Journal of the Mechanical Behavior of Biomedical Materials 85 (2018) 43–50

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

Resistance from agar medium impacts the helical growth of *Arabidopsis* primary roots

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ARTICLE INFO

Keywords:
Plant biomechanics
Helical roots
Agar stiffness
Resistance

ABSTRACT

Agar is widely used in studies of root growth since it can be mixed at different concentrations to impact mechanical impedance. At high concentrations (1.2–1.5%), growth of *Arabidopsis* roots has been found to be wavy, but little research has explored this behavior based on a quantitative understanding of mechanical behavior. To this end, agar media with concentration ranging from 0.5% to 1.2% were prepared to produce gradient resistance during root penetration, and Young's moduli and penetrometer resistance were tested. *Arabidopsis* roots were then cultivated in these agar media with gradient stiffness. The result showed that Young's modulus increased linearly with the increase of concentration of agar media. For *Arabidopsis* primary roots, it was preferred to develop a helical pattern in agar media with concentration from 0.5% to 1.0%. As stiffness of agar increased, the percentage of helical roots and helix diameters in each agar medium declined; root lengths and auxin distributions showed variety. We demonstrate that the size of helical deformation decreases with agar stiffness as expected by theoretical analysis based on a combination of growth-induced mechanical buckling. In conclusion, the resistance from agar media impacts the properties of root helix, and helical roots growth is driven by growth force. Growth force and external mechanical forces contribute to root phenotypes in *Arabidopsis*.

1. Introduction

Plant organs, such as roots, shoots, tendrils and runners, often display movements along the direction of elongation, rather than growing straight.

Darwin recorded many basic observations of plant growth and development during his experiments. He grew various plants on the surface of inclined, hard-agar plates in the dark. On these plates, the roots of plants displayed a waving growth behavior, and Darwin accounted for the root movement that was produced from growing along a three-dimensional spiral path (Darwin, 1989). *Arabidopsis*, a commonly studied model plant, has gained significant interest in phytology and bioengineering research focused on probing the interactions between plants and the environment due to its unique root growth behavior of waving, skewing and fascinating coil patterns. When *Arabidopsis* seedlings were cultivated on an impenetrable agar surface that was not parallel to the gravity vector, the roots showed waving and skewing (Buer et al., 2000; Rutherford and Masson, 1996; Yang et al., 2015) while roots grown on a vertical plate exhibited a shallow wave (Gleeson et al., 2012; Okada and Shimura, 1990). On an inclined plate, the *Arabidopsis* roots grew with noticeable skewing accompanied with waving. In this circumstance, increasing the angle between the plate and gravity vector led to a greater root deviation from the vertical direction (Oliva and Dunand, 2007; Roy and Bassham, 2014). When slanted plates were nearly horizontal, coils occurred during *Arabidopsis* root development (Piconese et al., 2003; Simmons et al., 1995). In our previous experiments, *Arabidopsis* primary roots had a helical line movement when elongating freely in a soft medium (0.5× Murashige and Skoog basal salts pH 5.8 with KOH, 1.0% sucrose and 0.5–1.0% agar) (Yan et al., 2015; Yang et al., 2012).

Although these movements, which can follow various trajectories ranging from ovoid to irregular, were generally known by ancient plant scientists (Baillaud, 1962), a central question in developmental plant biology that remains is whether and how mechanical forces signal plant behavior and thereby, regulate morphogenesis (Hamant et al., 2008). Taking inspiration from Darwin's indication, Simmons et al. (1995) suggested that the observed root pattern resulted mainly from right-handed circumnutational helical movement. The roots first flattened on the plane of a hard agar surface and then resulted in waves, coils, slanting and torsions (Simmons et al., 1995). Mechanical forces play an

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https://doi.org/10.1016/j.jmbbm.2018.05.018
Received 6 July 2016; Received in revised form 31 January 2017; Accepted 9 May 2018

important role in plant morphogenesis, whether it be the waving root as it respond to physical barriers on the agar surface, the helical deformation of root by agar resistance (Migliaccio et al., 2009). Mechanical forces have dramatic effects on the final shape of *Arabidopsis* primary root and the internal biochemical signals.

The previous studies took an architectural perspective and considered auxin to understand the correlation between physical forces and root distortion and meristem activities that impact root development. Auxin is an essential hormone known to provide directional and positional information for plant growth and development. Auxin directional transport and asymmetric distribution play an indispensable role in root architecture (Rashotte et al., 2001; Swarup et al., 2005). Plant roots grow by continuous cell division at the root meristem and elongation at elongation zone (Wilson et al., 2013).

To explore the contribution of mechanical forces in plant morphogenesis, studies using agar and phytagels medium have intensified (Hamant et al., 2010). Agar and phytagels were commonly used to regulate the stiffness of root growth medium and to research the root mechanical response (Bengough et al., 2011; Jin et al., 2013). Studies on this topic were emerging to investigate the effects of local environment, such as moisture content or mechanical impedance on individual roots (Bengough et al., 2011; Jin et al., 2013). Recently, considerable insight into understanding root growth patterns in gel medium has come from physical analysis (Bengough, 2012; Niklas, 2014).

