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The Electromagnetic potential gradient ,Ca^{2+} and Starch-Statoliths on the Uneven Growth of Plants

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ABSTRACT. *Effect of electromagnetic potential gradients on the uneven growth of shoot apex of Phaseolus vulgaris was investigated. Phaseolus vulgaris plants were fixed to a 3 - D clinostat with microelectrodes which applied an electromagnetic potential gradient (E.M.P.G.). The effect of gravity on the plant fixed to the 3-D clinostat was eliminated by rotating the plant at 1-resolution per minute (rpm). The applied E.M.P.G. was noted to replace gravity. This denotes that the gravity signal felt by the plant may be transuded through E.M.P.G. From the experiments carried-out, Calcium ions (Ca^{2+}) and starch-statoliths were rejected as signal perceptors. Results proved that there was a trophic movement even when Ca^{2+} ions were blocked in travelling un-evenly by an inhibitor EDTA. At the same time, roots which did not have starch-statoliths showed response to gravity. These results indicate that the gravity signal could be replaced with an E.M.P.G. and at the same time it is neither Ca^{2+} ions nor starch –statoliths that perceive the gravity signal. Thus, it may be possible that the physical stimuli are transferred to a biological signal through an E.M.P.G.*

Abbreviations: 3-D – three dimensional, E.M.P.G.-Electromagnetic potential gradient.

INTRODUCTION

All life on earth has evolved under the influence of gravity (Arias 1999). For the last many years scientists tried to find out the mechanism of plant movements. However, literature do not give a definite answer tothis question. From the information gathered so far, it is evident that electromagnetic potential gradients (E.M.P.G.) are the cause of plant movements (Behrens, *et al.,*1982; Weisenseel *et al.,*1992). Experiments have been done only for phototropism and for gravitropism. No other plant movement mechanism has been studied in detail. From this research publication, the authors wish to draw the attention of the modern scientists to exploit the mechanism of plant movements.

Plants, in particular, have evolved in response to the earth's gravity. Earliest research on plant movements were carried out by Charles Darwin, which dates back to the early 19th centuary. Plant physiologists have been trying to solve this problem since then. The latest information reveals that auxin is transported from one side of the stimuli or away from the stimuli. Gravity will cause the auxins to concentrate on one side of the shoot. The mechanism which causes the auxins to travel is not developed yet. The gravity stimulus is somehow transduced or changed, into a physiological stimulus through an undetermined mechanism and chain of biological signals (Lucinda *et al*., 2000).

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In the early 1980's the prevailing theories explained tropic movements and afterwards a theory called Cholondy-Went theory was established (Evans and Ishikawa, 1997). According to this theory, shoot movement is controlled by auxins. In gravitropism, the gravity signal leading to accumulation of auxin on to the lower side of the shoot results in the plant apex bending away from the earth. Up to date no explanation has been given to determine the cause of this mechanism. Information gathered so far denotes that a difference in stimuli causes the uneven distribution. How it operates has not been established. Therefore, this research was carried out to establish the mechanism of gravitropism by exposing plants to electromagnetic potential gradients. It has been reported that Ca^{2+} ions and starch-statoliths play a major role in gravitropism. Thus, in the present the study, effect of Ca^{2+} ions and starch statoliths on un even growth was also investigated. When examined under the light microscope it was observed that sections of roots of *Phaseolus vulgaris* had starch-statoliths. To prove that roots without starch-statoliths also show gravitropism, the plant *Tinospora cordifolia* (Rasakida) was selected where roots grow faster and clearly show gravitropism.

MATERIALS AND METHODS

Experiment 1. Effect of eletromagnetic potential gradient and Ca^{2+} ions on uneven growth of shoot apex of *Phaseolus vulgaris*

Phaseolus vulgaris seeds, obtained from the Department of Agriculture were soaked overnight in distilled water (50 ml) in a container with a circumference of 25 cm and with a height of 20 cm. After overnight soaking, water was drained off. The seeds were kept between Whatman-1 filter papers. Seedlings with a height of 25 cm were obtained after 2 weeks. The seedlings were transferred to plastic containers (5 cm height) with garden soil. The isolated seedlings were allowed to grow 2-3 days in the container with garden soil. Once the seedlings matured, 10 ml of 50mM EDTA (Ca^{2+} inhibitor) solution was sprayed on one set of seedlings and left 72 hrs.

