Induction of Primary Root Curvature in Radish Seedlings in a Static Magnetic Field

Akira Yano,1* Eiko Hidaka,2 Kazuhiro Fujiwara,3 and Mitsuo Iimoto2

1Graduate School of Science and Technology, Chiba University, Matsudo, Chiba, Japan
2Faculty of Horticulture, Chiba University, Matsudo, Chiba, Japan
3Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan

Primary roots of radish (Raphanus sativus L.) seedlings were exposed to an inhomogeneous static magnetic field generated by a permanent magnet, during continuous rotation on a 0.06 rpm clinostat, thereby reducing the unilateral influence of gravity. The roots responded tropically to the static magnetic field with the tropism appearing to be negative. These roots responded significantly (P < 0.05) to the south pole of the magnet. The significant tropic response was found for a magnetic flux density of 13–68 mT, for a field gradient of 1.8–14.7 T/m, and for the product of magnetic field and field gradient of 0.023–1.0 T²/m. A small, but insignificant, response of the roots to the north pole has also been found. Bioelectromagnetics 22:194–199, 2001. © 2001 Wiley-Liss, Inc.

Key words: permanent magnet; magnetic field gradient; root elongation; tropism; magnetotropism

INTRODUCTION

Strong inhomogeneous static magnetic fields affect very weak para- or diamagnetic substances [Ueno and Harada, 1982; Beaugnon and Tournier, 1991] and therefore might directly affect living organisms. An outstanding example is levitation of living organisms, overcoming gravity by a strong magnetic field [Berry and Geim, 1997]. Static magnetic fields in the order of μT or mT may also affect certain processes of living systems. However, it has not been clarified how such weak static magnetic fields are able to affect organisms with the exception of magnetite based orientations [Blakemore, 1975; Walker et al., 1997].

The effects of static magnetic fields on the growth and development of plants have often been investigated. For example, some studies addressed the effects of static magnetic fields on tropism of plant roots. Audus [1960] showed that curvature of cress roots was induced in a 22 T²/m (product of field intensity B and gradient ∇B) in homogeneous static magnetic field. Kuznetsov and Hasenstein [1996] conducted similar experiments in flax and Arabidopsis thaliana and showed that a 10³ T²/m static magnetic field caused intracellular displacement of starchy amyloplasts in statocytes of the root cap and has been explained by a difference in diamagnetic susceptibilities between starch and cytoplasmic matrix. Since deposition of amyloplasts are widely believed to trigger gravitropism of plants [Kiss et al., 1989; Kiss and Sack, 1989; Sack, 1997], magnetically induced displacement of amyloplasts might explain the curvature of plant roots towards the direction of the displacement of amyloplasts [Kuznetsov and Hasenstein, 1996]. Other reports, however, suggested the effect of the earth’s magnetic field on the distribution and curvature of plant roots [Pittman, 1962, 1964; Woolley and Pittman, 1966; Kato, 1990]. In these studies, there must be no displacement of amyloplasts due to the weak static magnetic fields, but possibly some unknown effects might exist.

In the present study, radish (Raphanus sativus L.) roots were exposed to an inhomogeneous static magnetic field during continuous rotation on a clinostat in order to reduce the unilateral influence of gravity. The effect of a static magnetic field on the curvature of roots was investigated in terms of intensity and gradient of the magnetic field, product of the magnetic field and the field gradient B∇B, and direction of the magnetic field.

MATERIALS AND METHODS

Seedlings of radish (Raphanus sativus L.) were used, since a preliminary experiment showed that these

*Correspondence to: Akira Yano, Graduate School of Science and Technology, Chiba University, 648 Matsudo, Matsudo, Chiba 271-8510, Japan. E-mail: yano@midori.h.chiba-u.ac.jp

Received for review 3 June 1999; Final revision received 16 May 2000
seedlings elongated primary roots straight in a gel. Gellan gum (Wako Pure Chemical Industries, Osaka, Japan) gel without additional nutrients was used for its remarkable transparency, and roots in the gel could be observed very clearly. The gellan gum is a polysaccharide comprised of glucuronic acid, rhamnose, glucose, and α-acetyl moieties and is commonly used for in vitro culture of higher plants [Pierik, 1997].

