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REVIEW

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# Investigating plant responses to microgravity and adaptations in gravisensitive environments

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## Abstract

Plants are crucial because they give us food and oxygen. With the idea of living on other planets and taking long trips in space, we need to understand and explore the way how plants can survive in these strange places. However, while the gravity response on earth's surface has been extensively studied in plants, in space, where the gravity is very weak, things get confusing. One of the intriguing and essential subjects for space life is understanding how plants can sustain themselves in microgravity conditions. To investigate this, various clinostat devices and the CRISPR/Cas9 technique are crucial tools for exploring the functioning of PIN-formed protein and related signal transduction pathways. In this review, we aim to provide researchers with a brief overview of the mechanisms of CRISPR/Cas9, which can be immensely helpful when using this method alongside clinostat machines. Our primary goal in this review is to address the missing gaps in existing literatures, focusing on how plants perceive gravity and experimental approaches applicable for studying their responses to microgravity, both on earth and in space.

**Keywords** Gravity perception, Stimulated microgravity, Long term, Short term, PIN3

## Introduction

Gravity plays a vital role in plant growth and morphogenesis [1]. For a plant to carry out a variety of physiological and biochemical processes effectively, it must be of the appropriate size and shape. As a result, plant life is primarily reliant on the control of growth for size and morphogenesis for form. Specific genetic modules have a significant role in regulating development and morphogenesis in both animals and plants. However, the environment around plants also provides cues that affect growth and development, including light, temperature, water [2], and gravity [3]. Gravity is regarded as a unique environmental signal because it is always present on Earth in the same direction and magnitude [4]. Gravity has been widely considered as one of the most reliable and constant signals for plant development and continued existence [5]. However, since microgravity is a fundamental aspect of orbital flight in space, we believe that plant development and morphogenesis will be significantly affected in space [6]. Recent study suggests that the gravity level on Mars is strong enough to stimulate the flow of auxin, which is not in case of Moon where gravity is weaker and produces greater changes [7]. Plant cultivation in space, also known as "space farming" is crucial for the development of long-term human space travels. Plants play a pivotal role in bioregenerative life-support systems (BLSS) by providing essential factors

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and nutrients, including oxygen and valuable vitamins. They can also help to regulate atmospheric humidity and recycle carbon dioxide, which can upset the balance in space environments [8, 9]. The presence of gravity is essential for plant growth and development, as it is one of the key environmental factors that influence them. Microgravity has been studied in many different living organisms, including mammals and bacteria. It is generally considered a stressor for these organisms, but the effects can vary in the species. The null or altered gravity have two effects on organisms, one direct and another is indirect, the direct effect is referred to changes in the physiological process within organisms, e.g., the alteration in the mechanisms of biological reactions such as the way cell produce energy or the way protein fold, the indirect effect is the physical changes to the environment surrounding the organisms that may impact its physiological processes such as the liquid or gasses behave in the microgravity [10]. The indirect effect can be mitigated with proper ventilation and adequate water treatment systems, as recently done in Veggie and APH facilities on the ISS [11–13]. The impact of microgravity on plants has been a subject of great interest to scientists. Particularly in the context of space research, a major concern is the potential side effects of microgravity on plant growth and development, including alterations in soil water diffusion that affect hydration and disruptions in gas exchange vital for plant survival. Analyzing experimental results in microgravity is challenging due to the difficulty in distinguishing between these effects. Nevertheless, efforts have been made to replicate microgravity conditions on earth to gain better understanding of their potential impact on plant growth. When plants are exposed to microgravity, such as in space, they can undergo changes in their growth patterns, cell structures, and overall weight distribution. In microgravity, plants may experience bending stresses and specific mechanical stimuli that are not random. These forces can impact their growth and development, shaping their physical structure, and how they interact with their surrounding [14, 15]. Consequently, more research is needed to identify various molecular and technical approaches to accomplish the role of gravity on both the earth's surface and in orbit, which are good for plant survival in earthly and extra-terrestrial life. Based on this study, we suggested to researchers from around the world to use the CRISPR/Cas9 genome-editing technique to overcome the effects of gravity and microgravity in plants. We also work in this area of research separately and aim to contribute good research for the scientific community in the near future. In our previous work, we investigated the impact of both artificial and normal gravity on rice plants and identified the differential expression in the *OsPIN* genes [16]. However, the present review investigates the significance of normal gravity and microgravity effects on plants on Earth and during space travel, exploring the use of CRISPR/Cas9 technique in application far beyond cultivation. To achieve this, it is essential to identify the optimal genomic targets within the plants that can withstand harsh conditions such as extreme drought, salinity, heat, microgravity, and space.

## Effects of gravity on plants

For a plant to develop its root system in the direction of gravity, anchor itself in the ground, and grow upwards toward the sun, it requires the stimulus of gravity acting as a guiding force. Understanding "up" and "down" is essential for plant existence on earth [17]. In addition, it is necessary for photosynthesis, which produces food and oxygen, and therefore for all life on Earth. Studying the effect of gravity on orientation and growth in plants is facilitated by the ability to cultivate them. Through this method, significant advancements have been achieved in comprehending how plants sense gravity and respond through gravitropism [18]. The alter gravity condition disrupts the meristematic competence in cells located within the root apical meristem [19, 20]. Gravity has been a constant force shaping the evolution of plants, consequently impacting all aspects of their growth, development, and morphology. Furthermore, gravity plays a fundamental role in various physical phenomena, including buoyancy, convection, and sedimentation. The process of sedimentation of amyloplasts under gravity and microgravity condition is described in Fig. 1C. These phenomena, in turn, indirectly govern crucial aspects of plant growth, such as gas exchange, cellular respiration, and photosynthesis, which can be influenced by alternations in buoyancy. According to data from numerous studies, the phytohormone, auxin, has been identified as the factor that initiates a cascade of functional events leading to the alteration of meristematic cell proliferation, growth, and subsequent disruption of meristematic competence [21]. This hormone serves as a primary regulator of the delicate balance between cell proliferation and cell differentiation within meristems, forming the foundation for the significant role of meristematic tissue in plant development [22]. In addition, auxin exerts influence over various aspects of plant and development, encompassing the regulation of cell cycle progression as well as the coordination between cell growth and cell division [23]. Taking a broader perspective, auxin plays a critical role in regulating the link between stimuli detected by the plant and the subsequent cellular responses to these stimuli [24]. The molecular aspects of auxin and the genes associated with auxin efflux and influx facilitation under gravity conditions are described in ""Molecular,