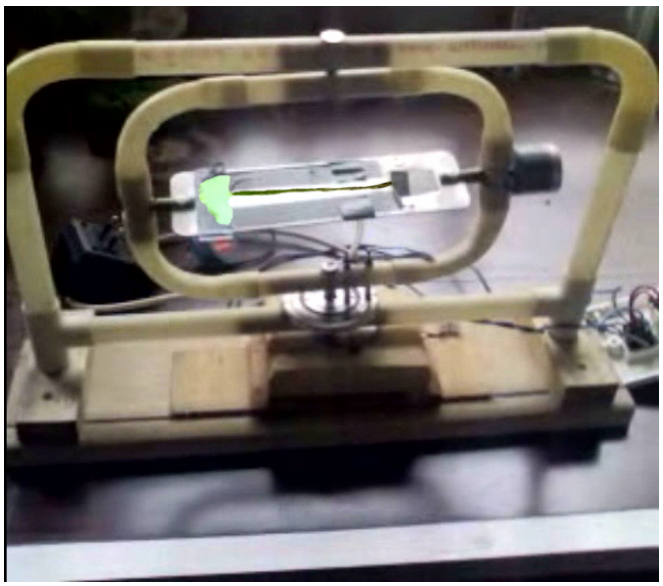

Plate1. 3-D Clinostat with *Phaseolus vulgaris* plant

The pot with the seedling was fixed to a 3-D clinostat (Plate1) which was manufactured in the Department of Physics, University of Peradeniya, Sri Lanka. The 3-D clinostat was designed and constructed as reported previously (Arias, 1999). The sample stage was 20 cm x4 cm. The main power of rotation was provided by two geared stepping motors. The frame was attached to a second frame which was further rotated perpendicular around a horizontal axis by the second motor. Then, the 3-D clinostat was rotated in two directions with the seedling, without E.M.P.G. without gravity (T1), with E.M.P.G. without gravity(T2), with gravity with E.M.P.G.(T3) and with gravity without E.M.P.G.(T4) for 72 hour. The rpm was 1 and the electric field applied was <8 Vcm^{-1}. Afterwards the curvature gradients of the seedlings were measured. The experiment was repeated 5 times.

Experiment2. Effect of starch-statoliths on un-even growth of *Tinospora cordifolia* (Rasakida) roots.

Roots of *Phaseolus vulgaris,* and *Tinospora cordifolia* were taken for the experiment. Cross sections were prepared and stained with iodine dissolved in potassium iodide and observed through a light microscope. In *Phaseolus vulgaris* roots, starch-stattoliths were clearly observed under light microscope while *Tinospora cordifolia* roots did not contain any starch-statoliths. Therefore, it was decided to observe *Tinospora cordifolia* root sections using a Scanning Electrone Microscope (SEM). To observe under SEM, roots of *Tinospor cordifolia* were taken. Cross sections were prepared and stained by using a solution of Iodine dissolved in potassium iodide to observe starch-statoliths. The sections were oven dried for 15 minutes at a temperature of 70 ^{0}C to evaporate the moisture. The prepared sections were coated with Gold and photographs were taken using a Scanning Eletron Microscope (SEM) as a permanent record.

RESULTS & DISCUSSION

The results of the present study indicated that plants grow horizontally when they were fixed to the 3-D clinostat and rotated (without gravity and E.M.P.G.) (Table.1). This may be due to the fact that the effect of gravity is eliminated when an E.M.P.G. of 8Vcm^{-1} is applied and rotated with rpm of 1 (T1). However, when the effect of gravity is absent, E.M.P.G. is felt by the plant and as a result the plant bends towards the (+)ve electrode or the anode at a degree of $30^0.978' \pm 1^0.706'$ (T2). When the plant is subjected to both gravity, without rotating at 1 rpm and E.M.P.G. of 8Vcm^{-1} (T3) the two factors act together on the plant causing less curvature compared to the Treatment 2 ($15^0.016' \pm 0.173'$). The total effect of gravity is felt when the plant is subjected only to gravity without an E.M.P.G. As a result, the plant showed the highest curvature of $90^0.017' \pm 0.168'$. Statistical analysis showed significant difference between treatments at 5% probability level (Table 1).

