

# Ionic Currents Associated with Primary Root Growth of Sunflower Seedlings and the Effect of External pH

Panote Thavarungkul,<sup>1,\*</sup> Chulaphorn Muangdit<sup>1</sup> and Karnnika Sunbhanich<sup>2</sup>

<sup>1</sup> Biophysics Research Unit: Biosensors and Biocurrents, Department of Physics, <sup>2</sup>Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.

\* Corresponding author, E-mail: tpanote@ratree.psu.ac.th

Received 31 Oct 2002

Accepted 24 Mar 2003

**ABSTRACT:** The current-patterns along horizontally growing intact sunflower (*Helianthus annuus* L.) seedling roots were investigated using a two-dimensional vibrating probe system. Currents entered the root cap and the meristem and left along the remaining part for most of the shorter roots (10-15 mm). For longer roots (15-20 mm) currents entered the root tip, left the main elongation zone and entered the root again in the post elongation and the root hair zones. These currents entered the meristem with a density of about 1 to 9  $\mu\text{A cm}^{-2}$  and left with a density of about 0.3 to 5  $\mu\text{A cm}^{-2}$  in the elongation zone. The inward currents in the post elongation and the root hair zones were smaller, i.e., about 0.1 to 2  $\mu\text{A cm}^{-2}$ . The results suggest an association between current pattern and root growth. Ion-substitution and pH experiments showed that  $\text{H}^+$  ions were the major contributors to the root currents. Increased root elongation rate at low pH was associated with increased current density and the reversal of the current direction from outward to inward in the main elongation zone. The findings support the acid growth hypothesis.

**KEYWORDS:** acid growth, extracellular currents, *Helianthus*, root elongation, vibrating probe.

## INTRODUCTION

A wide variety of plant and animal systems are known to generate steady transcellular ionic currents that can be detected extracellularly with a vibrating probe.<sup>1</sup> In multicellular plant tissues, the most studied systems were the roots.<sup>1</sup> This is because they generate large ionic currents, grow on simple media and have an existing growth polarity, and their growth can be easily monitored. The role of these currents in roots is still not clear. They have been suggested to be involved with the growth and polarity regulation,<sup>2-6</sup> gravity-induced bending response<sup>7,8</sup> and repair of wound.<sup>9,10</sup> These authors arrived at these conclusions because these effects were associated with the change in either the pattern and/or magnitude of the endogenous currents.

Although growing roots are ideal for investigating these developmental currents, only a few species have been studied in detail. The planting of sunflower (*Helianthus annuus* L.) in Thailand has been increasing and the investigation of this plant seems appropriate. Our long-term aim is to examine in detail relationships between these currents and growth in sunflower roots and root hairs under various root environments. Roots and root hairs were selected since they take up water and minerals from the soil and are important for the nutrition of plants. From the studies of these currents

in roots it is becoming clear that the effect of ionic environment and plant growth regulators,<sup>2,4-6</sup> as well as toxic elements,<sup>11</sup> on growth and development were accompanied by a change of these currents. Therefore, these currents may also be used as an indicator of the effect of these substances. The first step in our research is to investigate the pattern and ionic basis of these currents around primary roots of this plant and their association with growth.

## MATERIALS AND METHODS

### Plant Material

Sunflower seeds *Helianthus annuus* L. (Cargill 3322) were provided by Professor Paisarn Laosuwan, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand. They were surface sterilized in 6% (w/v) hypochlorite for 15 min then washed thoroughly in flowing water. Seeds were sown in 10-cm-diameter petri dishes between double layers of paper towel soaked in artificial pond water (APW) pH 6.0. The petri dishes were placed vertically, with seed's radicle end down, in the dark at  $26 \pm 2$  °C. After 2 days, seedlings that had developed straight roots were selected for experimentation. In the first set of experiments 15-20 mm long roots (obtained about 48 h after the seeds

were sown) were selected and decapped with a scalpel blade to prevent gravitropic response. However, after 3-4 h of measurement in a horizontal position the root started to bend downward and further measurement was not possible. This might be because the root cap tissue was not completely removed for fear that the root might be wounded. Since the root cap cells had not been totally removed, the remaining cells could cause root curvature, although at a slower rate than when the root cap was still intact.<sup>12</sup> From our observation, shorter roots have lesser response to gravistimulation, therefore, shorter roots of 10-15 mm in length (obtained about 43 h after the seeds were sown), with cap were used for the rest of the experiments. All measurements were carried out before any bending due to gravity was observed.

### Root Morphology

Two-day old sunflower seedling roots show four different regions. A root cap of about 0.3 mm lies at the tip protecting the meristematic tissue that extends to about 2.2 mm from the tip. The elongation zone begins about 2.2 mm behind the root tip and reaches up to the root hair zone approximately 7.0 mm from the tip.

### Immobilization of Seedling for Vibrating Probe Measurement

After the seed shell was removed from the cotyledons of the selected intact seedling its hypocotyl was placed between two pieces of perspex and fastened by silicone grease. The bottom piece of perspex was then attached to the bottom of a petri dish (60 mm diameter, 10 mm high) by a thin layer of silicone grease. The entire root protruded horizontally out of the perspex holder. The seedling was covered with 15 ml of APW and left to equilibrate for 45 min before starting the measurement.