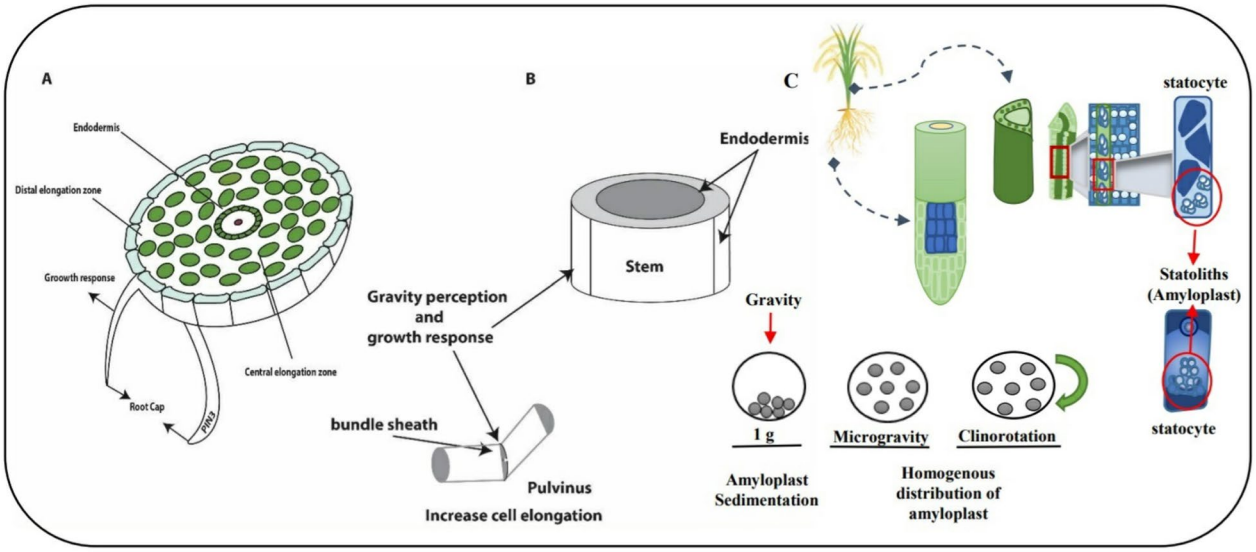


**Fig. 1** Schematic representation of advanced plant structures. Graviperception takes place in specialized gravity-sensing cells (statocytes) in the root cap. These statocyte cells are most probably present in the endodermal region of the plant cells, where starch granule amyloplast sedimentation occurs under stimulated gravity conditions. **A** The PIN-formed protein also contributes to the facilitation of auxin efflux and influx under stimulated gravity conditions, and **B** shows that different parts of the plants perceive gravistimulation, adopted from Kolesnikov et al [25]. whereas **C** shows the amyloplast position in the roots and shoot part of the plant. It also describes the amyloplast sedimentation in normal gravity, microgravity, and simulated microgravity conditions

cellular, and physiological aspects of plants under gravity conditions" section.

## Gravity, microgravity, and hypergravity

Gravity is a natural fact, in which material objects attract each other with a force of inversely proportionate to the square of the distance between them and proportional to the masses of each object. Every planetary body has a gravitational field surrounding it that attracts all other things toward it. At the earth surface, the magnitude of the gravitational field is described as an acceleration of 9.81 m per second ($m/s^2$) and commonly termed as "1 g". In comparison to gravity on Earth"s surface, microgravity is relatively small. It is defined as one millionth of gravitational force on earth's surface and is symbolized as "μg" (where the Greek mark "μ" represents one millionth). In mathematics, g equals 106 g; however, microgravity describes the acceleration of less than 1 g. Hypergravity is defined as an acceleration force that exceeds the gravitational pull at the earth's surface, that is greater than 1 g. It can be simulated in a laboratory using a centrifuge, which is capable of replicating the acceleration and deceleration forces experienced by spacecraft during takeoff and landing [26]. In addition, hypergravity experiments support microgravity research by assisting in the discovery and comprehension of gravity-related phenomena [27, 28]. Plants exhibit a consistent response to gravity known as gravitropism. However, gravity remains relatively constant throughout much of the earth, with only a negligible variation. For instance, Mount Everest, the highest point on earth, has a slightly lower acceleration due to gravity compared to sea level, with just a 0.3% difference. In contrast, microgravity in space is considered the most significant abiotic stress for plant [29]. Zero gravity refers to the absence of gravity, which is significantly different from the gravitational conditions experienced on earth, particularly in space [30].

## Microgravity-generated conditions

### Real microgravity conditions

Short-term gravity can be generated in balloons (30–60 s), drop towers or drop shafts (2–10 s), parabolic flights of aircraft (20–25 s), or sounding rockets up to 15 min) (Fig. 2). Gravity is reduced by approximately 16.5% (16.20 $m/s^2$) on the Moon and 38.0% (3.721 $m/s^2$) on Mars compared to Earth [31]. These approaches are suited for systems that must respond quickly. To investigate the long-term effects of microgravity, satellite or human-tended space labs must be used. The creation of space stations realized the ideal of humans spending an extended period in space. The Russian MIR space station, which hosted over 100 astronauts and cosmonauts, orbited the Earth at an altitude of 300–400 km. Since 1998, the International Space Station (ISS) has been continuously orbiting the Earth, offering accommodation for