The above results indicate that the vector gravity is transduced through E.M.P.G. since it was possible to replace the force of gravity by E.M.P.G. Thus it may be possible that the external stimulus gravity has been transduced to the plant by E.M.P.G.

Table 1. Effect of electromagnetic potential gradient on growth of *Phaseolus vulgaris.* The degree of curvature is denoted at different treatments.

Replicate No	Absence of gravity & absence of E.M.P.G (T1)	Absence of gravity & presence of E.M.P.G (T2)	Presence of gravity & presence of E.M.P.G. (T3)	Absence of E.M.P.G & presence of gravity (T4)
1	$1^0.07^1 \pm 0.014^1$	$31^0.25^1 \pm 0.272^1$	$15^0.02^1 \pm 0.004^1$	$90^0.01^1 \pm 0.007^1$
2	$1^0.05^1 \pm 0.034^1$	$30^0.12^1 \pm 0.858^1$	$15^0.01^1 \pm 0.006^1$	$90^0.02^1 \pm 0.003^1$
3	$1^0.15^1 \pm 0.034^1$	$30^0.13^1 \pm 0.848^1$	$15^0.00^1 \pm 0.0016^1$	$90^0.01^1 \pm 0.007^1$
4	$1^0.15^1 \pm 0.066^1$	$32^0.24^1 \pm 1.262^1$	$15^0.03^1 \pm 0.014^1$	$90^0.02^1 \pm 0.003^1$
5	$1^0.10^1 \pm 0.016^1$	$31^0.15^1 \pm 0.172^1$	$15^0.02^1 \pm 0.004^1$	$90^0.025^1 \pm 0.008^1$
Mean Value (Xi)	$1^0.104^1 \pm 0.0328^1$	$30^0.978^1 \pm 1^0.706^1$	$15^0.016^1 \pm 0.173^1$	$90^0.017^1 \pm 0.168^1$

It has been reported that the transduction of the gravity signal could be converted to a biological signal through either Ca^{2+} ions (Ishikawa and Evans, 1992), starch-statoliths (Sack, 1991), pH gradient (Evans e*t al.,* 1980), auxins (Evans, 1991; Konings;1995), and electrical properties (Weisenseel *et al*., 1997). Therefore, in the present study effect of Ca^{2+} ions on transduction of gravity signal was investigated. However, results showed no significant difference between the control and plants treated with EDTA (Table 2), showing that Ca^{2+} ions have no effect on curvature. In contrast to these results, for more than a decade it has been assumed that there is strong relationship between Ca^{2+} and gravitropism. It is also uncertain that the observed redistributions of wall Ca $^{2+}$is sufficient to induce bending and there is at present no direct evidence from measurement or imaging that changes in Ca^{2+}, initiate or transduce gravitrophic signals (Sinclair and Trewavas; 1997).

Table 2. Effect of Ca^{2+} ions on the un-even growth of seedlings of *Phaseolus vulgaris*

Curvature(Treated) 50mM EDTA sprayed and left for 72hrs	**Control**
$45^0.2^1 \pm 0.010^1$	$45^0.5^1 \pm 0.020^{1\backslash}$
$44^0.3^1 \pm 0.0015^1$	$44^0.4^1 \pm 0.010^1$
$46^0.2^1 \pm 0.020^1$	$46^0.2^1 \pm 0.015^1$
$44^0.3^1 \pm 0.025^1$	$43^0.4^1 \pm 0.012^1$

Effect of Starch–Statoliths on gravitropism.

The starch statolith model states that intracellular sedimentation of starch particles act as gravity sensors. In agreement with this reduced gravitrophic sensitivity has been reported in mutants without starch (Sack, 1996).

