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A Biomechanical Characterization of Plant Root Tissues by Dynamic Nanoindentation Technique for Biomimetic Technologies

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Abstract. In this work we present a study on mechanical properties of *Zea mays* primary roots. In order to have an accurate overview of the root structure, three different regions have been analyzed: the outer wall, the inner part, and the root cap. We used a dynamic nanoindentation technique to measure the elasticity modulus of root tissues in correspondence of different distances from the root tip. A sample holder was built to test the tip and a method conceived to separate the outer wall of the root from the inner part. As determined by dynamic nanoindentation, we measured the storage modulus of plant roots over 1–200 Hz range. We found that the values of the storage modulus along the outer wall are higher with respect to the central core. Moreover, the inner core and root cap seem to be similar in terms of elasticity modulus. This study aims to shed light on the mechanical properties of roots that significantly affect root movements and penetration capabilities. The gathered data on mechanical response and adaptive behaviours of natural roots to mechanical stresses will be used as benchmarks for the design of new soft robots that can efficiently move in soil for exploration and rescue tasks.

Keywords: Plant root tissues · Mechanical properties · Nanomechanics · Root-inspired robots

1 Introduction

It is well known that plant roots are an excellent example of efficiency for soil penetration and exploration with optimal adaptation strategies. Specifically, roots can adapt their growth direction through their soft tissues. Therefore, the penetration capabilities in conjunction with the flexibility of root structure can inspire new design and fabrication of soft robots [1]. In this work we present a preliminary study on the mechanical

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properties of *Zea mays* primary root, at level of its outer wall, inner part, and cap. For this aim, we used a dynamic nanoindentation technique.

2 Materials and Methods

2.1 Planting and Sample Preparation

We tested 3/4-day old *Z. mays L.* seeds. The seedlings were grown on filter paper with tap water and kept into a growth chamber at 25 °C. We measured the mechanical properties in correspondence of three different regions of the root at different frequencies. The nanoindentation experiments were carried out at room temperature employing an iNano indentation system (Nanomechanics, Inc.) and using the 'Dynamic Flat Punch Complex Modulus' test with a routine method for biomaterials [2–4]. We exploited indenter tips having 109 and 198.5 μm diameter. All the root regions were investigated in 1–200 Hz frequency range. To avoid root dehydration, the nanoindentation measurements were carried out in distilled water. The measurements were made on roots (~1 cm in length) at different distances from the tip (2, 3, 4, 5, 6, and 7 mm) in correspondence of the wall, the inner core, and the root cap (Figs. 1 and 2). To avoid any movements of the root during the tests, we fixed the samples on the bottom of the holder by means of attack (Loctite). Due to the complex geometry of root tip, an *ad hoc* sample holder with two inclinations was built to perform tests in the root tip area (Fig. 1b). In order to extract the root core, we made a circumferential incision of the root at the base of the seed, thus the outer wall can be easily separated from the inner core (Fig. 1d).

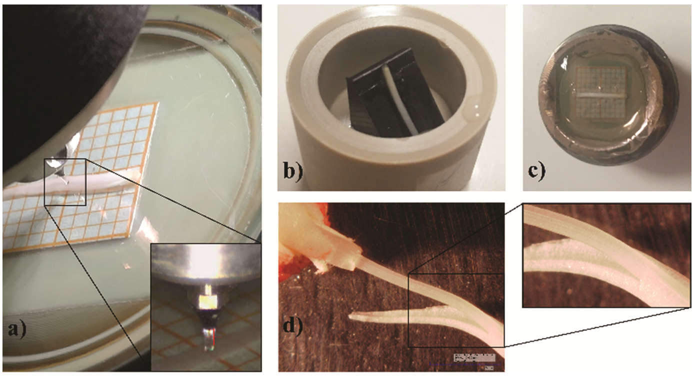

Fig. 1. View of a nanoindentation experiment. (a) A nanoindentation test. The zoom shows the tip of the indentation system; (b) and (c) a sample holder used to test mechanical properties of root tissues near to the cap, and in outer and inner areas, respectively; (d) separation procedure of the outer wall from the inner core.

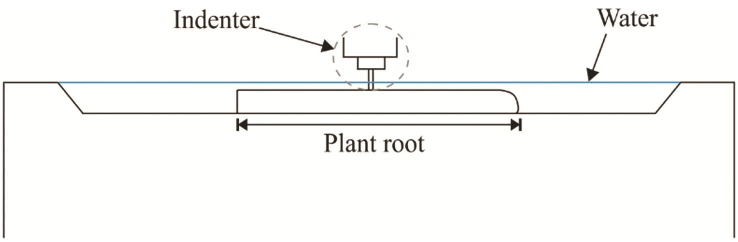

Fig. 2. Schematic of the setup used for testing along the root at different distances from the tip.

2.2 Statistical Analysis

We performed one-way ANOVA to quantify the storage modulus (E) changes with respect to the distance from the tip and at different tissue level (i.e. outer and inner tissues, inner tissue and near the cap) for each frequency. Moreover, we exploited two-way ANOVA (without interaction) to test the effect of the distances and tissues for each frequency.

The statistical analysis is at the 95% confidence level.

3 Results

3.1 Nanoindentation Measurements: Results of Statistical Analysis

We obtained significant E difference between inner and outer tissue measurements at all distances from the tip for each test frequency, except for the inner core measurements at 1 Hz frequency ($p > 0.05$). The one-way ANOVA analysis pointed out significant differences ($p < 0.05$) at 2 mm (10 ÷ 200 Hz), 3 mm (200 Hz), 4 mm (35 ÷ 200 Hz), 5 mm (1 ÷ 200 Hz), and 7 mm (1 ÷ 200 Hz) distance. On the contrary, the comparison between the inner core measurements at each distance from the tip and the measurements near the cap showed no significant differences ($p > 0.05$) for each frequency. Moreover, the two-way ANOVA results showed significant E differences ($p < 0.05$) within the group at all the distances and the group of the tissue levels (i.e. inner core and outer wall).