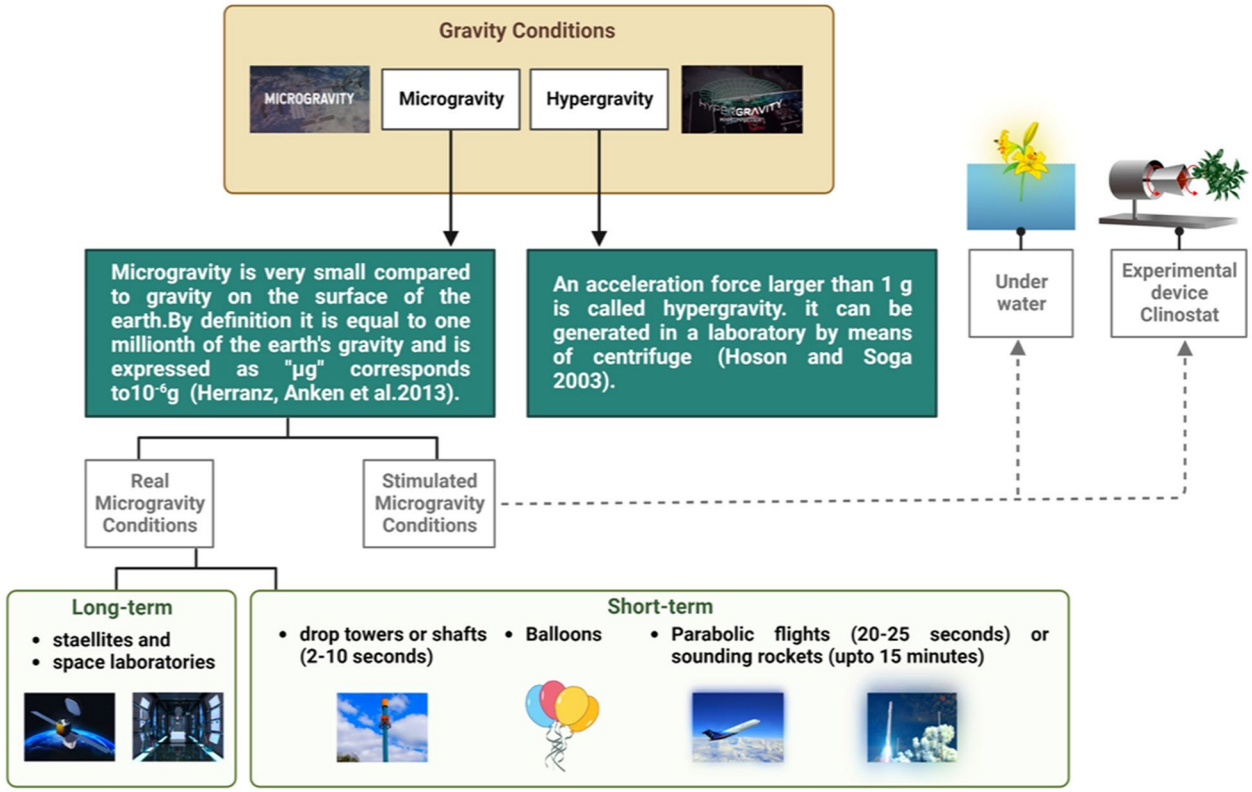


**Fig. 2** The evaluation of gravity conditions for the earth or orbit. The stimulated gravity conditions for both long and short term while using different platforms. Source: [27, 33]

up to six astronauts and serving as laboratory for conducting systematic microgravity research [32].

#### Simulated microgravity environments

Multiple ground-based facilities and equipment have been developed by scientists to achieve the condition weightlessness. In a pool, bouncy offsets gravity, producing a simulated microgravity environment that makes it ideal for astronauts to receive underwater training (Fig. 2). On earth, scientists can generate microgravity using specialized device like a random position machine or clinostat. These machines move along specific trajectories, often random, to counteract the effects of gravity [34]. Magnetic forces have also been found to serve as a valuable substitute for microgravity in ground-based experiments [35]. They can cause levitation of cellular organelles, including statoliths in roots, hypocotyls, rhizoids, and bacteria [36–38]. Although, these platforms do not eliminate gravity itself but continually alter its direction [15]. In zero gravity, the absence of convection becomes problematic as it hinders the movement of gasses around tissues, which can affect gas exchange. An example of the influence of gravity can be seen in amyloplasts, where stored food (starch) is involved in gravity perception. However, long space missions present challenges for seed performance, nutritional content, and plant flavor due to hostile space conditions. Simulating gravity, such as through rotational artificial gravity using centrifugal force, can help mitigate gravitational stress. The Stanford torus systems, rotating at 1 rpm with a diameter of 1.8 km and a mass of round 10 million tons, is an example of such an approach [39]. Moreover, a clinostat is an investigational device that can balance the gravity direction nearby single or dual turning axes. However, studies carried out in environments that mimic microgravity must be supported by research done in actual microgravity [32]. Drop towers offer short but high-quality microgravity exposure (up to 5–10 s) at a relatively low cost (around 6,000–10,000€ per drop) and boast a quick turnaround time (2–3 drops per day). However, their drawbacks include the limited duration and potential for up to 50 g of landing acceleration, rendering them unsuitable for all experiments, Nevertheless, they are increasingly popular for biological research, particularly for repaid molecular assessments like phosphoproteomics and the study of secondary messenger signaling during the initial stages of microgravity response [40]. A recent study suggests that reduced gravity and the lack of

