A new *Arabidopsis thaliana* root gravitropism and chirality mutant

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**Abstract**

A new *Arabidopsis thaliana* (L.) Heynh. root mutant, named *clg1*, was isolated from the Feldmann–Du Pont T-DNA insertional mutagenesis collection. It is characterized by primary roots that make early right-handed coils, show increased right-handed slanting, and reduced positive gravitropism. The mutant roots however did not exhibit increased resistance to IAA, but only a modest increment of resistance to the auxins 2,4 D, and NAA, and to the auxin transport inhibitors TIBA and NPA. By contrast, the mutant roots showed a notable resistance to plant hormone ethylene (given as ACC). *clg1* appears to be new, since it complements the most known auxin and gravitropism mutants, maps to chromosome 5, and shows a phenotype largely different from that of the known ethylene mutants. The increased right-handed slanting (chirality) can possibly be a consequence of the reduced gravitropic response, since gravitropism and slanting are competitive growth-direction leading forces. The increased resistance to ethylene, seems to indicate that this phythormone plays a role in the gravitropic response of roots (as already proposed for shoots), and possibly in the regulation of the connected signal transduction pathway. The gene involved in the *clg1* mutation, which is recessive, was mapped, as above reported, to chromosome 5, close to the visible marker *tt3*. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords**: Arabidopsis; Roots; Gravitropism; Right-hand slanting

1. **Introduction**

As is the case for many arguments in plant biology, in the last few years the research on root growth and development was notably fostered and renewed by the use of the model system *Arabidopsis*. In particular, it has recently been discovered that the roots of this plant do not grow straight along the gravitational vector, but instead follow a complex pattern that seems to be the result of forces of a different nature. These are essentially circumnutation, positive gravitropism, and thigmotropism [1–5].

Many scientists contributed to this research: Maher and Martindale [6] and Mirza [7] first reported the coiling habit of *Arabidopsis* roots; Okada and Shimura [2,8] showed the waving habit of the roots down tilted hard-agar plates; Simmons et al. [3,4] characterized the slanting and the right-handedness of the roots; Rutherford and Masson [9] elucidated many fine aspects of the process.

Fundamental to this research was the recurrent discovery of interesting mutants, the first of which, *aux1*, showing resistance to auxin and no gravitropic response, was described by Maher and Martindale [6]. It was followed by the description by Estelle and Somerville [10], and Lincoln et al. [11] by a full set of new mutants, the most significant of which are *axr1*, *axr2*, and *axr3*, showing increased root resistance to auxin and reduced gravitropism. The description of these mutants was followed by the isolation of *agr1* by Bell and Maher [12], also characterized...
by reduced root gravitropism, but not by resistance to auxin, and by the mutants named **wav** by Okada and Shimura [2,8], because of the anomalies shown in the root waving pattern. Later **wav5** was determined to be allelic with **aux1**, and **wav6** with **agr1**, **eir1**, and **pin2**; auxin and ethylene mutants. Simmons et al. [3,4] thereafter isolated **rgr1**, also agravitropic in the roots, which was later shown to be allelic with **axr4** [13]. Rutherford and Masson [9] isolated **sku1,2,3,5**. When its roots were grown down a tilted hard-agar surface, they showed modifications in the wavy pattern, and more recently, Marinelli et al. [14] isolated **I-6C**, a mutant that shows inverted (left-handed) root slanting. In the last few years some of the above genes were also cloned, and resulted in the encoding of elements involved in the transport and metabolism of auxin [15–20]. The ensemble of the data gathered in these years, led at the same time to the formulation of different theories regarding the root growth patterns, which are somewhat discordant. In particular, Okada and Shimura [2] hypothesized that the wavy pattern was the result of the cumulative effects of positive gravitropism and thigmotropism, the root tip moving from side to side on an inclined plate after the impact with the agar surface. Simmons et al. [3], and Migliaccio et al. [21] saw instead the process as the resultant of circumnutation and positive gravitropism, connecting the waving with the well known tendency of roots of many plants to grow circumnutating to one hand [1,5,22]. Rutherford and Masson [9] substantially backed the Okada and Shimura hypothesis, even acknowledging that circumnutation should be involved in the process.

