Review of: "Continuous tracking of gravistimulated roots in a chambered coverslip by confocal microscopy allows first glimpse on mechanoadaptation of cell files during curvature initiation"

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The idea of sensing gravity as a guidance system for appropriate plant growth and development had been realized long back by Julius von Sachs (1832-1897), pioneer plant physiologist, who had foreseen the involvement of chemical messengers (hormones) in the differential cellular extension required for recoursing plant organs. In the following period, plant growth modulation steered by environmental or exogenous cue drew attention of several plant scientists including Darwins (Charles and Francis) who in 1980s did elegant experiments finally leading to the discovery of auxin. But the differential growth of plant organ could be better explained by Cholodny-Went hypothesis through unequal distribution of auxin. However, as regard to the nature of stimulus, distinction of perception of light between two sides of a plant organ is sharp and clear compared to the differential sensing of gravity vector, though differential distribution of auxin is ultimately held responsible for both the cases. Linking starch-statolith hypothesis with PIN-mediated auxin distribution made it possible to explain gravistimulated unequal growth.

Though the studies on plant movement have been taken up since the historical times, follow up of gravistimulation on the dynamics of movement at cellular level seems to be relatively new. Present article includes a thorough photographic (confocal microscopy) documentation of the movement of cell files in *Arabidopsis thaliana* roots subjected to external gravitropic stimuli sufficient for initiating curvature movement. Notably, the growth medium was designed to exclude exogenous carbon source to avoid any possible interference in root trait establishment and the roots of the vertically grown seedlings were shaded (using “D-root” system) from direct illumination to prevent light-escape growth of root and associated alterations in nutrient uptake. The 6-7 days grown seedlings were transferred to chambered cover slips which were rotated suitably to induce gravitropic stimuli and imaging was performed with a confocal microscope with vertical stage.

Similar to the approach of von Wangenheim et al. (2017), in the present article the author tried to visualize the mechanoadaptation of cell files at 3-D level in the curvature initiating zone by demonstrating the bending of root through time-lapse photographs, starting from 0 to 60 min. It is evident from the representative root (*eir1-4 PIN2::PIN2:mcherry*) that certain curvature is formed towards the gravitropic vector after 60 min of gravistimulation. It is to be noted that although elongation of the epidermal cells of the upper side is visible, however, cells of the lower side do not seem to have elongated significantly (Fig. 1C and Fig. 2B). Lateral root cap (LRC) cells can be observed to have elongated along with the epidermal cells. The author then attempted to recognize the minute events of curvature initiation after gravistimulation (through a representative root expressing PIN2:GFP), whereby sliding of one epidermal cell file over its neighbouring cell file can be observed to take place during this period (Fig. 3). Such shift is reported to have started at the meristematic zone gradually moving towards distal elongation zone, which is difficult to understand in terms localized cell extension, since epidermal cell elongation, particularly associated with gravitropic bending, is probably restricted to the distal elongation zone.

Thus, the article provides photographic substantiation and detailing of the curvature initiation phenomenon but without any mention about the mechanism of the process, although it seems extremely difficult to fine tune the molecular mechanism correlating the differential 3-D positioning of the epidermal as well as neighbouring cells in the curvature zone. Previous works on gravistimulated curvature movement of root tips have identified clear involvement of reactive oxygen species (ROS) in the process (Joo et al., 2001; Singh et al., 2017). It has been demonstrated by Singh et al. (2017) that auxin, being accumulated on the lower side of the growing axis in response to gravity, reduces ROS production by inhibiting NADH oxidase activity, whereas both NADH and NADPH oxidase enzymes, located on the upper side of the axis, generate ROS uninterruptedly owing to the lower level or absence of auxin in the region. Resultantly, cell walls of the outer cells of the upper side of the axis become relaxed/loosened leading towards elongation growth (and curvature) of the apical region.

Specific comments:

- Abstract should emphasize more on the work done and salient outcomes or findings instead of introductory remarks It is scientifically more accurate to use “autonomic” root movement instead of “autonomous” root movement.
- “Sample preparation and mounting for microscopy” should have been placed under “Materials and Methods” section instead of “Results and Outlooks” section.
- While describing the results, observations should have been narrated in passive voice instead of active voice.
- There is ample scope of improving the language and phrasing taking care of grammar. The word “manifold” has been used in several places (Abstract, Introduction, Results and Outlooks) with different implications or sense, “shootwards” is repeated consecutively in “Introduction” (Line No. 9) and the word “extend” has been used in place of “extent”.

References cited:

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