convective forces have been shown to facilitate the formation of protein crystals more efficiently. This improved crystallization process significantly contributes to our ability to more readily determine the intricate structures of proteins, thereby accelerating progress in drug development [41]. It has been reported that the microgravity simulator rotating-wall vessel (RWV) is capable of achieving high-density, 3D cell cultures, exemplified by the growth of BHK-21 cells to 1.1×107 cells/ml. It provides a low-shear, well-oxygenated culture environment, making it suitable for various cell types, including normal and neoplastic cells [42]. A recent study reported findings regarding the effects of microgravity simulator random positioning machine (RPM) on Fusarium, including increased growth, spore production, and germination, while biofilm production was reduced under RPM exposure [43]. Previous study reported that the water immersion method is used in medical science to simulate microgravity. Contrary to most land plants, rice is exceptional because its coleoptiles grow faster underwater than in the air [44]. The enhanced growth is a result of increased cell elongation in the submerged environment despite limited oxygen availability [45]. It has been reported that the rice coleoptiles (*Oryza sativa* L. cv. Sasanishiki) reached a maximum length of 81.2 mm on day 5 when grown underwater, whereas in the air, they reached only 12.4 mm, this difference could be possibly attributed to the buoyancy effect [46]. A recent study shows that the alter gravity enhances the amino acids profile, but this effect can be reversed after 11 days of microgravity. These changes suggest a protein degradation process and the conversion of specific amino acids into glucose and ketoleucine, particularly in microgravity [47]. However, studies still not yet fully understand how organisms detected gravity and initiate subsequent response. Cellular mechanisms operate at various levels, including transcriptomic, proteins, metabolisms, and ions. Metabolomics, a relatively new field, is highly sensitive and can produce strong effects in response to minor cues, often much more significant than what is observed in genomics or proteomics. For instance, disease-related metabolic markers can change by 100 to 10,000 times, while protein markers typically only changed by 1 to 10 times in response to stimuli [48–50].

## Clinostats

In the late 1800s, Sir Thomas Knight, Sachs, and Ciesielski believed that gravity was the most important factor to plant growth and development [51]. Consequently, many types of clinostats were developed over time to study and identify the effects of gravity on organisms [10, 52–54]. A clinostat is a machine that aids in the rotation of specimens around one or more axes, resulting in differential rotational speed and direction [55]. Various kinds of clinostat have been working to investigate the growth and development of plants to address fundamental problems in the field of gravitational biology. The machine clinostats are classified into various varieties based on their rotational speed and direction: clinostats with two or three axes of rotation, as well as clinostats with a single axis that rotates slowly (1–4 rpm) or quickly (50–120 rpm). The system is referred to as a random positioning machine when the rate of rotation for the axes fluctuates. Furthermore, magnetic levitation has been employed for these instruments to balance gravity [56].

## Role of the rotating clinostat in growth and morphogenesis

Microgravity can be created through free fall or parabolic flight for a relatively short period of time, which is usually insufficient to cause noticeable changes in plant development and morphogenesis [57]. The microgravity effect was produced using a device that has a horizontal axis, which compensates for the unilateral impact of gravity. A horizontal clinostat is commonly thought to be convenient; however, it has some limitations, such as steady airflow or solution flow and the fixed trigger of the lateral sides of the materials [27, 33]. A three-dimensional (3D) clinostat has two rotating axes at right angles to avoid these risks [57]. In most plant materials, three-dimensional clinostats with randomly rotating motors have little effect on growth-regulating factors over a short period of time [33, 57]. In addition, long-term rotation created a cellular structure that may result in excessive growth [27, 53, 58]. These data could be attributed to the clinostats incapacity to eliminate the static component of gravistimulation, as the device solely enables dynamic gravistimulation in a clockwise direction [53, 58] (Fig. 3).

Alternately, the clinostat rotation significantly altered plant development. Various plant materials have been observed to undergo automorphogenesis on clinostats, according to earlier research by Sachs and Pfeffer [33, 57, 59]. Automorphogenesis involves alterations in the growth orientation or unconfined curvature of organs [53, 57]. Previously, it was discovered that clinostat rotation at 12 rpm changed the direction of root and shoot growth, with shoots growing downward toward the earth's surface and roots growing upward toward the direction of light. This improved the amino acid profile of rice seedlings compared to the control [16]. On a 3D clinostat, plant roots exhibit an initial growth pattern toward the tips of root primordia, after which they diverge toward irregular direction [57]. In addition, the growth pattern of several species, including pea, maize, rice, and garden cress, occurs at random during the early and late growth phases. Contrarily, plant roots that have grown in

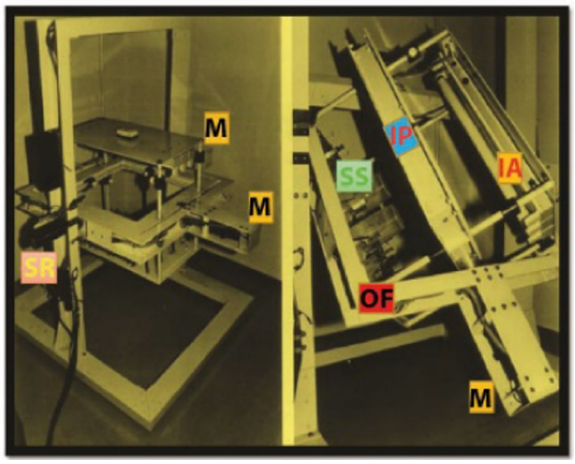


**Fig. 3** The 3D clinostat device showing the outer supporting frame (left) is long 1.40 m. Illumination apparatus (IA), inner frame (IF), motor with an encoder (M), outer frame (OF), slip ring (SR), and sample stage (SS). Source: [53]

the clinostat exhibit automorphic curvature. This automorphic curvature has no dorsiventrality, and it occurs in an erratic direction [60].