The nature of the root pattern seems therefore still unclear, and more research, mutant analysis, and the cloning of the involved genes are surely necessary. With this in mind we started our search for new **Arabidopsis** mutations of the root growth pattern, that led us to the isolation of **clg1**, a mutant that does not appear to have been already described, that shows reduced gravitropism, and probably, as a consequence, high tendency to produce coils and increased slanting to the right-hand. This paper is about the characterization of this mutant, that we hope can become a new tool to investigate growth and tropic movements in **Arabidopsis** roots.

### 2. Materials and methods

#### 2.1. Plant material and growth conditions

The T-DNA mutagenized seeds (collection Feldmann–Du Pont), as well as the mutants **aux1-7**, **axr1-3**, **axr2** were provided by the Nottingham **Arabidopsis** Stock Center (Nottingham, UK). Seeds of **A. thaliana** (L.) Heynh., ecotype Wassilevskija, as well as the **rgr1** and **wav6** mutants were obtained from D. Söll laboratory (Yale University, New Haven CT, USA).

Before plating, the seeds were sterilized by treating them for ten min with a solution made up of 50% commercial bleach (brand name Clorex) and 0.01% (w/v) SDS, followed by four washes with sterile distilled water. The seeds were then plated in horizontal rows, in petri dishes, on a medium made up (except in some cases where 0.8 and 1% agar were used) of 1.5% agar, 1% sucrose, and 0.5 MS basal medium, enriched with Gamborg’s vitamins (from Sigma, n. M0404), adjusted to pH 5.7 with KOH. To synchronize germination, dishes were left in a cold room (4°C) for 2 days before moving them to the growth room.

Plants were at first grown on dishes for 10–14 days in a growth room (in white fluorescent light at 80 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and 25°C), and then transferred in Arasystem pots (5 cm) on universal soil (Einheitserde-Humuswerke, type VM; Gebr. Patzer, Sinntal-Jossa, Germany), in a Conviron climatic chamber, in white fluorescent light at 200 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The plants were grown keeping them at first in short days (8/16 h light–dark periods) to promote rosette growth, and, after 3 weeks, in long days (16/8 h light–dark periods) to promote flowering. Humidity in the Conviron was 65%, temperature 23°C. The pots were watered three times a week: two with tap water, and one with a nutrient solution (0.5 mg/l Mannalin-A). At the time of seed maturation the plants were moved to a greenhouse, where they were kept until seed collection.

#### 2.2. Mutant selection

Of the 49 pools of 100 lines that constitute the Feldmann collection, 48, for a total of 20 758 single seedlings, were submitted to the first
screening. This consisted of the visual examination of the growth direction (slanting and gravitropism) and of the general behavior of the primary root, looking for modification of the above characters.

Seeds were germinated and grown in petri dishes, on agar medium containing kanamycin (50 mg/l). The dishes were kept vertical in the light for 3 days, then part of them were rotated 90° with respect to the vertical to check for the gravitropic response, and part were inclined 60° on the horizontal plane to check for slanting. The dishes thereafter were kept in the above settings for about 1 week. Plants resistant to kanamycin, showing an anomalous root growth pattern, were selected, grown to maturity and selfed. The second generation was then submitted to a new screening on a medium deprived of kanamycin, together with the wild-type as a control. As a result of the screening we made, a few interesting root mutants were isolated, of which we started to study the most promising one. This was named clg1 for the characteristic of making very early coils, even on vertically oriented agar plates, and for showing increased right-handed slanting.

2.3. Gravitropism and slanting determination

To quantify the gravitropic response of the roots, clg1 seeds were grown in square dishes between two 1% agar layers to avoid the production of slanting, which can interfere with gravitropism. The dishes were kept vertical throughout the experiments, for 3 days in the light, then moved to dark for 7 days, and rotated by 90° in a clockwise direction. The angle made by the roots with the first direction of growth was measured at 2 h intervals over an 8 h period, and after 24 h of gravistimulation. Angle determination was made by a protractor on line marks traced on the bottom of the dishes.

Root slanting was measured similarly by a protractor, making reference to a vertical line parallel to the gravitational vector traced on the bottom of the plates. Dishes were kept vertical for 3 days, and then inclined 60° over 7 additional days, in light conditions.