The results of the nanoindentation tests along the three root regions are reported in Tables 1, 2 and 3.

Table 1. Storage modulus (mean ± SD) of the root cap for all frequencies (1, 3, 10, 15, 35, 85, 200 Hz). A total of 7 indentations on 4 roots were performed.

Freq. (Hz)	E (MPa)
200	4.23 ± 1.53
85	3.9 ± 1.29
35	3.65 ± 1.22
15	3.39 ± 1.15
10	3.27 ± 1.13
3	2.81 ± 1.01
1	2.49 ± 0.93

Table 2. Storage modulus (mean ± SD) along the inner core at different distances from the tip (2, 3, 4, 5, 6, 7 mm) for all frequencies (1, 3, 10, 15, 35, 85, 200 Hz). The measurements number for each distance is reported.

Freq. (Hz)	E (MPa)					
	2 mm (no. 9)	3 mm (no. 12)	4 mm (no. 14)	5 mm (no. 12)	6 mm (no. 7)	7 mm (no. 5)
200	4.54 ± 1.74	4.63 ± 1.53	4.21 ± 1.15	3.33 ± 1.15	3.23 ± 1.19	2.77 ± 0.83
85	4.29 ± 1.69	4.39 ± 1.45	3.97 ± 1.12	3.17 ± 1.08	3.04 ± 1.15	2.61 ± 0.85
35	4.13 ± 1.67	4.23 ± 1.41	3.83 ± 1.1	3.04 ± 1.06	2.93 ± 1.14	2.49 ± 0.84
15	3.98 ± 1.64	4.08 ± 1.37	3.69 ± 1.09	2.91 ± 1.06	2.83 ± 1.12	2.38 ± 0.84
10	3.9 ± 1.63	4 ± 1.34	3.63 ± 1.09	2.85 ± 1.06	2.78 ± 1.11	2.33 ± 0.85
3	3.54 ± 1.54	3.65 ± 1.27	3.3 ± 1.05	2.58 ± 1	2.54 ± 1.05	2.1 ± 0.84
1	3.25 ± 1.5	3.38 ± 1.25	3.09 ± 1.07	2.4 ± 0.98	2.38 ± 1.04	1.9 ± 0.85

Table 3. Storage modulus (mean ± SD) along the outer wall at different distances from the tip (2, 3, 4, 5, 6, 7 mm) for all frequencies (1, 3, 10, 15, 35, 85, 200 Hz). The measurements number for each distance is reported.

Freq. (Hz)	E (MPa)					
	2 mm (no. 5)	3 mm (no. 13)	4 mm (no. 8)	5 mm (no. 22)	6 mm (no. 14)	7 mm (no. 15)
200	7.32 ± 2.22	5.99 ± 1.7	5.65 ± 1.92	5.49 ± 1.3	4.36 ± 1.26	4.82 ± 1.14
85	7.01 ± 1.99	5.64 ± 1.69	5.43 ± 1.92	5.27 ± 1.26	4.16 ± 1.2	4.6 ± 1.1
35	6.63 ± 1.89	5.31 ± 1.61	5.18 ± 1.87	5.01 ± 1.22	3.95 ± 1.15	4.39 ± 1.1
15	6.23 ± 1.75	4.99 ± 1.51	4.94 ± 1.83	4.75 ± 1.19	3.74 ± 1.1	4.17 ± 1.11
10	6.03 ± 1.65	4.83 ± 1.46	4.82 ± 1.81	4.62 ± 1.18	3.64 ± 1.07	4.07 ± 1.11
3	5.23 ± 1.39	4.15 ± 1.22	4.32 ± 1.66	4.05 ± 1.1	3.21 ± 0.96	3.62 ± 1.06
1	4.66 ± 1.27	3.63 ± 1.08	3.93 ± 1.51	3.65 ± 1	2.94 ± 0.86	3.35 ± 0.99

4 Discussion

The E smaller values at the inner tissue with respect to the outer tissue suggest that plant roots could be made by a stiff coating around a soft core, which works as a 'soft skeleton'. This would represent a cushioning layer that contributes to protect the root cap from external abrasion and friction, together with mucilage secretion from the same area. On the other hand, plant roots penetrate soil by growing at the apical region, thus a stiff tissue near the tip could enhance the movements into a medium. Moreover, the E higher value at distances close to the tip and the tissue softness just behind the tip could explain the adaptive behaviour during penetration. Since soils could have several barriers, e.g. rocks, roots may activate a response to circumvent them, when the penetration is not the optimal strategy at low energy cost. Yet, roots buckle to avoid obstacles, e.g. a tip-to-barrier angle, was observed in [5].

5 Conclusions

In this work, we used the dynamic nanoindentation technique to study the mechanical properties of root tissues. Since new cells are continuously created in the apical region, the cellular differentiation is at an early stage close to the root tip. Therefore, the results of the mechanical tests could reveal root penetration strategies during growing from the tip. Our results suggest that roots could consist of an internal 'soft skeleton' and a stiffer outer wall. This combination of soft and stiff materials may enhance plant roots to simultaneously penetrate and adapt to soil constraints. Future developments of these preliminary results will be the study of the whole plant tissues in various plant species to better understand the role of mechanical tissue properties in penetrating soil at different impedance.

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