## Gravity perception models

Various theories have been proposed to explain how higher plants perceive gravity. According to [61] in plants, there are two major hypothesis related to graviperception are the protoplast-pressure model and starch statoliths that are describe here. Both the ideas about protoplast and statoliths have their own importance and evidence [62, 63]. The protoplast pressure idea suggests a few things (1) in many unicellular organisms that respond to gravity, such as Euglena, and in certain cells of the alga Chara, sedimenting particles that typically settle in response to gravity are notably absent. (2) The density of the surrounding fluids affects the way cytoplasmic streaming responds to gravity in the internodal cells of the Chara. (3) Based on inhibitor studies, there is a potential that molecules similar to integrin's can detect variation in protoplast pressure. (4) The density of the external medium also seems to influence how gravitropism functions in the roots of *Oryza sativa*. (5) Starch-deficient Arabidopsis mutants demonstrate the ability to perceive gravity. The contemporary support for the protoplast pressure hypothesis is rooted in the research conducted by [64], which investigated gravitropism in wheat coleoptiles, as well as the study by [65], which explored cytoplasmic streaming in intermodal cells of Chara. Interestingly, during the late nineteenth century when gravitropism was first studied, several German botanists also put forward a protoplast model for gravity perception, as discussed in a review by [66]. The key point made by proponents of the protoplast pressure hypothesis is that research findings from gravitropism studies involving starch-deficient mutants (e.g., studies conducted by [67–69]) align with the statolith theory but fail to definitively distinguish between the gravitational pressure and statoliths theories of gravity sensing as suggested by [70]. However, it is important to note that most of the evidence supporting the protoplast model comes from studies of cytoplasmic streaming in specialized giant internodal cells of characean algae as described by [65, 66]. This evidence may not fully represent the more widespread gravitropism observed in various plant groups. There is, however, one study on gravitropism in rice roots that lends support to the protoplast model as documented by [70] There are still arguments in support of Nemec and Haberlandt's concept, which predictably combines statoliths with gravity perception [71]. This hypothesis explains how gravity simulates the specialized cells called statocytes in axial plants. Amyloplasts, which have a diameter of 1.5 to 3 μm and serve as the source of stimulation, move too rapidly toward the distal end of the cell to effectively interact with specific cellular components. As a result, the amyloplasts are known as "statoliths" and their purpose is to act as sensors of the gravitational pull. The starch statolith model [72–74] is the most widely accepted hypothesis and is supported by numerous facts. Previous studies have demonstrated a close relationship between the location of statoliths and the location of gravity sensing. During gravistimulation, amyloplasts in statocytes redistribute or sediment as opposed to other cell organelles. In addition, plant components such as roots, shoots, pulvini, and gynophores exhibit a reduced ability to react to gravitational stimuli when amyloplast lack starch as depicted in Fig. 1. This is evidenced by weak gravitropic response observed in the starch less mutants of various plant species such as *Arabidopsis thaliana* (e.g., pgm1), rice (*Oryza sativa*), and *Nicotiana tabacum* [68, 75–84]. It was previously reported that small prokaryotic organism such as bacteria may not be able to respond to gravity due to the Brownian motion [85–87]. However, recent studies have observed changes in membrane fluidity in response to gravity, shedding new light on this consideration and potentially change the previous view [88]. Various slime molds, fungi, and fern structures exhibit distinct gravitropic responses, including gravitaxis, gravitropism, and negative gravitropism [89]. It has been reported that plants use gravity to grow up and down using pathways involving PIN proteins and calcium, they help plants to grow better in space and tackled food challenges on earth [90]. Polar auxin transport after plant reorientation results in asymmetric distribution, leading to differential

growth and bending. PIN1 and PIN7 exhibit distinct polarizations in pro-embryos and adult plants based on cell type and developmental stage, influencing direct auxin flow [91] (Fig. 4).

## Lipid signaling

### Phosphoinositide-specific phospholipase C

The phosphoinositide-specific phospholipase C (PI-PLC) hydrolyzes phosphatidylinositol-4,5-5biphosphate (PI4,5-P2) to generate inositol-1,4,5-triphosphate (IP3) and diacylglycerol [92]. The involvement of IP3 in the release of calcium from intracellular reserves is unique and specific to cellular functions [93]. Several studies support the idea that PI-PLC and its by-product IP3 play a key role in gravity signaling. Molas and his colleague have observed that the level of biphasic IP3 exhibit two distinct phases of fluctuation in response to gravity. During the initial 10 to 15 s of gravity rotation, there is a significant increase in IP3 levels observed in the lower section of maize (*Zea mays*) and both upper and lower sections of oat (*Avena sativa*) pulvini, constituting the primary phase.

Following 30 min of gravistimulation, the second phase of dynamics was noted, characterized by a notable surge in IP3 levels in the elongating lower portion of pulvini, leading to a high level of IP3 [94, 95]. The entire Arabidopsis inflorescence stem responded to short-term gravity by increasing IP3 levels in two phases. The occurrence of the second IP3 peak in the underside of the pulvini signifies a consistent role of IP3 generation in the transmission of gravitational signals [96]. Contrastingly, the beginning of their twisting reaction coincides in timing with the duration of the second IP3 phase in plants [94, 96]. The sustained elevation of IP3 through a prolong duration that depends on PLC activity may cause metabolic asymmetries that result in the proliferation of each pulvinus half cells differently [97]. The exact mechanism by which gravity activates the PI-PLC is unknown. The increase in IP3 levels in response to gravitational stimuli is independent of auxin transport [95, 97]. The inhibition of calcium channels by lanthanum ions and protein phosphatases 1 (PP1) and 2A (PP2A) by okadiac acid results in the hindrance of IP3 accumulation and the