### Experimental Media

APW was used through-out the experiments. It consisted of  $1.0 \text{ mol m}^{-3} \text{ NaCl}$ ,  $0.1 \text{ mol m}^{-3} \text{ KCl}$ ,  $0.1 \text{ mol m}^{-3} \text{ CaCl}_2$ , and  $1.0 \text{ mol m}^{-3} \text{ MES}$  adjusted to the required pH with Tris. In the investigation of natural currents APW was adjusted to pH 6.0. Media in the pH experiments were APW with three different pH values, *i.e.*, 4.0, 6.0 and 8.0. Media used during ion-substitution experiments were equimolar where  $\text{K}^+$  and  $\text{Ca}^{2+}$  were replaced with  $\text{Na}^+$ ,  $\text{Na}^+$  with  $\text{K}^+$  and  $\text{Cl}^-$  with  $\text{SO}_4^{2-}$  and adjusted to pH 6.0. The resistivity of APW was  $(4.9 \pm 0.4) \times 10^3 \Omega \cdot \text{cm}$ .

### Measurement of Ionic Currents

Extracellular currents were measured with a two-dimensional vibrating probe system (Applicable Electronics, Forestdale, MA, USA). The preparation

and the calibration of the probe were as described previously.<sup>13</sup> Experiments were performed inside a grounded Faraday cage. The petri dish containing the seedling was placed on the stage of an inverted microscope (CKP, Olympus, Tokyo, Japan) that was used to view the electrode's position and its plane during current calibration. A stereo microscope (SZ-III zoom stereo microscope, Olympus, Tokyo, Japan) with a larger field of view was used to observe the root from above. This microscope was used to determine the spatial dimensions of the root, as well as to position the electrode during measurements. The two-dimensional vibrator assembly was mounted on a mechanical 3D micromanipulator (MM-333, Narishige, Tokyo, Japan) where the position of the electrode was adjusted manually.

The APW solution around the root was grounded by two platinum/platinum-blackened electrodes a few centimeters away from the seedling. During the measurements, the vibrating electrode was positioned about  $150 \mu\text{m}$  from the root surface for about 30 seconds. It was vibrated with the amplitude of  $25 \mu\text{m}$  in each direction, *i.e.*, along the X and Y axes, with a frequency of a few hundred Hz. Between each measurement, the electrode was moved to a position more than  $2000 \mu\text{m}$  away from the root for a reference, *i.e.*, zero current reading, for another 30 seconds. For each position, there were two voltage gradient signals, one for the X and the other for the Y direction. The net current vector for the position was then calculated from the current of X and Y directions obtained from the voltage gradients.

The ionic currents were measured on both sides along the length of the root starting from the root tip up to the root hair zone 10-11 mm away. Each position was  $500 \mu\text{m}$  from the previous measurement. The temperature during the experiments was  $26 \pm 2 \text{ }^\circ\text{C}$ .

### Ion Composition of Currents

To find the ions that were involved in the natural currents two approaches were used. First, to investigate the involvement of  $\text{H}^+$ , the magnitude and patterns of the currents were mapped in media of different pH values (4.0, 6.0 and 8.0). For each root the magnitudes and directions of currents at two pH values were compared. The measurements started with the root bathed in normal APW pH 6.0 medium. After removing this medium the petri dish was rinsed twice with distilled water and rinsed two more times with the new medium, either pH 4.0 or 8.0. Care was taken not to touch or change the position of the root. The seedling was then covered with 15 ml of the new medium and left to equilibrate for 45 min before measurement was resumed. During this time the probe was recalibrated. After the measurement the procedures were repeated by changing the media back

to pH 6.0 where the final set of measurements was carried out. This was to compare them to the measurements made at the beginning to see whether the change in the ionic environment would have a longer lasting effect on the currents.

The second approach, the ion-substituting experiments, was to measure the currents when the components of the ions of APW (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Cl<sup>-</sup>) were selectively removed from the bathing medium. The changing of the bathing media and the time-scale of these experiments were as in the pH experiments. In both the pH and ion-substituting experiments the currents were measured on one side of the root only to reduce the time needed. This was to avoid long term effects of the change in ionic environment.

**Root Elongation Rate Measurements**

Before each vibrating probe measurement the position of some natural mark near the root tip was noted on the stereo microscope eyepiece micrometer scale. This was compared to the position after the measurement. To calculate the root elongation rate the distance between the pre- and post-measurement positions was divided by the time span. To correlate the effect of pH on root growth, relative elongation rate at a different pH with respect to the elongation rate at pH 6.0 of the same root was determined.

**RESULTS**

**Current Pattern around Growing Roots**

Two sets of experiments were carried out. In the first set, the roots (15-20 mm long) were decapped before the currents were measured. This was to

prevent the horizontally growing root from bending due to gravitropic response. However, it was observed later that shorter roots have a lesser response to gravistimulation. So, a second set of experiments was done with shorter capped roots (10-15 mm long).

