1 **TORSIONS-DRIVEN ROOT HELICAL GROWTH, WAVING AND SKEWING IN *ARABIDOPSIS***

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5 **Abstract**

6 Helical growth broadly exists in immobile plants to support their limited movement, and *Arabidopsis* seedling
7 root exhibiting natural left-handedness helical growth is considered as a simplified model for investigating this
8 interesting behavior. Efforts have been made for understanding the mechanism of root helical growth and
9 consequent root waving and skewing on tilted and impenetrable surface, and several models have been
10 established. Here, previous reports are reviewed and a straightforward torsions-driven mechanism has been
11 emphasized, and additional experiments have been performed to fill up the gaps of this theory in our study.

12 This study implies that, torsions originating from handedness of both cortical microtubules and cellulose
13 microfibrils play central role in root handed helical growth. Different from torsions directly provided by handed
14 assembled cortical microtubules, torsions originating from right-handed assembled cellulose microfibrils are
15 relaxed by their cross-linking with pectin within cell wall, but only exhibited when their cross-linking is
16 interrupted due to damaged cell wall integrity. To topologically relax these torsions, supercoils of cortical
17 microtubules and/or cellulose microfibrils exhibiting as oblique alignments are formed in root cells, which alter
18 the orientation of root cell files and generate handed helical roots. Working together with gravitropic response,
19 relaxation of torsions originating from helical roots drives roots to elongate with handedness, which therefore
20 produces waved and skewed roots on tilted and impenetrable surface.

21 **Natural root helical growth, waving and skewing in *Arabidopsis***

22 Immobile plants develop various strategies to perform limited movement, among which, helical growth is one of
23 the most efficient. Helical growth broadly exists in plants, and *Arabidopsis* root exhibiting natural but slight
24 left-handed root helical growth provides us a simple model for understanding the mechanism of handed growth
25 (Fig.1A) [1; 2; 3; 4]. Coupling with gel-root interaction and gravitropic response, their helical roots wave and

26 skew with specific angles on tilted and impenetrable surface, which are surprisingly ecotype-dependent
 27 behaviors [1; 5] (Fig.1B-1D). In our study, the direction of root skewing is determined by looking downward
 28 from upside of petri-dish, which therefore opposite to our figures taken from downside of petri-dish through the
 29 medium.

30
 31 **Figure.1** Natural root helical growth, and root waving and skewing on tilted and impenetrable surface. (A)
 32 Five-day-old seedling root of *Ws* ecotype *Arabidopsis*, and the helical growth was pointed by white arrow; bar,
 33 0.2mm. Seven-day-old seedling roots from *Ws* (B) and *col-0* (C) ecotype *Arabidopsis* on 30° tilted 1/2MS
 34 medium containing 1.5% agar; bar, 5mm; and their skewing angles were shown in (D); n=20, and three
 35 biological repeats are performed; standard deviations are indicated as error bars. .

36 Cortical microtubule-determined root helical growth in *Arabidopsis*

37 The cortical microtubules-dependence is the most studied, and believed to be the main cause of root helical
 38 growth in *Arabidopsis* [2; 4; 6; 7; 8; 9; 10; 11; 12]. The microtubule is a hollow cylindrical structure with the
 39 diameter of 25nm generally surrounded by 13 straight protofilaments assembled by α - and β -tubulin molecules,
 40 of which typical length is several micrometers [13; 14] (Fig.2A). Both *in vitro* and *in vivo* data suggest that,
 41 microtubule from mammalian cells could be assembled by 11-16 protofilaments with a 3-4 start helix, but only
 42 microtubule with 13 protofilaments and a 3-start helix (13-3) could perfectly maintain all protofilaments parallel
 43 to the axis [15; 16; 17; 18; 19] (Fig.2C). To maintain the lattice integrity, microtubule assembled by 11-3, 12-3,

44 15-4, 16-4 protofilaments would cause right-handedness (Fig.2B), but 14-3 protofilaments would cause
 45 left-handedness, of which process is interestingly dynamic and reversible (Fig.2D) [15; 16; 18; 20]. Except
 46 straight microtubules with 13-3 protofilaments, left-handed microtubules with 14-3 protofilaments is
 47 predominant *in vitro* [16].

48 Shortly after mitosis, cortical microtubules are transversely aligned closely beneath to plasma membrane through
 49 an identified mechanism, which then determine cell anisotropic expansion through acting as cytoskeleton and
 50 guiding cellulose microfibrils [21; 22; 23]. If cortical microtubules with 14-3 protofilaments present in
 51 *Arabidopsis* as *in vitro*, transversely aligned left-handed cortical microtubules would exhibit right-handed
 52 supercoils to relax the torsions when stretched by the cytoplasm in rapidly elongating root cells (Fig.2E), which
 53 would be shown as obliquity of cortical microtubules (Fig.2F) [7]. The oblique cortical microtubules guiding the
 54 synthesis of cellulose microfibrils would alter the directional elongation, and form left-handed root cell file
 55 rotation (Fig.2G) which eventually cause right-handed supercoil of root (Fig.2H).

