Electrical signal from root to shoot in *Sorghum bicolor*: induction of leaf opening and evidence for fast extracellular propagation

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Abstract

We have observed earlier that primary leaf opening in *Sorghum* is a light-dependent process. We now show that giving a short photo-exposure to the roots alone also induced leaf opening over a similar time scale. However, any injury to the primary root inhibited the leaf formation. To check the propagation rate and response in this plant, the excitable properties and capability of conduction of electrical stimulus were investigated by extracellular recordings. *Sorghum* seedlings (5–7 days) were examined using non-damaging electrical stimuli. We demonstrate that seedlings when stimulated in one organ, the root region, produced a characteristic response, which could be recorded further up from the stimulating region in another organ, the shoot tissue. The minimum period of stimulation was 150 μs and threshold stimulus intensity was 100 μA. The general characteristic electrophysiological properties of the seedlings and the extracellular propagation of electrical signal suggest that *S. bicolor* exhibit typical excitable properties comparable to neural tissues. Moreover, electrical stimulus given to the root medium could overcome the requirement of photo-exposure to induce primary leaf formation in etiolated seedlings. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Stimulus; Threshold; Strength–duration relation; Rheobase; Chronaxie

1. Introduction

The development and adaptation of plants depends on communication between the environment and the cell, between cells in a tissue and between organs. In the last few years there has been increasing evidence for a novel signaling mechanism, involving root–shoot communication, for efficient regulation of plant growth and development. There is evidence that information from roots, regarding the soil water content, regulates stomatal behaviour, leaf growth, rate of respiration and photosynthesis [1,2]. The root–shoot interactions may also play an important role in other aspects of plant development. Tripathy and Brown [3] showed that light, when perceived by roots, inhibited chlorophyll accumulation in leaves of wheat seedlings.

Although so far hormones, cytokinins and abscisic acid (ABA) were considered as the likely candidates for the message flowing from the roots to the leaves, evidence also supports the involvement of electrical signals. Stimulation of *Salix viminalis* roots by providing nutrients, hormones or pH-changes caused changes in action potentials in the leaves [4]. These were followed by changes in the rate of photosynthesis and respiration. Similarly, it was shown that sudden osmotic stress applied to the roots of sunflower generated an electrical signal that resulted in a decrease in the stomatal conductance.

The existence of an electrical or fast regulatory mechanism has not been well established in higher plant systems [5]. The earlier electrophysiological studies in plants were limited to single cells of...
characean algae [6–9] and leaf movements of *Mimosa pudica* [10,11], *Aldro* 6 *anda* 6 *esculosa* [12] and *Dionaea muscipula* [13]. Lately there is some evidence for electrical activity in plants as a mechanism for signal propagation and for regulation of various physiological and biochemical responses [14,15]. Mechanically stimulated depolarizing transients have been shown to be involved in regulating the elongation growth of the stem in *Luffa cylindrica* [16], affecting phloem transport in *Mimosa pudica* [17], initiating protein synthesis in tomato [18] and increasing the rate of respiration of ovary during pollination in *Hibiscus rosasinensis* [19]. The characteristics of action potentials have been studied in shoots of *Lupinus angustifolius* [20,21], *Helianthus annuus* [22] and *Salix viminalis* [23].

In this study we provide evidence for root–shoot interactions during primary leaf formation (emergence and expansion) in *Sorghum bicolor*. We identified the involvement of a fast (possibly electrical) signal as the basis of rapid inter-organ communication. In view of this an attempt was made to investigate whether young *Sorghum* seedlings exhibit excitable properties and could conduct electrical impulses from the root to the shoot.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of *Sorghum bicolor* var. PC-6 were obtained from the Indian Agricultural Research Institute, Regional Center, Karnal. All experiments were carried out at 26 ± 1°C in total darkness or continuous light as per requirement. The treatment and growth conditions are same as described earlier [24]. Seedlings (5–7 day old) grown in distilled water alone were used for these experimental studies [24].

2.2. Treatment

To study the effect of a short light-exposure on primary leaf formation, 5 day old etiolated seedlings of uniform height and morphology were used. Any exception to this is mentioned specifically in the text. As required, either the root or shoot region of the etiolated seedlings was irradiated once with light, for 5 min, followed by continuous darkness. To study the effect of light exposure on roots, the shoot portion was carefully covered by two layers of foil (under safe light) before irradiating the roots. Similarly the roots were covered before irradiating the entire shoot portion of etiolated seedlings.