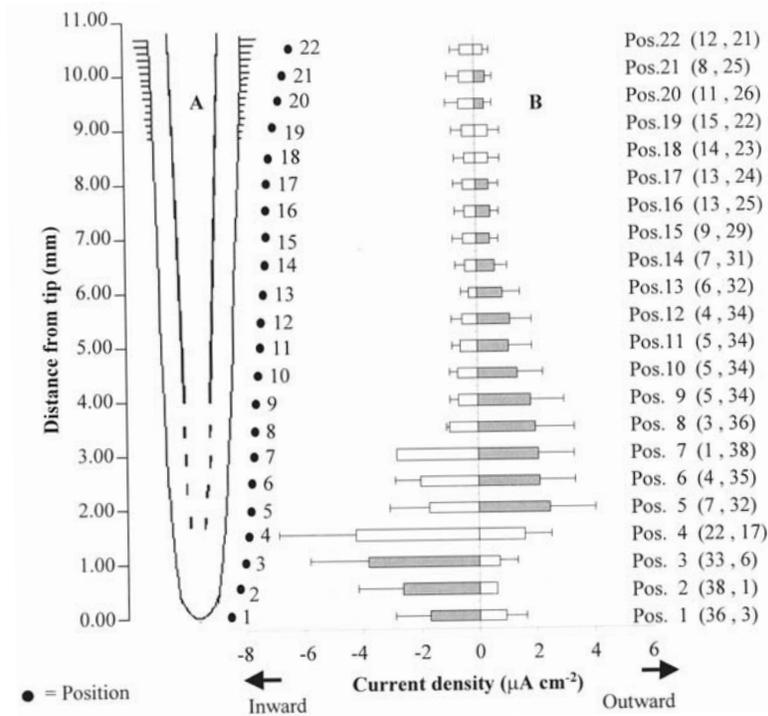
Endogenous ionic currents were measured along the horizontally growing primary roots of 39 decapped and 30 capped roots. The results of the magnitude and the outward or inward direction of these currents for the decapped and capped roots are summarized in Figs 1 and 2, respectively. The currents at the tip were significantly inward. In this region there were three positions of inward current for the decapped and four for the capped roots. The one extra position of inward currents in the capped root was the root cap. In the elongation and root hair zones the currents were significantly outward. Three current patterns emerged:

**Pattern I:** Currents entered the tip of the roots, *i.e.*, the meristematic tissue of the decapped root (Fig 3), and both the root cap and the meristem of the capped roots. These currents left the root in the elongation and root hair region. The average current densities are shown in Table 1. This was the main pattern observed in the capped roots (18 out of 30 roots) while only 5 out of 39 decapped, longer roots, had this pattern.

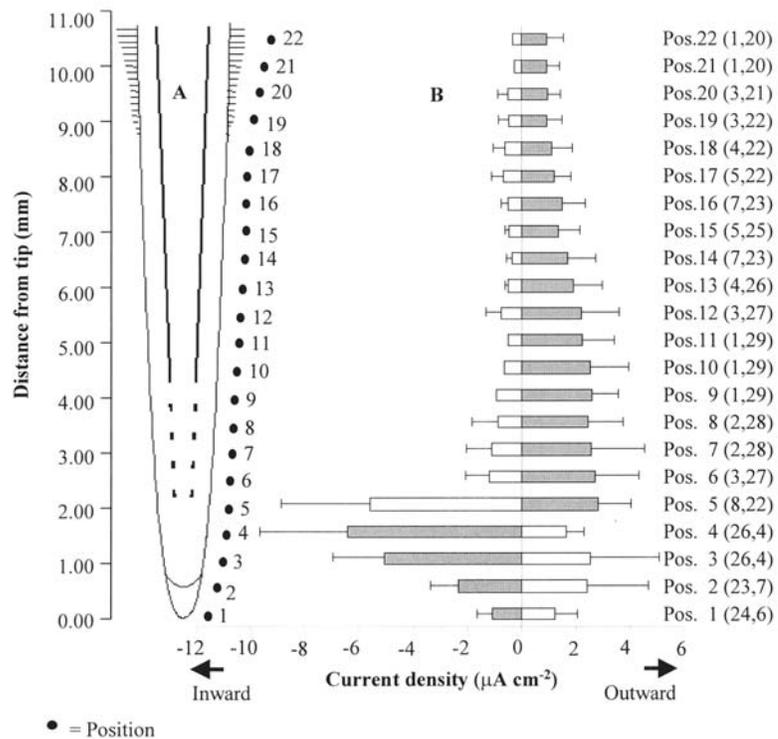
**Pattern II:** Currents entered the tip of the roots (Fig 4), left the root in the main elongation zone, and entered the root again in the post elongation and the root hair region. Table 1 shows the average of the current magnitudes of this pattern. This pattern occurred most frequently in the decapped roots, *i.e.*, 17 roots, and only 3 of the capped roots. Though this was the pattern found in nearly half of the decapped

**Table 1** Range of average current densities along the roots of current patterns I, II and III.

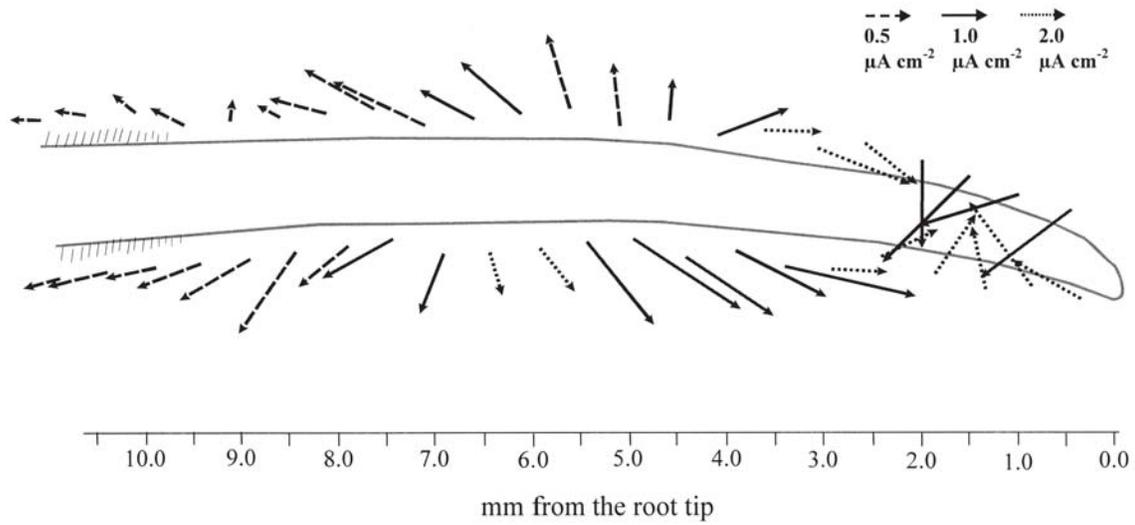
Root zone	Average current density ( $\mu\text{A cm}^{-2}$ )							
	Pattern I		Pattern II		Pattern III			
	Both sides		Both sides		On one side		On the other side	
	Decapped	Capped	Decapped	Capped	Decapped	Capped	Decapped	Capped
Root cap	-	inward	-	inward	-	inward	-	inward
Meristematic	inward	1.2-6.2	inward	1.4-8.8	inward	1.0-8.8	inward	1.0-8.8
	1.7-3.0		1.3-5.1		1.0-4.1		1.0-4.1	
Main elongation	outward	outward	outward	outward	outward	outward	outward	outward
			1.7-2.2	2.5-4.7			0.3-2.4	1.5-3.6
Post elongation			0.4-1.6	1.1-3.8			inward	inward
Root hair			0.7-2.0	0.3-1.8			0.1-0.8	0.1-0.5