In this study, we reported a kind of circumnutation: root helical growth inside 0.5–1.2% agar media. To verify the hypothesis that resistance inhibits helical growth of *Arabidopsis* primary roots in agar, we quantified mechanical properties of agar media and the geometric factors of helical root, formulated a simple mechanical model for the *Arabidopsis* root architecture. The root helix movement resulted from internal growth force and agar resistance. This is consistent with the results in previous paper that *Arabidopsis* root waving and coiling represented a flattened spiral growth pattern (Migliaccio and Piconese, 2001; Migliaccio et al., 2013). The root helical growth in soft agar is in line with the root waving on hard agar surface. This indicated the reason underlying root waving in *Arabidopsis*.

2. Methods and materials

2.1. Mechanical properties of agar media

The growth medium consisted of 0.5× Murashige and Skoog basal salts with Gamborg's B5 vitamins (Sigma M-0404), 1.5% sucrose and 0.5–1.2% agar and was adjusted to pH 5.8 with KOH. The agar media were cast into rectangles (length = 40 mm, width = 20 mm, height = 12 mm). These rectangular samples were used to measure the elastic modulus and penetration resistance, and each parameter was measured in triplicate for each sample. Under the conditions of 23 ∘C and 50% humidity, axial compression tests were performed on an Electro PULS E1000 (Instron, USA. ± 1000 N dynamic load capacity). Loading rate was 1 mm per min. Finish the test when cross-head displacement reached 1.5 mm. Specimens of each agar concentration were tested to obtain the Young's modulus.

Further gel experiments were carried out to test the relationship between agar concentration and penetration resistance by a penetrometer with a 30° semi-angle cone angle. The diameter of cone base was 2 mm. Penetrometer resistance was calculated through dividing the maximum force required to push the penetrometer into gel at 10 mm per min by the cross-sectional area of cone base. In each agar concentration, three penetrations were repeated.

2.2. Helical root culture

All experiments in this study used *Arabidopsis thaliana*, ecotype *Columbia* (Col-0) and DR5-GUS mutant. MS medium for root growth was the same as earlier-mentioned. The melted, sterilized agar media of 0.5–1.2% agar concentration were poured into petri dishes (diameter = 9 cm, glass) up to a thickness of 12 mm. When the agar cooled and hardened, it was cut along the chord (8 cm) with a scalpel, and the small part was discarded. The petri dish was placed vertically , which enabled the chord to be positioned horizontally. Ecotype *Columbia* (Col-0) and DR5-GUS seeds were surface sterilized with 50% commercial NaOCl bleach and 0.01% SDS, followed by three sterile water washes. Fifteen to twenty seeds per petri dish were sown 1 mm deep into agar media and in a horizontal row. Primary root was subjected to a resistance force when elongating through agar, and the direction of resistance was opposite to the growth direction (Fig. 1). The plates were sealed with a microporous surgical tape. Growth conditions also included a 16-h-light/8-h-dark cycle (100 μmol m^{-2} s^{-1}, cool white fluorescent and incandescent bulbs) and 75% relative humidity at 22 °C for 7 days.

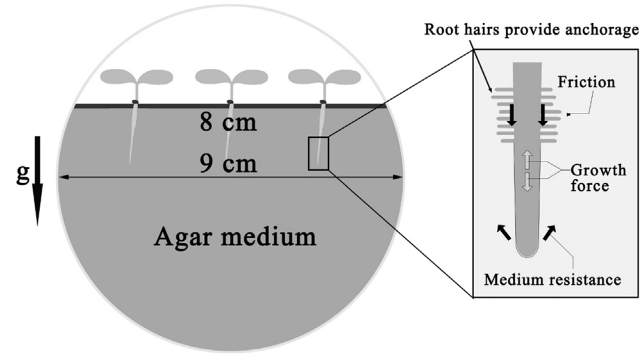

Fig. 1. A schematic of how the *Arabidopsis* plants were grown in agar medium. The inset illustrates schematically the force analysis of root tip when *Arabidopsis* were growing in agar medium and agar concentrations leaded to different resistance during root penetration.

2.3. GUS staining and microscopy

DR5-GUS mutants have been used in many experiments to demonstrate the root patterning as affected by auxins. In order to visualize auxin distribution, we used a DR5-GUS mutant according to Ulmasov et al. (1997). DR5-GUS seedlings were incubated overnight in solution containing 100 mM phosphate buffer pH 7.2, 0.5 mM $K_3[Fe(CN6)]$, 0.5 mM $K_4[Fe(CN6)]$, 10 mM EDTA, 20% methanol, 0.01% Triton X-100 and 2 mg/ml X-gluc. Seedlings were then cleared and analyzed microscopically. For analysis of DR5-GUS reporter expression, seedlings were induced at 37 °C and stained for GUS as previously described (Ivanchenko et al., 2010; Ulmasov et al., 1997).

2.4. Phenotypic and statistical analyses

Images of roots (n = 90 in every culture condition) were taken for each of the individual plates. Thirty roots were tested for each stiffness, and experiments were typically repeated for three times. For clear visualization of the root growth pattern in agar media, 7-day-old roots were observed using an Olympus microscope. Microscopy was available since agar medium was transparent.

To avoid error in measurement of root length through two-dimensional photographs (root could not always grow in a plane), roots were separated from agar medium after experiment and shaped straight on the slice with 1/2 MS liquid nutrition, then the length was measured from root apex to hypocotyl along the central axis of the primary root with microscope. Measurements were made by Image J software. Statistical analyses were performed by Microsoft Excel software.