The red light (λmax 650 nm, 500 μW cm⁻²) was obtained by filtering the light from four 100 W tungsten lamps through CBS filters (Carolina Biological Supply Co., USA) and white light (2600 lux) through white fluorescent tubes. The characteristics of light sources were according to Sharma and Sopory [25].

For electrical recordings, individual seedlings were stimulated with a low intensity electrical pulse (described below). For following the effect of electrical stimulus on primary leaf formation, stimulus of low intensity (0.5–1mA, 5–10 s) were provided simultaneously to a group of 20–25 seedlings, by placing the electrodes in the root medium, to ensure a uniformity in treatment.

2.3. Measurement of response

The changes in the morphology of shoot tips were monitored after every 24 h beginning from the day of treatment up to day 5 after the treatment [24]. In the case of seedlings growing in the absence of light, the shoot tips were observed very carefully under dim green safe light. The stage of leaf formation and number of seedlings showing the changes in each set were recorded. Besides this, percentage leaf formation was calculated as:

\[
\text{Percentage leaf formation} = \frac{\text{No. of seedlings showing formation of the first leaf}}{\text{Total no. of seedlings in the set}} \times 100.
\]

For each set a minimum number of 20 seedlings were kept. Each experiment was done in triplicate and repeated at least three times and the average values were used for analysis. The readings were taken at intervals of 24 h. In the case of etiolated seedlings, precautions were taken to perform the experiments in dim light, as the response was found to be extremely sensitive to light.
2.4. Extracellular recording of electrical impulse

A schematic diagram of the recording setup is shown in Fig. 2. A pair of stainless steel insulated (except at the tip), wires 1–1.5 mm apart served as stimulating electrodes. The lower end of seedling (2–3 cm from the tip of the root) was placed on the electrodes such that it touched the bare surface. The stimulating electrodes were connected to a Grass S44 stimulator through a Grass PSIU6 isolation unit. A stainless steel insulated (except a small portion at the tip) wire approximately 100 μm diameter was used as the recording electrode. It was made to touch the shoot region (3–4 cm from the seed) with the help of a micromanipulator. The signal was amplified (AM systems) and displayed on an oscilloscope (TDS 420, Tektronix, USA) and a hard copy of the record taken on a printer connected to the latter.

The repeatability of the signal was seen for a minimum of three times on at least three seedlings. The data presented in Fig. 3 is a representative recording of the signal.

3. Results and discussion

3.1. Root-shoot interaction in primary leaf formation

We have observed earlier that *Sorghum* leaf formation (emergence from coleoptile sheath and subsequent expansion) is a light-dependent process [24]. There is no leaf formation in seedlings in the absence of light even after 10–11 days of growth. However, a short but saturating pulse of light given to the shoot portion of 5 day old seedlings resulted in primary leaf formation. The leaf started emerging from the coleoptile sheath within 1 h of receiving light irradiation and in approximately 24 h a partially open primary leaf was obtained.

Interestingly, injury to the root inhibited light-induced leaf formation in etiolated seedlings (Table 1). A 5 min photo-exposure could induce primary leaf formation in 5 day old etiolated seedlings but could not induce primary leaf formation if the root tip of the seedlings was cut/removed (Fig. 1A–g). If such seedlings were placed under continuous light (for up to 48 h), the tissue became green, but the leaf remained enclosed within the coleoptile sheath (Fig. 1A–h). In control plants transferred from darkness to light, primary leaf was obtained within 6–10 h. However, primary leaf formation was delayed by more than 48 h in *Sorghum* seedlings grown and germinated in continuous light if root was cut (Fig. 1A–b) and even in this case a fully expanded leaf was not obtained. This suggests the involvement of root–shoot interactions in the formation of primary leaves.

Primary leaf formation could also be induced in etiolated seedlings when the roots were exposed to a 5 min pulse of white light or red light, and the kinetics of leaf formation followed a similar temporal relation, as observed on giving light irradiation to the shoot portion only (Fig. 1B). This indicated that the signal must be communicated rapidly to the shoot tips, suggesting the presence of a fast, possibly electrical, signaling pathway. Considering that the stimulus was given to the root tissue while the response was measured in the shoot region, the possibility exists of a pathway for rapid conduction of signals in the form of an electrical impulse as one of the viable mechanisms for communication between the two main organs of the seedlings. There is other evidence that indicates long-range communication between the root and shoot, facilitating regulation of normal growth and development. Earlier studies implicated phytohormones, cytokinins and ABA, as likely candidates for transmission of the signal [1]. However recent studies have demonstrated that in addition electrical transients may be involved [15]. The regulation would thus involve an interaction between the electrical and hormonal messages.