Root elongation was also evaluated in light conditions, after the 3rd day from germination, by measuring under a stereomicroscope, the root growth over a 5 day period.

2.4. Response to hormones

Seeds were germinated and grown for 5 days in the light, in vertically set petri dishes. Thereafter, same size seedlings were transferred to a new medium containing the hormones, and grown for 7 days in the dark. Root elongation was measured over the last 7 days, and from the measurements the growth relative to controls was evaluated. Data are mean values of at least 10 samples ± SE.

Hormonal substances, e.g. IAA (indole-3-acetic acid), 2,4D (2,4 dicloro phenoxyacetic acid), NAA (naphtalene acetic acid), TIBA (2,3,5 triiodobenzoic acid), NPA (N-(1-naphtyl) phtalamic acid), were administered by dissolving them in ethyl alcohol, and by dispersing a minimal amount of the substance in the media (alcohol less than 0.1%, w/v). Ethylene was administered as ACC (1-amino cyclopropane-1-carboxylic acid) dissolved in water.

The response of shoots to ethylene and auxins (hook formation) was measured on plants germinated and grown totally in the dark on 0.8% agar, visually determining the hook shape and assigning it to one of three classes: regular, exaggerated, or absent.

The environment temperature was kept in all the above experiments constantly at 25°C.

2.5. Genetic analyses

Crosses were made on the apical part of a flowering stalk carrying the immature buds following standard techniques [4].

All data were submitted to the χ², or the Student’s t-tests, to check for confidence with the expected segregation ratio.

Pictures were taken by means of a Nikon camera on Ektachrome 160 T Kodak film.

3. Results

3.1. Genetics of clg1

clg1 was selected through a new screening of the 4900 T-tagged Arabidopsis lines of the Feldmann–Du Pont collection as reported in the previous section. This mutant is characterized by a tendency to make early right-handed coils, by increased right-handed slanting, and by slow
Fig. 1. Growth habit of clg1 primary roots compared with wild-type Ws (ecotype Wassilevskijia). Plants grown on hard-agar plates (1.5%), set 60° inclined with respect to the horizontal plane.

Table 1
Chromosome mapping of clg1a,b

<table>
<thead>
<tr>
<th>Marker phenotype</th>
<th>F2 total</th>
<th>clg1</th>
<th>ch1-1</th>
<th>er1</th>
<th>gl-1</th>
<th>cer2-2</th>
<th>tt3-1</th>
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</thead>
<tbody>
<tr>
<td>Chromosome location</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Number screened</td>
<td>605</td>
<td>110</td>
<td>22</td>
<td>30</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N° marker phenotype</td>
<td>110</td>
<td>20</td>
<td>22</td>
<td>30</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>18.1</td>
<td>18.1</td>
<td>20.0</td>
<td>27.2</td>
<td>19.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>X² per 3:1 ratio</td>
<td>2.73</td>
<td>1.47</td>
<td>0.31</td>
<td>2.05</td>
<td>2.05</td>
<td>36.66</td>
<td></td>
</tr>
</tbody>
</table>

a A chromosome mapping line with visible markers ch1, er1, gl1, cer2, tt3 (ABRC seed stock CS3078) was crossed to clg1 (pollen donor) and grown to generate the F2 progeny for mapping.
b Low values result from deliberate conservative clg1 scoring.

Gravitropic response (Fig. 1). The first two characteristics, in contrast with the wild type, are visible not only on inclined, but frequently also on vertically oriented hard-agar plates. This set of root characteristics persisted after three generations produced through selfing and two crossings with the wild type, indicating the monogenetic nature of the traits. Moreover, the cross with the wild-type showed, through the suppression of the mutation in the F1, and the segregation in the F2, the recessivity of the characters, and the Mendelian transmission of them. The mutation did not show however, cosegregation with the resistance to kanamycin in the F2 population originated from the kanamycin resistant F1 individuals. A segregation consistent with a ratio 9:3:3:1 was seen. Consequently, the involved gene did not result tagged.