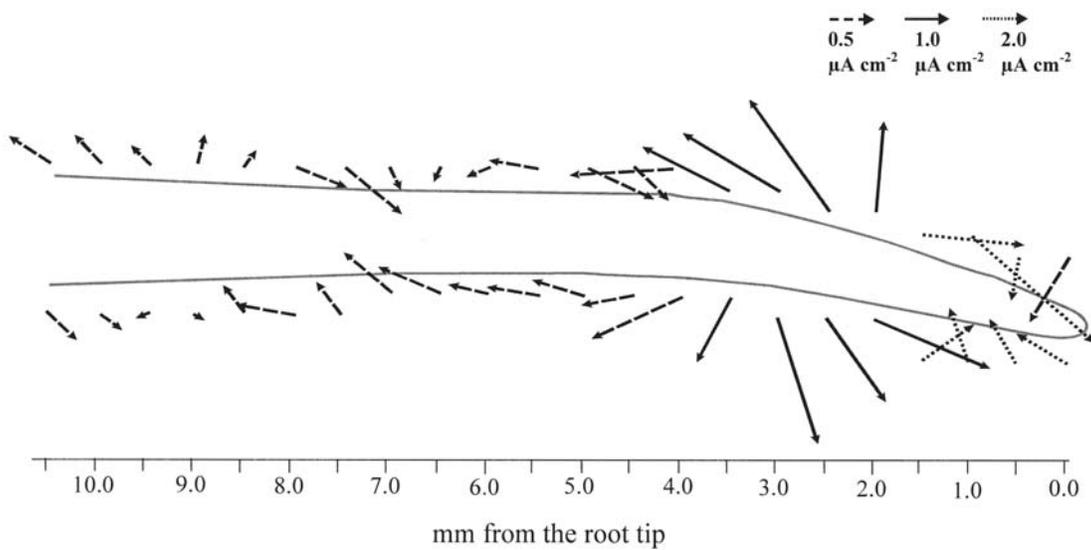
**Fig 1.** Direction and magnitude of endogenous current measured along 39 decapped intact primary roots of sunflower seedlings growing horizontally in APW. A) Schematic drawing of the root and the measurement positions approximately 150  $\mu\text{m}$  from the root surface. B) Directions of current flow, inward or outward, and current densities ( $\pm\text{SD}$ ). Shaded bars indicated statistically dominant directions of current flow ( $P < 0.05$ ). The first number in the parentheses of each position is the number of roots having inward current and the second the number with outward current.



**Fig 2.** Direction and magnitude of endogenous current measured along 30 capped intact primary roots of sunflower seedlings growing horizontally in APW. A) and B) are as in Fig 1.



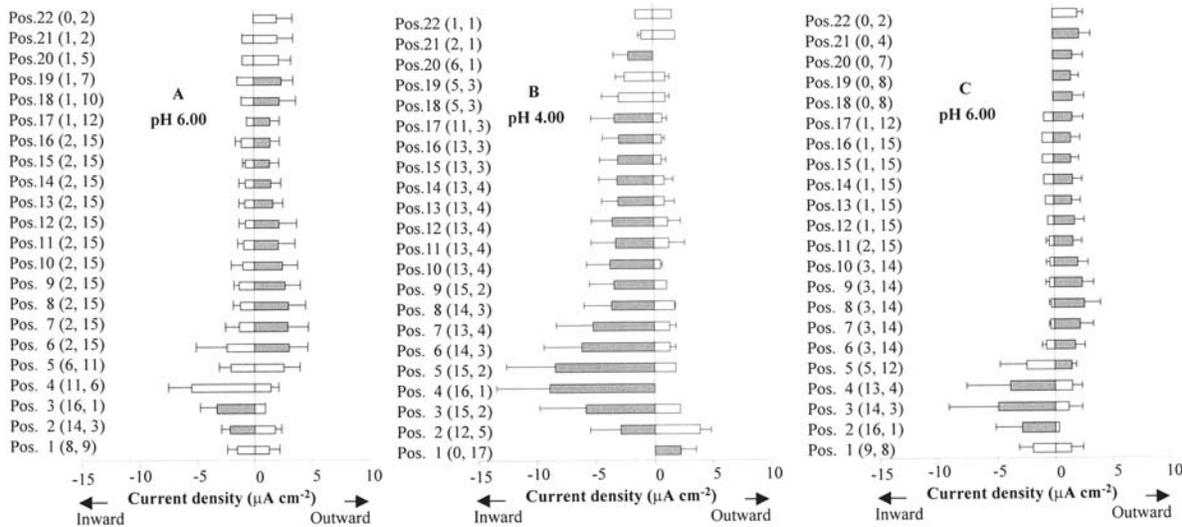
**Fig 3.** An example of pattern I current densities. Currents entered the tip of the roots and left the root in the elongation and the root hair regions. The arrows showing the magnitude and direction of the currents.