All experiments were performed in a growth cabinet (Model MIR-253, Sanyo, Osaka, Japan) at a constant temperature of 24 ± 1 °C and relative humidity of 75 ± 5%. Fifty seeds were positioned on the gel with the embryos downward. After 24 h, 20 germinated seedlings with the root length of 2 ± 1 mm were chosen and each seedling was placed in the center of the surface of 14 g/l gellan gum gel in a glass test tube (25 mm in diameter, 120 mm in length). The test tubes were vertically positioned for the roots to grow straight towards the bottom of the tubes. Subsequently, seedlings were grown for 15 h in the dark. Six seedlings elongating a straight primary root toward the bottom of the test tube were then chosen and used for the following experiments.

At the start of each experiment, the root tip was positioned 13.5 mm above the center of a cylindrical Nd-Fe-B permanent magnet (17 mm in diameter, 2.5 mm in width) that was fixed on the wall of the test tube (Fig. 1). A rectangular coordinate system (x, y, z) (unit: mm) was defined in the space of the test tube as shown in Figure 2. The center of the magnet surface on the test tube has been defined as \( M_0 (0, -12.5, 0) \). The magnetic flux densities in the test tube were determined by using a Hall effect magnetometer (Model HGM-8200, ADS, Tokyo, Japan), its probe (Type F-1, active area of 0.165 × 0.165 mm) being fixed on a micromanipulator to position the Hall element of the probe in the test tube, stepwise 1.0 mm. Distribution of the magnetic flux densities on the y–z plane is shown in Figure 3. The maximum magnetic flux density was 236 mT at the surface of the magnet. In the test tube the product of the magnetic field and the field gradient \( B \nabla B \) was found to be between 0.002 and 1.5 T²/m. Four similar magnets with identical shape and magnetic properties were used in each experiment. The differences of magnetic fields generated by the four magnets were at the level of geomagnetic fields.

In duplicate experiments, a magnet was fixed on a test tube with the north pole directed to the roots (named treatment N) and two other magnets were each fixed on a test tube with its south pole directed to the roots (named treatment S). Two brass disks having an identical shape to the magnets were fixed on the remaining two test tubes, which were magnetically untreated controls. These six test tubes were placed on a clinostat with the magnet or brass facing the outside surface.
of the clinostat. Two test tubes without plant material were also placed on the clinostat to remain the weight balance for a stable clinostat rotation (Fig. 4).

The outside of each test tube was then wrapped with white paper. The clinostat was continuously rotated at 0.06 rpm. The distance from the center of the clinostat to the center of each test tube was 17 cm. Cotyledons of radish seedlings were illuminated from the opening of the test tube by a fluorescent lamp (photosynthetic photon flux density: 22 μmol/(m²·s)) to maintain the stem elongation direction straight toward the opening of the test tube by phototropism [Hart, 1990].

Photographs were taken from both the x and y axes after 24 h of clinostat rotation. The position of the primary root defined as \( R_{zi} (x_{zi}, y_{zi}, z_i) \) was then determined from the photograph. The distance \( d_{zi} \) from the line \( M (0, -12.5, z) \) to each root position \( R_{zi} \) was calculated for every 1 mm increment of the \( z \) coordinate (Fig. 2). \( \delta d \left( = d_{zi} - d_{zi-1} \right) \) was then calculated for each root and used as an index for the extent of root curvature with respect to the magnetic field. Experiments were repeated ten times. Analysis of variance (ANOVA) was performed on \( \delta d \)'s of treatment N, treatment S, and control within each \( z_i \) (the data were not pooled between other \( z \)'s). If statistical differences were found by ANOVA, the least significant difference test was used to identify pairs of means of \( \delta d \)'s that were significantly different. \( P < 0.05 \) was defined as the level of significance.

