, J. Ueda, K. Miyamoto, R. Yamamoto, T. Hoson, S. Kamisaka, Effect of simulated microgravity on auxin polar transport in inflorescence axis of *Arabidopsis thaliana*, Biol. Sci. Space 9 (1995) 331–336.
[43] I. Ottenschlager, P. Wolff, C. Wolverton, R.P. Bhalerao, G. Sandberg, H. Ishikawa, M. Evans, K. Palme, Gravity-regulated differential auxin transport from columella to lateral root cap cells, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 2987–2991.
[44] C.M. De Moraes, J.C. Schultz, M.C. Mescher, J.H. Tumlinsoni, Induced plant signaling and its implications for environmental sensing, J. Tox. Environ. Health 67 (2004) 819–834.
[45] M. Dicke, J. Bruin, Chemical information transfer between plants: back to the future, Biochem. Syst. Ecol. 29 (2001) 981–994.
[46] V. Shulaev, P. Silverman, I. Raskin, Airborne signalling by methyl salicylate in plant pathogen resistance, Nature 385 (1997) 718–721.
[47] E.E. Farmer, C.A. Ryan, Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 7713–7716.