**Fig 4.** An example of pattern II current densities. Currents entered the tip of the roots, left the root in the main elongation zone, and entered the root again in the post elongation and the root hair regions. The arrows showing the magnitude and direction of the currents

**Table 2.** Elongation rates of sunflower primary roots. The current density is the average peak inward currents at position 3 or 4.

Root type	Current pattern	Number of roots	Seedlings' age (h)	Initial length (mm)	Elongation rate ( $\mu\text{m min}^{-1}$ )	Current density ( $\mu\text{A cm}^{-2}$ )
Capped	I	11	$43 \pm 3$	$13 \pm 2$	$2.2 \pm 0.8$	$7.5 \pm 1.3$
Decapped	II	10	$47 \pm 3$	$17 \pm 2$	$11.0 \pm 3.5$	$3.5 \pm 1.4$
Decapped	III	12	$48 \pm 2$	$17 \pm 2$	$14.3 \pm 7.5$	$4.3 \pm 2.7$



**Fig 5.** Directions of current flow, inward or outward, and current densities ( $\pm$ SD) measured in APW at A) pH 6.00, changed to B) pH 4.00 and returned to C) pH 6.00. Shaded bars indicated statistically dominant directions of current flow ( $P < 0.05$ ). The first number in the parentheses of each position is the number of roots having inward current and the second the number with outward current

**Table 3.** Relative elongation rates of sunflower capped primary roots at different pH with respect to the elongation rates at pH 6.0 of the same root.

Set	Number of roots	pH	Relative Elongation Rate
1	10	6.0	$1.0 \pm 0.6$
		4.0	$2.9 \pm 1.2$
		6.0	$1.2 \pm 0.9$
2	7	6.0	$1.0 \pm 0.5$
		8.0	$0.5 \pm 0.4$
		6.0	$1.0 \pm 0.4$
3	3	4.0	$3.5 \pm 2.7$
		6.0	$1.0 \pm 0.6$
		8.0	$0.8 \pm 0.3$

roots, the second region of inward current in the root hair zone was not significantly shown in Fig 1. However, at positions 18 and 19 in the root hair region there were some ambiguities in the current direction. When only the results with pattern II were tested the significantly inward direction showed up in position 18, 8.5 mm from the root tip in the root hair zone ( $P < 0.05$ ).

**Pattern III:** This is a combination of the above two patterns occurring in the same root (Table 1). On one side, currents entered the tip of the root and left the remaining part of the root as in pattern I. Along the other side, currents entered the tip of the root, left the root in the elongation zone, and entered the root again

in the root hair region as in pattern II. The numbers of decapped and capped roots that showed this pattern were 16 and 7, respectively.

In addition, one of the decapped and two of the capped roots were found to have currents leaving the root tip and entering the elongation zone.

#### Relationship between Elongation Rate and Ionic Currents

Elongation rates of 11 capped and 22 decapped roots are shown in Table 2. Seedlings with decapped roots that were older and had longer roots grew their roots 5 to 7 times faster than the younger seedlings with shorter capped roots. To find out whether the elongation rate increased with the current density, elongation rate *versus* the peak inward current density at position 3 or 4 were plotted for each current pattern, *i.e.*, pattern I of the capped roots and patterns II and III of the decapped roots (graphs not shown). The results did not show any significant correlation between the magnitude of the peak inwards currents and the elongation rate.

#### Relationship between Currents, pH and Root Growth

To test the involvement of  $H^+$  in root currents three sets of experiments were carried out on capped roots. In the first set the currents were measured from the same root in APW of pH 6.0, 4.0 and 6.0, respectively. A similar procedure was used with pH 6.0 and 8.0. For the third set the current measurement started with pH 4.0, then went to 6.0 and finally to 8.0. Increasing the external pH reduced the current density as well as the elongation rate of the root (Table 3). Fig 5 shows the results obtained with pH 6.0 and 4.0. Decreasing the