RESULTS

At the start of the magnetic field exposure (at \( z = -13.5 \)), the mean root position \( (x \pm SE, y \pm SE) \) (unit: mm) of treatment N, treatment S, and control were \( (-0.4 \pm 0.28, -0.1 \pm 0.27), (0.1 \pm 0.29, -0.4 \pm 0.25) \) and \( (-0.2 \pm 0.28, 0.3 \pm 0.25) \), respectively. Two seedlings of treatment S elongated its roots outside the gel and one control seedling did not grow and were removed from the analysis. In case a root touched the test tube during the magnetic field exposure, the position of the roots were determined until the roots almost contacted the test tube. The root length (mean ± SE) of treatment N, treatment S, and control were 30.1 ± 1.77, 29.0 ± 1.96, and 29.9 ± 2.21 mm, respectively. No significant differences in root length among the treatments and no visible disorders were observed in roots and shoots.

Deviations of roots elongating away from stronger magnetic fields were found, as shown especially in the top view of treatment N and the side view of treatment S of Figure 5. Positive \( \delta d \)'s of treatment N and treatment S were found from \( z = -11 \) to +15 (Fig. 6, see Table 1 for the number of data used), indicating that roots curved away from the line \( M \) on which the magnet was fixed, while control experiments showed \( \delta d \) close to zero. At position \( z = 0 \) (the center of the magnet), the value of \( \delta d \) of treatment S showed a maximum among both treatments and was significantly larger than the value of \( \delta d \) of the control at \( z = 0 \) \( (P < 0.05) \); the difference was not significant for any other comparisons at any other \( z \)'s. Each root experienced different values of magnetic field

Fig. 3. Distribution of the magnetic flux densities on the y–z plane in the test tube (see also Fig. 2).

Fig. 4. Four test tubes with plant material and magnets, two test tubes with plant materials and brass plates and two test tubes without plant material for weight balance were placed on the vertically situated clinostat and rotated at a speed of 0.06 rpm.
intensity, gradient, and $B\nabla B$ depending on its position. The largest magnetic flux density that one root of treatment S experienced at $z = 0$ was 68 mT (14.7 T/m, 1.0 T$^2$/m) and the lowest flux density that another root of treatment S experienced at $z = 0$ was 13 mT (1.8 T/m, 23 mT$^2$/m).

**DISCUSSION**

At position $z = 0$ the $\delta d$ of treatment S was the largest and was significantly larger than controls ($P < 0.05$), indicating that roots significantly curved away from stronger magnetic fields when roots passed the region where $B\nabla B$ was 0.023–1.0 T$^2$/m. Using a 2 rpm clinostat, Audus [1960] showed a magnetically induced curvature of cress roots that were exposed to a $B\nabla B = 22$ T$^2$/m magnetic field. Kuznetsov and Hasenstein [1996] also showed that a $10^3$ T$^2$/m magnetic field induced curvature of flax roots. In the present study, curvature of radish roots was induced by magnetic fields weaker than the fields used in these two studies mentioned. Kuznetsov and Hasenstein [1996] showed that magnetically induced displacement of amyloplasts in statocytes of root cap cells triggered the curvature of roots. In other words, the nature of the magnetically induced curvature might reflect the statolith hypothesis in gravitropism. The gravitropism is a well-known physiological phenomenon in plants, although the mechanism is not yet completely clear. For example, sedimentation of amyloplasts (starch-containing plas-
Magnetotropism (statolith hypothesis) [Kiss et al., 1989; Kiss and Sack, 1989; Sack, 1997] has been widely recognized to trigger gravitropism, however Caspar and Pickard [1989] showed gravitropism in a starchless mutant of Arabidopsis. Furthermore, Wayne et al. [1992] suggested that roots respond to gravity by sensing a gravitational pressure exerted by the protoplast. It seems meaningful to consider the magnetically induced tropism with respect to the displacement of amyloplasts. The magnetic force $F_m$ [$N$] acting on amyloplasts can be estimated from the following formula [Audus, 1960; Kuznetsov and Hasenstein, 1996]:

$$F_m = \frac{(\kappa_a - \kappa_b) \cdot V}{\mu_0} \cdot B \nabla B$$

(1)

where $\kappa_a$ (= $-10 \times 10^{-6}$) is the diamagnetic susceptibility per unit volume of starch, which is the major content of amyloplasts, $\kappa_b$ (= $-9.0 \times 10^{-6}$) is the diamagnetic susceptibility of water, which is regarded as the magnetic equivalent of cytoplasm, and $V$ [$m^3$] is the volume of an amyloplast. In the present study we found the most significant tropic response occurring for $B \nabla B$ in the range of 0.023–1.0 T$^2$/m. Hence, substituting 0.023 for $B \nabla B$ in Eq. (1), we can calculate the magnetic force acting on an amyloplast with a volume $V$ of $1.8 \times 10^{-2}$ $V$ [$N$]. Since rotation of the clinostat generated a centrifugal force $m r^2$ of $3.2 \times 10^{-5}$ $V$ [$N$] on the amyloplast, we suggest that the stronger magnetic force is the determining factor that caused roots to curve away from the stronger magnetic fields, i.e., towards the center of the clinostat rotation. However, this reasoning does not explain the different responses of roots to the north or south pole direction of the magnet.

At present, it does not seem possible to provide a satisfying explanation for some previous studies that also mention a different growth response of plants to magnetic north and south poles. Krylov and Tarakanova [1960] indicated that roots and stems of corn and wheat grew faster when their embryos were facing the south pole of a magnet rather than the north pole. They assumed that different magnetic pole orientations act differently on the enzymatic activity of a plant to grow. Ružić et al. [1993] also showed that the south pole stimulated growth of a chestnut more than the north pole. To consider the origin of this polar dependent response, a question may arise whether plants form magnetite like some other organisms [Blakemore, 1975; Gould et al., 1978; Walcott et al., 1979; Hanson et al., 1984; Walker et al., 1988; Chang and Kirschvink, 1989; Kirschvink et al., 1992]. So far, however, presence of magnetite in plants has not been documented [Lowenstam, 1981; Lowenstam and Kirschvink, 1985; Frankel, 1990].

Although displacement of amyloplasts might result in a root curvature, other experimental evidence exists which states that weak static magnetic fields, like the earth’s own magnetic field, can affect certain plant growth responses under unilateral influence of gravity. Pittman [1962] and Woolley and Pittman [1966] reported a clear tendency for north–south

### TABLE 1. Number of Roots Used for Analysis at Each z

<table>
<thead>
<tr>
<th>$z$ (mm)</th>
<th>-12 to -3</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment N</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>18</td>
<td>18</td>
<td>17</td>
<td>15</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>13</td>
<td>11</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment S</td>
<td>18</td>
<td>17</td>
<td>17</td>
<td>16</td>
<td>15</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19</td>
<td>19</td>
<td>18</td>
<td>17</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>15</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
orientation of winter wheat roots under the geomagnetic field. Pittman [1963] also reported that seeds of wheat, barley, oats, rye, and flax germinated faster and grew faster in 48 h when oriented parallel to the magnetic field than when perpendicularly orientated to the field. Hence, we would not totally exclude the possibility that curvature, as found in the present study, might be induced as the extension of such unaccountable effects of weak magnetic fields on plant responses.

In conclusion, the present study shows that primary roots of radish seedlings curved away from stronger magnetic fields when placed under conditions of omnilateral gravity stimulation. The root curvature was found significantly enhanced when roots were exposed in a gradient magnetic field in the vicinity of the south pole of the magnet.

ACKNOWLEDGMENT

We thank Dr. Yngve Hamnerius of Chalmers University of Technology for helpful suggestions while preparing the manuscript. We thank TDK Co. Ltd. for offering the magnets. We much appreciate comments made by the reviewers to improve this paper.

REFERENCES