Tests of allelism were made with most known and available mutants. Through crosses with aux1–7, axr1–3, axr2, wav6 (agr1, eir1, pin2), rgr1 (axr4), allelism was primarily excluded on the basis of the complementation seen in the F1 or F2. On the other hand, the map position of clg1 excluded also allelism with the above mutants, with the exception of agr1, that maps on chromosome 5. The complementation test we run however contradicts this possibility.

1 clg1, as reported, was complemented with the most common and available mutants that show a modified response to gravitropism or auxin concentration. In spite of this, we cannot exclude that it could be allelic with one of the many already described mutants. We did not think, however, it is useful to furthermore extend complementation tests that are time consuming and not always productive.
The mapping of clg1 was made by crossing it with the visible markers mapping line CS 3078 (originally DP 23, Patton and Meinke line), from the ABRC of Ohio State University. As reported in Table 1, the mutation showed linkage with the marker tt3, and thus it is located on chromosome 5, close to that marker. No recombinants were recovered among the 110 F2 clg1 individuals of the reported screen.

A control of other root characteristics, which frequently appear modified in similar mutants such as axr1, rgr1, showed that clg1 roots are wild-type as concerns the number of lateral roots, root hairs, and the number of statolysts in the root cap cells.

3.2. Gravitropism, slanting and elongation

The gravitropic response of clg1, reported in Fig. 2 (top), was clearly reduced, since about 30° of difference were observed with respect to the wild-type after an eight hour period. After 24 h, however, both the wild type and the mutant were for the most part 90° bent (data not shown). Therefore, in the mutant the gravitropic response appears only slowed down, but not reduced in absolute values.

The slanting to the right-hand, reported in Fig. 3, was increased in clg1, even though a widened distribution was observed with respect to the wild-type. The absolute mean values were 22.9° in wild-type and 48.2° in the mutant.

Root elongation (Fig. 2, bottom) was only slightly increased with respect to the wild-type
after 72 h (the Student’s $t$ test is barely significant for $P < 0.05$).

3.3. Effect of hormones

As can be seen from Fig. 4, $clg1$ roots did not show increase of resistance to IAA at all the tested concentrations, showed only a small resistance to 2,4 D (at conc. $10^{-7}$ and $10^{-6}$ M), and a moderate resistance to NAA at all the tested concentrations, but $10^{-6}$. By contrast, a high resistance to the plant hormone ethylene, given as ACC, was observed at all the tested concentrations.

In addition, from Fig. 5 it can be seen that the increase of resistance to the auxin transport inhibitors NPA and TIBA, at concentrations between $10^{-6} - 5 \times 10^{-5}$, of $clg1$ roots was very modest, and resulted slightly significant at the Student’s $t$-test.

4. Discussion

$clg1$ has been isolated as a root chirality and gravitropism mutant, by means of a screening that was principally directed to find new mutants showing variations in the degree of right-handed slanting of the primary roots, in comparison with the wild-type. In the case of $clg1$ the variation in the chirality is observable in the clearly increased right-handed coiling and slanting attitude of the

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We use the expression chirality when referring to the characteristic of *Arabidopsis* roots of slanting and producing coils to the right-hand, which apparently correspond to right-handed spirals flattened on an agar plate. The right side of the plant has been determined by observing, from the top of the plants, the coiling direction in the wild-type roots with respect to the direction of growth. This coiling hand corresponds to that of a common screw, which in physics is considered right-handed (3,9,21).
roots. The mutant primary roots produce coils very early (after 4–5 days) on inclined, or even on vertically set plates (Fig. 1), whereas these are seen sometimes only in old roots (2–3 weeks) in the wild type, and, in addition, the roots show an increased slanting to the right-hand (Fig. 3). These characteristics can be the consequence of a reduced gravitropic response, since gravitropism and slanting appear to be competitive forces, that lead the growth of the root toward different directions. And because reduced gravitropism was demonstrated in roots growing inside agar layers, e.g. in a condition in which any slanting and coiling are abolished, a primary reduction of gravitropism, and a dependent increase of slanting can be considered as a reason to explain clg1 root characteristics. In addition, observations made on roots growing on horizontally set plates (data not reported), a setting that should abolish gravitropism, did not show any clear difference in the coiling habit of clg1 with respect to the wild type. We can thus preliminarily conclude that the gain in slanting in clg1 probably depends on a reduction of

gravitropism, and not necessarily on an increased absolute right-hand deviation from the vertical of the roots. Nevertheless, this point can be settled only by abolishing the force of gravity that pushes also in the case of horizontally set plates the root tip down, and this can be obtained with the use of a clinostat or in microgravity. We can anticipate that we are already working and planning experiments in this direction.