56 Unfortunately, direct observations on very limited number of cortical microtubules hasn't exhibit us the presence
 57 of that with 14-3 protofilaments [8]. But considering the possibility that torsions provided by a small proportion
 58 of handed assembled cortical microtubules might be capable of re-orientating other non-handed cortical
 59 microtubules, a further quantitative study might be required for concluding or excluding the presence of
 60 left-handed cortical microtubules with 14-3 protofilaments.

Figure.2 Cortical microtubule-determined root helical growth in *Arabidopsis*. (A), partial typical microtubule polymerized by α - and β -tubulin molecules with 13 protofilaments and a 3-start helix; (B), right-handed microtubule; (C), typical microtubule and (D), left-handed microtubule due to various assemble of protofilaments are shown; (E), right-handed supercoil of cortical microtubule alignments; (F), oblique cortical microtubules guiding cellulose microfibrils determine the direction elongation of root cell; (G), left-handed root cell file rotation; and (H), right-handed supercoil of root.

The role right-handed assembled cellulose microfibrils play in root handedness

Backbone of *Arabidopsis* root primary cell wall is composed of cellulose microfibrils cross-linking by polysaccharide molecules, such as pectin and hemicellulose [24; 25] (Fig.3B). Comparing with the uncertain handedness of cortical microtubules, cellulose microfibrils synthesized by transmembrane cellulose synthase rosettes containing six cellulose synthase are determinately right-handed assembled [2; 26] (Fig.3A). Based the same theory described above, right-handed assembled cellulose microfibrils should cause left-handed supercoil of cellulose microfibrils alignments (Fig.3D) and therefore right-handed root cell file rotation (Fig.3E).

75 But this mechanism hasn't been clarified, whereas the cross-linking by polysaccharide molecules might be the
76 key. Generally, cellulose microfibrils are cross-linked by flexible polysaccharide pectin [24; 25], which might be
77 capable of relaxing the torsions originating from handed assembled cellulose microfibrils.

78 Actually, previous data has already exhibited us the clues: 1. Ca^{2+} is crucial for the cross-linking of cellulose
79 microfibrils by pectin, and high concentrated NaCl displacing Ca^{2+} from cell wall and therefore decross-linking
80 cellulose microfibrils would result in right-handed root helical growth [27; 28; 29]; 2. Mutation of receptor-like
81 kinase FER perceiving pectin decross-linked from damaged cell wall and activating Ca^{2+} export to enforce
82 cellulose microfibrils-pectin cross-linking also results in right-handed root helical growth [30; 31]. But further
83 experiments could clearly demonstrate this mechanism are required.

84

85 **Figure 3.** Cellulose microfibrils-determined root helical growth in *Arabidopsis*. (A), the synthesis and
86 right-handed assemble of cellulose microfibrils modified from [2]; (B), cross-linked and (C), decross-linked
87 cellulose microfibrils by pectin; (D), left-handed supercoil of cellulose microfibrils; and (E), left-handed root
88 cell file rotation.

89 **Elongation rate-dependent root helical growth**

90 Besides handed assembled cortical microtubules or cellulose microfibrils, another elongation rate-dependent
 91 mechanism has been revealed to be related to root helical growth in *Arabidopsis*. In *sku5* or *cob* knockout
 92 mutants, of which anisotropic cell expansion is interrupted, elongation of root cells is largely inhibited [32; 33;
 93 34; 35; 36; 37]. But possibly due to the pressure from epidermis and cortex, defected transverse expansion of
 94 their root inner structures is inhibited, which therefore cause the different elongation rate between outer and
 95 inner structures aggravating their original helical growth [6].

96 **Formation of root waving and skewing on tilted and impenetrable surface**

97 On tilted and impenetrable surface, helical root cell file rotation works together with gravitropic response to form
 98 waving and skewing roots, and several models have been established to explain their formation [1; 2; 3; 4; 38].
 99 In this study, this process is further observed, and the traces left on medium surface by impeded point and
 100 re-localized elongation zone (Fig.4A) reveal a simplified torsion-driven model: 1, root cap fails to enter tilted
 101 medium but is impeded; 2, due to the impediment of root cap, rapid elongation drives root to curve at elongation
 102 zone, and then to left the trace on medium surface shown in Fig.4A; 3, in the process of curvature, to relax the
 103 torsions provided by the left-handed root cell file rotation at elongation zone, modified right-handed root
 104 supercoil is formed (Fig.4B); 4, as long as the gravitropic response is activated by the curvature of root tip due to
 105 right-handed root supercoil, root tip curves downward again, and repeat the processes described above to form
 106 root waving and skewing.