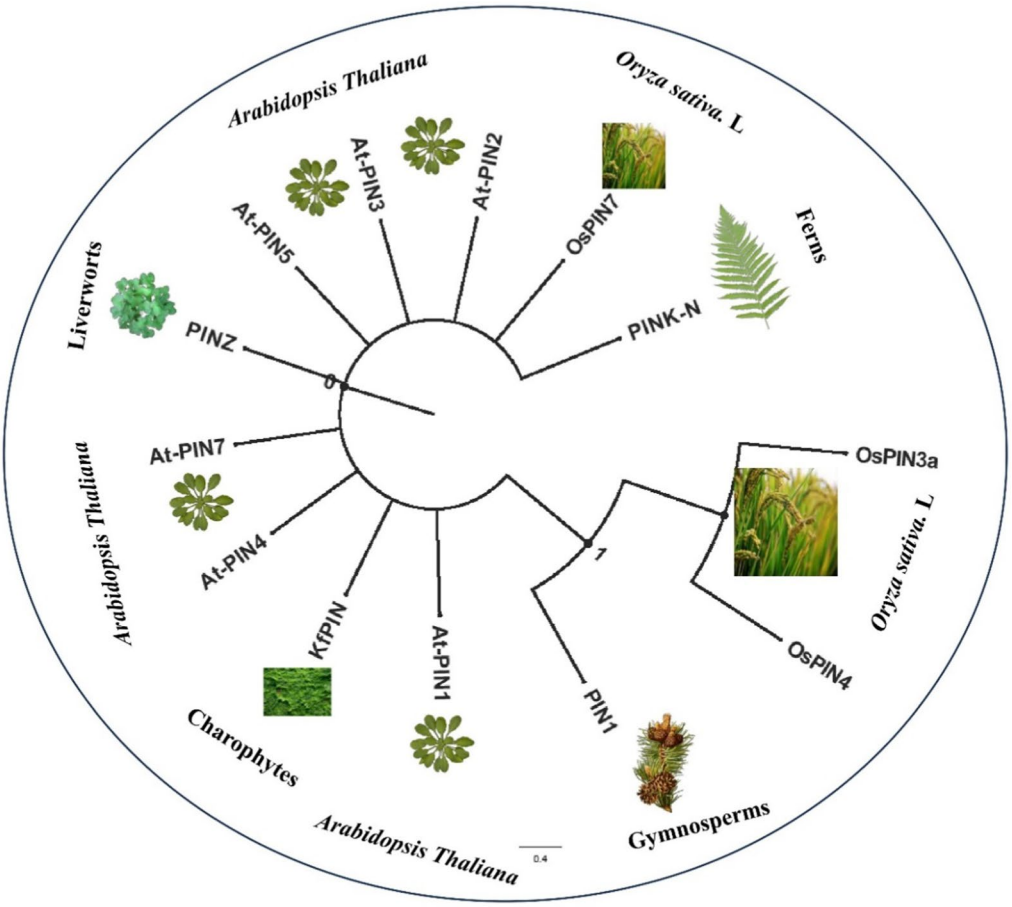


**Fig. 4** Schematic representation of a phylogenetic tree of genes involved in gravitropism in various taxonomical groups shows the subcellular localization of PIN transporter

corresponding gravistimulatory response [95]. A delay in the increase of IP3 levels occurs when the starch levels are lowered in the lower half of the shoot pulvinus statoliths. In addition, certain internode tissues divide of amyloplasts displayed no alterations in IP3 levels upon exposure to gravitational stimuli [94, 97].

This suggests that PI-PLC activation during gravity signaling may be impacted by amyloplast sedimentation, calcium, and protein dephosphorylation (Fig. 5). While long-term changes in IP3 levels were impacted, short-term variation remained unaffected by the Pi-PLC inhibitor, suggesting that different pools of PI-4, 5-P2, and related Pi-PLC contribute to different phases of IP3 production, with varying degrees of sensitivity to the inhibitor [97]. These findings might imply that various PI-PLC regulatory mechanisms and potentially multiple PI-PLC isoforms are implicated in gravity signaling.

## Role of calcium in signal transduction

Calcium ion serves as a vital second messenger in several signal transduction pathways, and the utilization of a calcium-sensitive luminous indicator called aequorin revealed a biphasic alteration in cytosolic calcium levels in *A. thaliana* upon exposure to gravitational stimuli. Moreover, petioles and hypocotyls showed this impact, while cotyledons did not. Studies have shown that the initial calcium peak remains unaffected by the plants orientation relative to the gravitational field. In contrast, the second peak is more intense and lasts for a longer duration (20–35 s), and is reliant on the seedlings' positional changes concerning the gravitational vector [98–100].

The modest level of fluorescence seen with aequorin suggests that the calcium variations in calcium levels may be restricted to specific cellular compartments or may be the result of a small group of cells that respond to gravity. Researchers discovered that in the single-celled spores of *Ceratodon* and *Ceratopteris richardii*, there was a transfer of calcium across cells, moving from the outside environment to the lower portion of the cells and from the upper region to the extracellular medium. This transfer of calcium reversed its direction after 25 s of exposure to gravitational stimulation [101]. After subjecting the creeping *Chrysanthemum morifolium* to 5 min of gravistimulation, it was found that there was an increase in calcium accumulation in the cytoplasm of stem endodermis cells. In addition, the cell walls of the repositioned cells had a higher concentration of calcium on their lower side as compared to their upper side [102].

Despite ongoing research, the precise mechanisms responsible for regulating calcium transport during gravity signaling remain unclear. However, studies have shown that the sensitivity of roots and hypocotyls to gravity can be enhanced by introducing external calcium. Conversely, inhibiting calcium-dependent ATPase, calcium channels, or mechanosensitive ion channels has demonstrated to impede gravitropism [101, 103–107]. Research into the mechanism linking variations in calcium to auxin transport mechanisms is still ongoing [102]. The transient fluctuation in ions can be converted through various mechanisms such as calmodulin, calcium-dependent protein kinases and phosphatases, phospholipases, or gene expression of calcium-sensitive proteins. Among the ways in which calcium influences gravitropism is by modifying the transport of PIN1. Moreover, experiments with transgenic plants that simultaneously overexpressed synthetic microRNAs targeting gene encoding Ca $[2]^{+}$-ATPases exhibited higher levels of cytosolic calcium. These plants displayed abnormalities in the basal localization of PIN1 in the root tip epidermal cells, which interfered with the proper growth of gravitropic roots [102] (Fig. 5). The protein kinase, PID, is a vital modulator of auxin transportation and its activity is negatively controlled by calcium. In root region, ARG1 and ARL2 proteins function within a pathway distinct from amyloplast sedimentation [108, 109]. It might be possible that these proteins regulate vesicle transport, potentially including PIN3-containing vesicles through the interaction with actin cytoskeleton. Proteins ARG1 and ARL2 directly interact with HSP70, potentially serving as molecular adapters that regulate folding, arranging, and assembly of components involved in gravity signaling pathways [110].

## Molecular, cellular, and physiological aspects of plants under gravity conditions

Several transporters responsible for auxin influx and efflux, including AUX1/LAX (AUXIN-RESTANT MUTATION 1/LIKE AUX1) proteins, ATP BINDING CASSETTE B/MULTIDRUG-RESISTANCE/P-GLYCOPROTEINS (ABCB/MDR/PGP), and PIN-FORMED (PIN) PROTEINS, are involved in generating and maintaining the gravity-induced asymmetrical distribution of auxin [111, 112]. The polarized distribution of auxin primarily results from the activity of PIN auxin efflux transporters, which are localized in a polar manner within the cell membrane and transport auxin solely in one direction [113]. The PID gene family, comprising PID, PID2, WAG1, and WAG2, is pivotal in plant development and auxin signaling [114]. PID mutants exhibit phenotypic similarities to PIN mutants, suggesting a functional link [115]. Initially it was identified as a negative regulator of auxin signaling, PID later emerged as a positive regulator of auxin efflux carriers. Overexpression of PID alters PIN protein localization, and a model by [116] and colleagues suggests that PIN proteins may initially lack polarization, and becoming polarized upon phosphorylation by PID.