external pH changed both the direction and magnitude of the current significantly. Initially, at pH 6, the currents entered the root at the tip and left along the elongation and root hair zone. The outward currents changed direction and became inward currents when the external medium was changed to pH 4. At this lower pH the mean peak inward current was  $8.9 \pm 4.5 \mu\text{A cm}^{-2}$ , having increased from  $5.5 \pm 2.0 \mu\text{A cm}^{-2}$  at pH 6. For each root the currents at each position were compared for the two pH values and the differences of all positions were tested using the paired t-test. Out of a total of 17 roots 13 showed a significant increase in the current magnitude ( $P < 0.10$ ). When the external pH was increased from pH 6 to pH 8 the direction of the currents remained approximately the same while the magnitude decreased slightly (graph not shown). The same results were obtained when the external pH was changed from 4 to 6 to 8, i.e., the magnitude of the currents decreased with increased pH while the directions of the currents of pH 4 and pH 6 were similar to those shown in Fig 5, and those of pH 8 were similar to pH 6. When the magnitude and direction of the currents measured at pH 6 before and after the pH changes (to either pH 4.0 or 8.0) were compared, the direction remained approximately the same while the magnitude decreased slightly, both after pH 4 and pH 8. It is possible that the change of pH in the environment may have some long term effects on the root. The possible effect of low pH was observed during the experiments, that is, surface of the roots exposed to pH 4.0 turned slightly brown after 30 minutes. Under normal set up the time the root exposed to pH 4.0 was about one hour before the solution was changed back to the normal pH 6.0 APW. However, after the change, the brown colour remained. Therefore, some cells might be slightly damaged and this would have some effect on the ionic currents. The damage must be slight since after returning to pH 6.0 the roots resumed their original growing rate (Table 3). The effect of the low pH was also observed in *Arabidopsis* root exposed to a low pH of 4.5 where damage occurred in the epidermis and in internal tissues.<sup>14</sup>

### Ion-substitution Experiments

To examine the correlation between the ions in the external medium and the currents and growth, ion-substitution experiments were carried out. Between 10 to 13 roots were tested for each ion. The overall pattern of endogenous currents did not change significantly for all ions. The magnitude of the currents at each position along the root were compared and tested for each root using paired-t test. Only the absence of  $\text{Cl}^-$  caused a significant ( $P < 0.025$ ) decrease of currents. When only the peak inward currents at the root tip was considered, similar results were

obtained, i.e., when there was no  $\text{Cl}^-$  the peak inward current decreased significantly, i.e., the mean value of the peak current reduced by about 48%.

## DISCUSSION

### Natural Ionic Currents

The ionic current patterns observed around primary roots of 2-day old sunflower seedlings clearly indicated that these currents entered the meristematic and the root hair zones. The magnitude of the currents differed from root to root and this is shown by large standard deviations in Fig 1 and Fig 2. On top of the different nature of each root, the differences may also be due to the temporal nature of these ionic currents where the magnitude changed with time.<sup>7,15</sup>

There were 3 current patterns in both the decapped and capped roots. However, the proportions of the roots belonging to each pattern were different. In the shorter (10-15 mm), capped roots, 60% of the investigated roots had pattern I currents. The currents entered the root cap and the meristem, and left other regions of the root. Most of the roots investigated so far had this pattern.<sup>3-5,9,10</sup> This may be because in these studies the currents were only measured for 3-5 mm from the root tip, in the meristematic and the elongation zones. On the other hand, in the longer (15-20 mm), decapped roots, current patterns II and III were observed in 85% of the roots where, at least on one side of the roots, the currents entered the meristematic and the root hair zones and left along the elongation zone. It is most likely that when the root grew longer the root hairs started to emerge, resulting in another region of inward currents. This is similar to what was found in *Avena*<sup>3</sup> where these currents were thought to be associated with the development of root hairs. The association between the length of the root and the current pattern was also observed in *Lepidium* where the current pattern in the same root changed from pattern I in a shorter root to pattern II when the root grew longer.<sup>7</sup>

From the total of 69 roots, with 39 being decapped and 30 being capped roots, there were 3 roots where the current pattern differed from the rest. The currents left the root tip and entered the elongation zone. One observation on these roots was that they were shorter than roots with other current patterns, i.e., about 11-12 mm in length compared to 13-15 mm. In other words, they grew slower and it is possible that slow growing roots may have a different current pattern. This has been found in the embryo of carrot, in which slow growing and fast growing lines had different current patterns.<sup>16</sup>

### Relationship between Elongation Rate and Ionic Currents

Roots with different length had different elongation rates. The decapped, older, longer roots had a 5-7 times higher elongation rate than the capped, younger, shorter roots. It has been reported that removal of maize root caps transiently stimulated root growth. Yet the growth rate of the decapped roots was only 1.6 times higher than that of the intact roots.<sup>17</sup> In another study, the removal of *Arabidopsis* cap cells altered only root curvature due to gravity without affecting growth rate.<sup>12</sup> Therefore, this 5-7 fold increase in the elongation rate observed in this study was not caused by removing the cap, but was probably due to the difference in age and original length of the root. Longer and older seedling roots grew faster because the size of the growth zone increased.<sup>18</sup> The interesting point to consider here is whether there is a correlation between the current pattern and/or magnitude and the root elongation rate.

In the faster growing decapped roots, the current patterns were mainly pattern II and III (33 out of 39 roots), where the currents entered the roots in two regions, namely at the meristem and the root hair zone. In most of the short capped roots (18 out of 30) that grew slowly, the currents only entered the meristem (pattern I). This was similar to what was found in *Lepidium* roots<sup>7</sup> where, within the same root, the growth rate was faster when the currents entered the meristem and the root hair zone than when the currents only entered the meristem. This finding further supports the correlation between current pattern and root growth.

The correlation between the magnitude of the current and the growth rate is another interesting feature to be considered. In intact roots of *Lepidium*<sup>7</sup> the currents had no correlation with the rate of elongation. Similarly, the embryos of carrot of two culture lines having different growth rates gave current density of the same order of magnitude, but the patterns of inward and outward currents were different.<sup>16</sup> On the other hand, within the same stage of embryos of oil palm,<sup>13</sup> current-density and growth were positively correlated, while the pattern of the currents remained the same. In the present work, the magnitude of the peak inward currents did not show a significant correlation with the rate of elongation. The ambiguity of this relationship might be because of the temporal feature of both the currents and growth rate. It has been found that in the same root, the magnitude and the pattern of the currents as well as the elongation rate changed with time.<sup>7</sup> Furthermore, within the same pattern the elongation rate also changed with time. Therefore it will be interesting to investigate the temporal variations of the elongation rate and the currents.