The slow gravitropic response, on the other hand, could indicate allelism of clg1 with some of the already isolated mutants that show a similar reduction of gravitropism. The complementation tests we ran (Table 2), however, and the mapping of clg1 to chromosome 5, close to the visible marker tt3, ruled out this possibility.

clg1 thus seems a new mutant, showing reduced gravitropic response, but only a moderate resistance to the auxins (mainly NAA). This is a condition that makes this mutant possibly more interesting, because, with the exception of agr1, which, however, was recently shown to encode for an auxin efflux carrier [16,18–20,23], and arg1

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**Table 2**

<table>
<thead>
<tr>
<th>Control</th>
<th>ACC (10^-4 M)</th>
<th>NPA (5 x 10^-5 M)</th>
<th>NPA (10^-5 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>wt</strong></td>
<td>0 13 0 18 0 0 0 0 11 0 0 7</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td><strong>clg1</strong></td>
<td>0 15 0 15 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
</tbody>
</table>

*Seedlings were grown in darkness for three days on 0.8% agar medium containing ACC, or NPA. Values represent the number of seedlings showing exaggerated (+ + +), normal (+) or absent (–) apical hook.*

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Fig. 5. clg1 and wild-type Ws root elongation from seedlings grown in presence of the auxin transport inhibitors TIBA and NPA. Elongation is expressed relative to the average root growth of the same genotype on medium without the inhibitors. Each value represents the mean of measurements of at least ten seedlings ± SE. Absolute mean values (± SE) of root elongation on hormone free media were: TIBA plot, wild-type = 1.37 ± 0.04, clg1 = 1.75 ± 0.05; NPA plot, wild-type 8.86 ± 0.34, clg1 = 10.25 ± 0.54; a = differences significant at the Student’s t-test for P < 0.05.
[24], all the other gravitropic mutants show a notable resistance to the auxinic substances and inhibitors of auxin transport. NAA however, is not a natural auxin and it is a easier permeant than IAA and 2,4 D. There is thus the possibility that clg1 could be impaired in a step of the gravitropic response pathway not or weakly connected with the action of auxin, possibly located downstream of the known auxin sensitive genes (AUX1, AGR1, AXR1, AXR2, AXR3).

clg1, in addition, showed clearly increased resistance to ethylene, a fact that was already observed in axr1, axr2, aux1 and agr1(eir1, Atpin2, wav6) [25], even though these mutants are also highly resistant to auxin, and thus the resistance to ethylene could be a consequence of that to auxin. Pickett et al. [26] affirm that they could not find out the real nature of the ethylene resistance, even though they suspect it can be in some way connected with auxin physiology. Ethylene resistance in clg1 supports a role for this hormone in the control of gravitropism not necessarily connected with auxin physiology. A role that has not been sufficiently clarified so far, especially as concerns roots. clg1, on the other hand, does not show the most common characteristics of ethylene insensitive mutants, such as variation in the production of lateral roots, absence of root hairs, and a modification in the seedling apical hook shape [25]. All these characteristics in fact appear wild-type in clg1 (Table 2).

Under the genetic point of view the gene involved in the clg1 mutation was recessive, transmitted through a Mendelian mechanism, and mapped to chromosome 5, close to the visible marker tt3 (Table 1). Close to this marker among the mutants that show characteristics similar to clg1 there is only agr1 on the map. The complementation test we made nevertheless indicated that these two mutants are not allelic.

A loss-of-function mutation is thus likely to be involved. The gene concerned, however, did not result tagged, and consequently the only way to isolate it is by means of positional techniques. We can anticipate that this part of the research will be attempted soon. In the meantime, we hope clg1 could be used as a new tool in investigating gravitropism and growth movements in plant roots.

Acknowledgements

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References