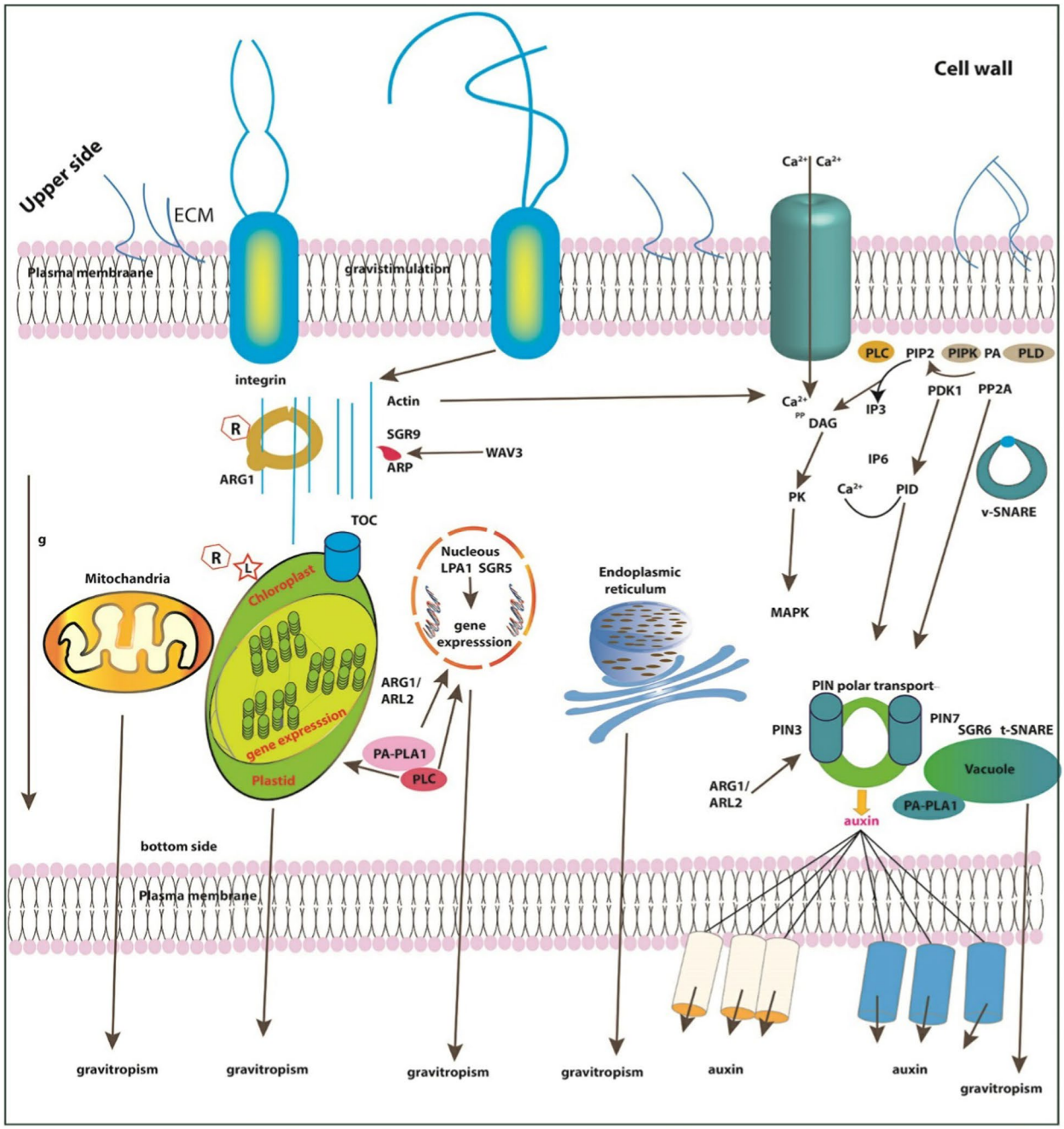


**Fig. 5** Schematic representation of known gravity signaling pathways in plant cells. Each cell organelle of plant cells is gravity sensitive which develops very complex signaling pathways described here: *DAG* diacylglycerol, *IP3* triphosphate, *IP6* inositol hexakisphosphate, *L* ligand, *ECM* extracellular matrix, *PA*-phosphatidic acid, *PA-PLA1* phosphatic acid-specific phospholipase $A_1$, *PIP2* phosphatidylinositol bisphosphate, *PIPK* phosphatidylinositol phosphate kinase, PK protein kinase, *PLC* phospholipase C, *PLD* phospholipase D, *PP* protein phosphatase, *PDK1* phosphoinositide-dependent protein kinase, *PID* serine/threonine-protein kinase PINOID, *R* receptor, *TOC* translocon of the outer envelope of plastids, and ARP actin-regulatory proteins. Source: Kolesnikov et al. [25]

WAG1 and WAG2 are functionally redundant with PID, implying similar roles in plant development and auxin signaling, altogether emphasizing the complexity of gene interactions in these processes [117, 118]. These three kinases contribute to similar functions in root development, particularly in processes such as apical hook opening and photoresponse [118–120].