### Relationship between Currents, pH and Root Growth

The increase in currents entering the root tip with decreased pH suggested that H<sup>+</sup> ions were the main component of the currents in sunflower roots. This finding supports earlier reports in which protons have been found to be the main contributors to root currents.<sup>2-7</sup> The outward currents in the elongation zone under normal pH found in the present work also corresponded well with the efflux of H<sup>+</sup> ions observed in corn roots using H<sup>+</sup> ion specific microelectrodes.<sup>15,19</sup> According to the "acid growth hypothesis" the excretion of proton from the protoplast into the wall space causes wall pH to fall, which induces an increase in the extensibility of the cell wall, leading to an increase in growth rate.<sup>20</sup> Since the elongation zone is the site of rapid and extensive cell elongation, the efflux of H<sup>+</sup> ions in this region supported this hypothesis.

When the external pH of the root was changed from pH 6 to pH 8 the currents and the elongation rate decreased. On the other hand changing the external medium to pH 4 increased the currents markedly. These changes were the same as in maize roots.<sup>4</sup> At low pH, besides the change in current magnitude the direction of the currents in the elongation zone also changed, from leaving to entering the root. These inward currents were most likely due to H<sup>+</sup> ions. The apparent influx of H<sup>+</sup> ions at pH 4 was similar to the large H<sup>+</sup> influxes in the elongation region of corn roots incubated in pH 4 solution.<sup>15</sup> This is probably because in this low pH buffered solution H<sup>+</sup> ions would move from outside into the root where the apoplast pH is higher (5.1-5.6 in maize root<sup>21</sup>) forcing the pH inside the root, especially in the elongation zone, to be more acidic resulting in more acid growth. This was supported by the fact that the elongation rate in the low pH also increased with inward currents, which were similar to those observed in maize roots.<sup>4,22</sup> It has been found that the increases in growth rate due to acid growth occurred in cells of the accelerating region of the elongation zone, and these were not due to changes in turgor, but to increased cell wall loosening.<sup>22</sup> The mechanism in which protons cause cell wall loosening is poorly understood. It has been suggested, using the "weak acid Donnan Manning" model, that H<sup>+</sup> displaces Ca<sup>2+</sup> in the cell wall during "acid growth".<sup>23</sup> These Ca<sup>2+</sup> ions may also interact with the enzymatic processes of wall loosening.<sup>23</sup> To date the most likely candidate involved with wall-loosening is an enzyme called expansin.<sup>24</sup> The mechanism of weakening is not yet known, but protons may play a role since expansins are more effective at acidic pH, which is consistent with their hypothesized role as catalysts of acid growth.<sup>24</sup>

### Ion-substitution Experiments

With the vibrating probe system, which is now called a vibrating voltage probe system, the information about the ions involved in the endogenous currents can be obtained by performing ion-substitution experiments or by addition of chemical inhibitors of channel and pump activity, which will enable the determination of the individual ion contributions to the net current detected. In sunflower roots, the removal of  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  from the external medium showed no effect on the pattern of the inward or outward currents. This was the same as what was found in roots of *Zea mays*.<sup>4</sup> When the magnitude of these currents was examined, the deletion of these ions did not give a consistent result. When the currents at all positions along the root were considered, the elimination of  $\text{Ca}^{2+}$ ,  $\text{Na}^+$  or  $\text{K}^+$  could either raise, reduce or produce no significant change in the current. When only the peak inward currents at the root tip were compared similar inconsistent changes were obtained. The absence of  $\text{Ca}^{2+}$ ,  $\text{Na}^+$  or  $\text{K}^+$  caused the peak current to either increase or decrease. These results were in contrast to the results obtained in corn roots where a net influx of  $\text{Ca}^{2+}$  and a net efflux of  $\text{K}^+$  were detected at the root tip together with  $\text{H}^+$  flux.<sup>19</sup> In this work only the depletion of  $\text{Cl}^-$  gave a significant decrease in the current magnitude. This is consistent with a  $n\text{H}^+/\text{Cl}^-$  symporter, where  $n$  is greater than 1.<sup>25</sup> That is, lowering the  $\text{Cl}^-$  concentration outside the root resulted in less influx of  $\text{H}^+$ , thus, less ionic currents.

From what has been described, protons seem to play a significant role in root currents. However, the ion-substitution technique could not clearly show the involvement of other ion components. Although the decrease or increase of current density in this type of experiment may indicate the involvement of the absent ion, it can also be the effect of the ion that is used in the substitution. The differences between the currents measured in normal conditions (APW pH 6.0) before and after the change in ionic environment also indicated that these approaches might have a lasting effect on the currents. Therefore, other alternative methods may be more appropriate. Systems using ion selective electrodes have been developed recently, such as the microelectrode ion flux measuring technique<sup>15</sup> or the vibrating ion-selective probe,<sup>26</sup> and these have proved to be a valuable addition in this type of investigation. Further research using ion selective electrodes would be a better alternative.

### ACKNOWLEDGEMENTS

This project was supported by the National Research Budget-Faculty of Science, Prince of Songkla University (PSU), Hat Yai, Thailand, Research Group

Development Fund-PSU, and by the Thesis Research Fund-Graduate School, PSU. We would like to thank Professor Paisan Laosuwan, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand for providing the sunflower seeds. We are grateful to Dr Ian A. Newman, Department of Physics, University of Tasmania, Australia, for the careful reading and for the discussion of this manuscript. We also thank Alan M. Shipley, Applicable Electronics, P.O. Box 589, Forestdale, USA, for his technical advice on the vibrating probe system.