Therefore in the present study, the effect of starch statoliths on gravitropism was investigated using two plant species, *Phaseolus vulgaris* and *Tinospora cordifolia*. When observed under light microscope *Phaseolus vulgaris* roots had starch-statoliths while no starch-statoliths were present in *Tinospora cordifolia* (Rasakida) even though they showed very high

gravitropism. This result implies that starch-statoliths may not play a major role in gravitropism in *Tinospora cordifolia* plants.

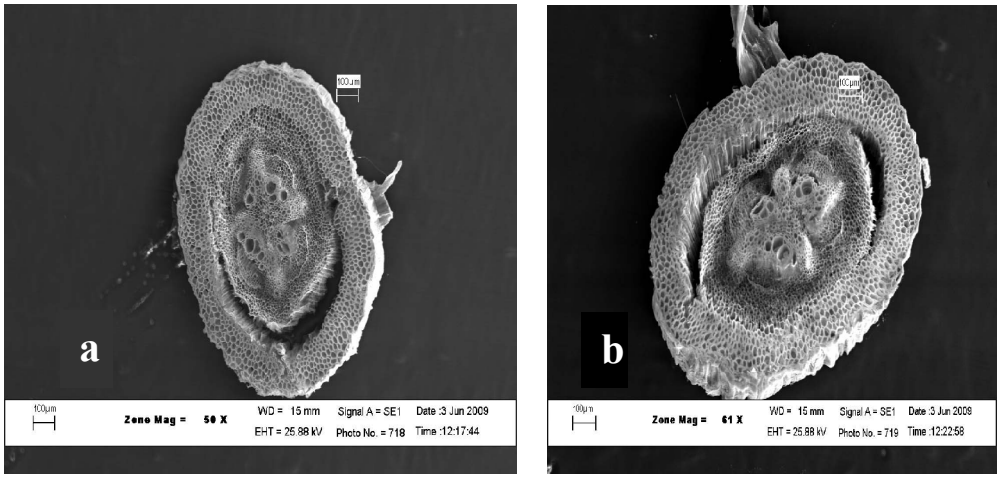

Plate 2. Scaned Electron Microscopic (SEM) images of roots of *Tinospora cordifolia* (Rasakida)

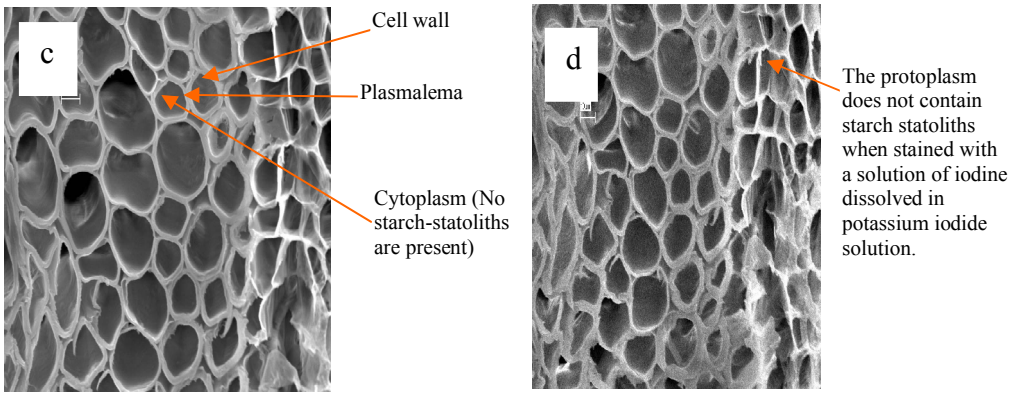

Plate3. Sections of *Tinospora cordifolia* (Rasakida) SEM structure. Detailed drawing showing no starch -statoliths

CONCLUSIONS

By using a rate of 1 rpm the effect of the gravitational field could be eliminated. The results denotes that the plant bends towards the anode, with an application of an E.M.P.G. of <8 Vcm^{-1} indicating that the effect of gravity could be replaced by an electromagnetic field. Thus it may be possible that the gravity stimulus is been felt by the plant after been converted to an E.M.P.G. At the same time, the observations denotes that Ca^{2+} ions and starch statoliths do not mediate as transduction in perceiving the gravity signal. However further experiments are needed to find out whether effect of pH gradient and auxins have any role to play in the un-even growth of plants.

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