Notably, all three kinases exhibit BFA-insensitive localization and play a role in phosphorylating PIN proteins, thereby affecting their subcellular distribution through processes that are insensitive to BFA [118]. However, PID2 has not been extensively studied. Evolutionary reconstruction and the observation that photoreactions are significantly more impaired in pid pid2 wag1 wag2, quadruple mutants compared to pid wag1 wag2 triple mutants suggest that PID2 may perform similar functions with the other three kinases [121]. It is worth noting that despite the changes in PIN protein distributions caused by PID-related phosphorylation, this modification can actually enhance the transport activity of PINs [122]. In the context of gravitropism, calcium ions play a crucial role as a prominent second messenger [105]. Another significant component involved in this process is the calcium-responsive kinases (CRKs), which are sensitive to calcium signals; CRK5, member of this kinase family exhibits localization to the plasma membrane (PM) and is responsible for phosphorylating PINs, thereby exerting control over various aspects plant development [123].In the root transition region of crk5-1 mutant plants, there is a distinct reduction in the presence of PIN2 within the upper plasma membrane (PM) of epidermal cells, while conversely, an increase in PIN2 abundance is observed in the apical PM of cortical cells. Interestingly, this phenomenon closely resembles the response seen in wild-type plants when subjected to a low concentration of BFA (Brefeldin A0). It is important to note that this shift in PIN2 localization does not coincide with any changes in the distribution patterns of PIN1, PIN3, PIN4, or PIN7 [123]. Furthermore, CRK5 is implicated in various PIN phosphorylation mechanisms, exerting its influence beyond a singular pathway. This kinase plays pivotal role in the regulation of hypocotyl hook development, potentially by modulating the phosphorylation state of PIN3. In addition, CRK5 exerts control over embryo development through its involvement in the phosphorylation of PIN1, PIN4, and PIN7. These diverse functions highlight the multifaceted impact of CRK5 on plant growth and development [124, 125]. Another way CRK5 is known to target specific phosphorylation sites, such as S252 or S253 of PIN1, S271 of PIN4, and S431 as well as S277/S278 of PIN7, it is noteworthy that there is currently no definitive in vivo or in vitro evidence to substantiate these specific phosphorylation events [125].

Together with CRK5, another principal contributor in the phosphorylation of most plasma membrane (PM)-localized PINs is CPK29, a protein kinase. Their collaborative action serves to control the polarity of PIN proteins, primarily by orchestrating BFA-insensitive recycling mechanisms [126]. The PIN1-4 and -7 proteins work together to regulate auxin distribution in the primary root [127]. These proteins are located in separatebut overlapping regions at the root tip [128]. The PIN1 helps the root meristem accumulate auxin from the shoot and is predominantly found in the lower membrane of vascular parenchyma cells. Furthermore, the gravitropic reaction was stopped by a mutant form of heat shock protein (hsp90) with abnormal PIN1 root expression or localization [129]. The PIN2 found in the upper region of root cap cells, moves auxin toward the root tip. However, in the root epidermal cells, PIN2 directs auxin toward the shoot. This asymmetric distribution of auxin mediated by PIN2 is necessary for the appropriate response to gravity in the root system [128]. The expression of both PIN3 and PIN7 is induced in the lateral root cap (LRC) columella cells of roots, which are present in the meristem area. In *A. thaliana*, FOUR LIPS (FLP) controls the transcription of PIN3 and PIN7 [130]. Moreover, the *OsPIN3*, *OsPIN4*, and *OsPIN7* genes appear to be involved in tropism, root meristem patterning, and the establishment of embryonic polarity [131].

The gravitropism phenomenon, which occasionally captures the interest of numerous researchers, depends on the plant cytoskeleton [132]. According to the tensegrity ("tension and integrity") hypothesis, the cytoskeleton of a plant can work as a receptor and propagator of the gravitropic stimulus [132]. The cytoskeleton system is made up of microtubules, filamentous actin, and various regulatory proteins. Furthermore, the use of inhibitors that prevent the formation of microtubule or actin in plants emphasizes the importance of the cytoskeleton in gravitropism. The role of actin network disruption did not always correspond to an agravitropic phenotype, raising questions about whether or not it might encourage statolith sedimentation and the gravitropic response [133–135]. Therefore, it is thought that the actin cytoskeleton is more crucial than gravity sensing for regulating the resting and sedimentation of statoliths [132]. A contemporary study revealed that during root gravitropism in *A thaliana,* AtCRK5 plays an important role in the stabilizing of reactive oxygen species (ROS) and nitric oxide (NO) [136]. During the gravitropic response of roots, the flow of auxin triggers an uneven distribution of both ROS and NO [137, 138], which affects PIN2 turnover and eventually causes auxin transport [133, 139]. Based on these findings, the hypothesis is that the auxin, ROS, and NO play a role in the fundamental response loop of the roots gravitropic response [136].

## Conclusion and opportunities for future research

On the earth's surface, plant growth and development rely on the vital stimulus of gravity. The prevailing theories purpose that gravitropism in plants resolves around the initial impact of gravity on statocyst sedimentation,

subsequently monitored by auxin redistribution, leading to differential cell growth responses. However, our understanding of how plants adapt to the challenges posed by microgravity conditions in space remains limited. In addition, the impact of this environment on the molecular mechanisms governing plant growth and development in response to gravity remains mostly unexplored. In recent years, the field has observed the emergence of various experimental techniques and technological platforms designed to unravel how plants adapt to microgravity. The utilization of these platforms has shed light on several longstanding mysteries. In this context, we highlight specific molecular signaling pathways that hold the promise of elucidating the mechanisms underlying plant responses to microgravity, and employing structural aspects of gravity signaling both on earth and in space. Among these pathways, unraveling the significance of kinase-dependent and -independent mechanisms that modulate the polarity of PIN proteins under space conditions is particularly intriguing. Furthermore, a comprehensive approach involves subjecting plants to various stress conditions, such as drought, salinity, temperature fluctuations, different gravitational forces including microgravity and hypergravity, using multidimensional clinostat devices. This approach allows for deeper understanding of the complexities of signal transduction pathways. Moreover, we believe that using CRISPR/Cas9 genome-editing technique along with clinostat devices, which hold potential applications for both terrestrial and extra-terrestrial life, will enable us to explore the effects of gravity, microgravity, and the facilitation of auxin efflux and influx on plants.

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**Data availability**
The data that support the findings of this study are available upon request from the authors, with the understanding that the data will be used solely for the purposes of scientific research and not for commercial or other non-research purposes. Restrictions may apply to the availability of these data, which were used under license for this study.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that the study was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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