107 In this process, cell wall mediates the relaxation of torsions, and application of pectinase solution from
 108 *Aspergillus niger* (P4716-Sigma-aldrich) reducing cell wall mechanic strength through digesting cell wall
 109 components strongly suppresses root waving and skewing (Fig.4G-4I), although their root helical growth is not
 110 significantly affected (Fig.4C-4F).

Figure 4. Formation of root waving and skewing, and the role cell wall play in this process. **(A)**, waved root tip from 7-day-old *Ws* ecotype *Arabidopsis* seedling by root incline assay, and traces left by impeded root cap and re-localized elongation zone are pointed by white arrows; bar, 1mm; **(B)**, formation of right-handed root supercoil originating from impeding of root cap and rapid elongation of root at elongation zone; 7-day-old *Ws* ecotype *Arabidopsis* root tips by root incline assay without **(C and F)** or with pectinase **(D and G)** are shown; bar, 0.5mm; their root waving and skewing are shown in **(E)** and **(F)**; bar, 5mm; and the skewing angles are indicated in **(G)**; n=20, and error bars are indicated.

The role gravitropic response plays in root waving and skewing

As described above, gravitropic response works to re-orientate the curved root tips due to relaxation of torsion from left-handed root cell file rotation, and to form root waving. Therefore, application of NPA blocking auxin polar transport and suppressing tropic response would significantly suppress the root waving, but form root supercoils (Fig.5A-5C).

On the contrary, *sku5* mutant exhibiting more severe left-handedness of root cell file rotation without altering

gravitropic response [32] (Fig.5D-5E) would overcome the influence from gravitropic response, and also form root supercoils (Fig.5F-5G).

It implies that both tropic response and helical growth participate in formation of root waving and skewing, which make it unreliable to determine the root helical growth simply through observing root waving and skewing, especially in those mutants affecting auxin polar distribution [39; 40; 41].

Figure 5. Gravitropic response in formation of root waving and skewing. Incline growth assay is utilized on *Arabidopsis* ecotype *Arabidopsis* without (A), or with 0.5 μM (B) and 1 μM (C) NPA blocking auxin polar transport; bar, 0.5 cm. Root tips of 5-day-old seedlings from (D) *Ws* and (E) *sku5-2* mutant allele, bar = 0.5 mm; and their root waving and skewing by incline growth assay are shown in (F) and (G); bar, 0.5 cm.

DISCUSSION

Our study emphasizes a useful and simplified mechanism to understand the helical behaviors of *Arabidopsis* roots which might also work on other organs and species. This model could be supported by the root helical behaviors found in various *Arabidopsis* mutants which have been well reviewed recently in [2] and re-classified according to the origins mentioned in our study: Class 1, mutations on tubulin molecules or MAPs altering handedness of cortical microtubules, such as *lefty1* and *lefty2* alleles modifying α -tubulin6 and α -tubulin4 respectively [9], and *loss-of-function* of a microtubule plus-end tracking protein *SPR1* [11; 42], which cause

respective helical behaviors; Class 2, *loss-of-functions* of cell wall integrity-related components, such as *FER* perceiving and recovering damaged cell wall integrity, which result in right-handed helical behaviors [30; 31]; Class 3, *loss-of-functions* of genes regulating cell anisotropic expansion, such as *COB* [35] and *SKU5* [32], which aggravate original helical behaviors; and Class 4, *loss-of-functions* of genes regulating auxin polar distribution, such as *AUX1* [39; 40; 43], *TWD1* [44] and *RCN1* [41; 45], which do not alter root helical growth, but alter other behaviors such as root waving and skewing. To understand the mechanisms of altered root helical behaviors in other mutants, further investigations are required.

PERSPECTIVES

To complete the model of torsions-driven helical behaviors in *Arabidopsis* root, urgent tasks are required: 1, quantitative data of the dynamic cortical microtubules assembled by different numbers of protofilaments; 2, convincing evidence of helical behaviors originating from right-handed assembled cellulose microfibrils.

METHODS

Plant materials

T-DNA insertion line *sku5-2* (FLAG_300H05) is in *Ws* ecotype *Arabidopsis* background.

Incline growth assay

Seeds of *Ws* and *sku5-2* mutant are sowed on 30° tilted 1/2MS medium containing 1.5% Agar, and then grow in green chamber under 16/8 hours light at 22°C for 7 days. In our study, the direction of root skewing is determined by looking downward from upside of petri-dish, which therefore opposite to our figures taken from downside of petri-dish through the medium.

Pectinase treatment

1mM pectinase solution from *Aspergillus niger* (P4716-Sigma-aldrich), which could reduce cell wall mechanic strength through digesting cell wall components, is added in 1L cooled 1/2MS medium, and then poured into petri-dishes.

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