### REFERENCES

1. Nuccitelli R (1990) Vibrating probe technique for studies of ion transport. In: *Noninvasive Techniques in Cell Biology* (Edited by J. Foskett and S. Grinstein), pp 273-310. Wiley-Liss, Inc., New York.
2. Weisenseel MH, Dorn A, and Jaffe LF (1979) Natural  $\text{H}^+$  currents traverse growing roots and root hairs of Barley (*Hordeum vulgare* L.). *Plant Physiol*, **64**, 512-518.
3. Miller AL and Gow NAR (1989) Correlation between profile of ion-current circulation and root development. *Physiol Plant*, **75**, 102-108.
4. Miller AL and Gow NAR (1989) Correlation between root-generated ionic currents, pH, fusicoccin, indoleacetic acid, and growth on the primary root of *Zea mays*. *Plant Physiol*, **89**, 1198-1206.
5. Miller AL, Smith GN, Raven JA, and Gow NAR (1991) Ion currents and the nitrogen status of roots of *Hordeum vulgare* and non-nodulated *Trifolium repens*. *Plant, Cell Environ*, **14**, 559-567.
6. Fromm J, Meyer AJ, and Weisenseel MH (1997) Growth, membrane potential and endogenous ion currents of willow (*Salix viminalis*) roots are all affected by abscisic acid and spermine. *Physiol Plant*, **99**, 529-537.
7. Iwabuchi A, Masafumi Y, and Shimizu H (1989) Development of extracellular electric pattern around *Lepidium* roots: its possible role in root growth and gravitropism. *Protoplasma*, **148**, 94-100.
8. Weisenseel MH, Becker HF, and Ehlgötz JG (1992) Growth, gravitropism, and endogenous ion currents of cress root (*Lepidium sativum* L.). *Plant Physiol*, **100**, 16-25.
9. Hush JM and Overall RL (1989) Steady ionic currents around pea (*Pisum sativum* L.) root tips: the effects of tissue wounding. *Biol Bull*, **176(S)**, 56-64.
10. Meyer AJ and Weisenseel MH (1997) Wound-induced changes of membrane voltage, endogenous currents, and ion fluxes in primary roots of maize. *Plant Physiol*, **114**, 989-998.
11. Ryan PR, Shaff JE, and Kochian LV (1992) Aluminium toxicity in roots. *Plant Physiol*, **99**, 1193-1200.
12. Blancaflor EB, Fasano JM, and Gilroy S (1998) Mapping the functional roles of cap cells in the response of arabidopsis primary root to gravity. *Plant Physiol*, **116**, 213-222.
13. Thavarungkul P (1997) Vibrating probe measurement of ionic currents around developing embryo of oil palm (*Elaeis guineensis* Jacq.). *J Exp Bot*, **48**, 1647-1653.
14. Koyama H, Toda T, and Hara T (2001) Brief exposure to low-pH stress causes irreversible damage to the growing root in *Arabidopsis thaliana*: pectin-Ca interaction may play an important role in proton rhizotoxicity. *J Exp Bot*, **52**, 361-368.
15. Shabala SN, Newman IA, and Morris J (1997) Oscillations

- in H<sup>+</sup> and Ca<sup>2+</sup> ion fluxes around the elongation region of corn roots and effects of external pH. *Plant Physiol*, **113**, 111-118.
16. Rathore KS, Hodges TK, and Robinson KR (1988) Ionic basis of currents in somatic embryos of *Daucus carota*. *Planta*, **175**, 280-289.
  17. Wilkins H and Wain RL (1974) The root cap and control of root elongation in *Zea mays* L. seedlings exposed to white light. *Planta*, **121**, 1-8.
  18. Beemster GTS and Baskin TI (1998) Analysis of cell division and elongation underlying the developmental acceleration of root growth in *Arabidopsis thaliana*. *Plant Physiol*, **116**, 1515-1526.
  19. Ryan PR, Newman IA, and Shields B (1990) Ion fluxes in corn roots measured by microelectrodes with ion-specific liquid membranes. *J Memb Sci*, **53**, 59-69.
  20. McQueen-Mason S (1997) Plant cell walls and the control of growth. *Biochem Soc Trans*, **25**, 204-214.
  21. Felle HH (1998) The apoplastic pH of *Zea mays* root cortex as measured with pH-sensitive microelectrodes: aspects of regulation. *J Exp Bot*, **49**, 987-995.
  22. Winch S and Pritchard J (1999) Acid-induced wall loosening is confined to the accelerating region of the root growing zone. *J Exp Bot*, **50**, 1481-7.
  23. Arif I and Newman IA (1993) Proton efflux from oat coleoptile cells and exchange with wall calcium after IAA or fusicoccin treatment. *Planta*, **189**, 377-383.
  24. Cosgrove DJ (1998) Cell wall loosening by expansins. *Plant Physiol*, **118**, 333-339.
  25. Felle HH (1994) The H<sup>+</sup>/Cl<sup>-</sup> symporter in root-hair cells of *Sinapis alba*. An electrophysiological study using ion-selective microelectrodes. *Plant Physiol*, **106**, 1131-6.
  26. Kühtreiber WM and Jaffe LF (1990) Detection of extracellular calcium gradients with a calcium-specific vibrating electrode. *J Cell Biol*, **110**, 1565-73.