The photosignal has been shown to mediate changes in membrane potentials in maize [26] and oat [27] coleoptiles, expanding leaves of pea [28].

Table 1

<table>
<thead>
<tr>
<th>Light Treatments</th>
<th>Primary leaf formation (%)</th>
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<tr>
<td>Root (intact)</td>
<td>Root (cut)</td>
</tr>
<tr>
<td>Dark</td>
<td>0</td>
</tr>
<tr>
<td>White Light (continuous exposure)</td>
<td>100</td>
</tr>
<tr>
<td>White light (5 min exposure)</td>
<td>100</td>
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Injury to the root inhibited light-induced leaf formation in etiolated seedlings. The experiments were performed on 5 d seedlings and the percentage of leaf formation obtained after 48 h is shown.
in ion transport are thought to be part of a transduction chain that links a blue light receptor to inhibition of hypocotyl growth.

To look for the possibility of electrical conduction between the root and shoot, the excitable properties of 5–7 day old Sorghum seedlings were studied. One of the properties of excitable tissues is their characteristic response to a threshold stimulus and their ability to propagate an impulse if stimulated by a suprathreshold stimulus. This has been routinely studied in neural tissues. The electrophysiological property of an excitable tissue is that it follows the all-or-none law, has a threshold and the tissue has a characteristic strength–duration curve. Hence, we recorded surface potential (at the shoot), which is comparable to extracellular potential (Fig. 2), in S. bicolor.

3.2. Characteristics and modulation of the signal

Five day old seedlings, when stimulated electrically either in the root or epicotyl (shoot) region, produced a characteristic response, which could be recorded further up from the stimulating region in the shoot tissue. When the tissue was stimulated with an electrical square wave pulse of low intensity only a stimulus artifact appeared (Fig. 3A). The artifact indicates the time of stimulation. At a

and rosette leaf of Arabidopsis [29]. Light can cause rapid changes in the membrane potential of plant cells by altering the activities of ion pumps and channels in the plasma membrane. Some of the light induced changes in membrane potential of plant cells may transduce the photomorphogenetic signal e.g. blue light induces a large, transient membrane depolarisation in the hypocotyls of etiolated seedlings [30]. The underlying changes

![Fig. 1.](image1.jpg)  
**Fig. 1.** (A) Representative photograph to show the degree of light induced leaf formation obtained after cutting the root of light grown (a–d) and etiolated seedlings (e–h). The various panels represent (a) Light grown seedling with intact root; (b) Light grown seedling with cut root; (c) Light grown seedling transferred to darkness (for 24 h) after cutting the root; (d) Light grown seedling transferred to darkness (for 48 h) after cutting the root; (e) Etiolated seedling with intact root; (f) Etiolated seedling with cut root; (g) Etiolated seedling in which root was removed immediately after the 5 min red light exposure; (h) Etiolated seedling transferred to continuous light after cutting the root. (B) The time course of response obtained on providing a 5 min light exposure to shoot and root tissues of 5 d etiolated seedlings is compared. WL, white light; RL, red light.

![Fig. 2.](image2.jpg)  
**Fig. 2.** A schematic diagram of the experimental set up for stimulation and recording of response in Sorghum bicolor. The Stimulator (S44) is connected through the current isolation unit (PSIU6) to a pair of stimulating electrodes, which are in contact with the root surface. The signal was amplified and displayed on the oscilloscope (CRO-TDS420) and hard copy taken as print out.
higher intensity of stimulation the ‘response’ was recorded (Fig. 3B). The response was recorded at a latency of 400 µs and the rate of propagation of the signal was calculated to be $270 \pm 20$ m s$^{-1}$. It is difficult to comment from this study if the signal was an action potential or a hyperpolarising potential. As the signal was recorded after some delay it is unlikely to be an induced current such as that of the artifact. According to standard neurophysiological knowledge it may be an action potential, since it is likely to be propagatory. Since there is as yet no definite evidence that there are propagatory action potentials in plants, we have preferred to term it as a ‘response’.

3.3. Threshold

The response was not obtained until the stimulation strength was increased to 100 µA. Moreover the intensity of the response did not change significantly with a further increase in the stimulation strength. The response was slow and it did not faithfully follow a stimulation frequency of more than 1 Hz. Even during a 1 Hz stimulation the first response was greater than the subsequent ones. Stimulation at 0.5 Hz was best in producing uniform reproducible responses to every stimulus, as shown in the overlap response (Fig. 3C).

3.4. Modulation of the response

The response obtained on electrical stimulation could be modulated by factors affecting membrane properties, thereby indicating that it was a biological phenomenon. Cooling an area of the root between the stimulation and the recording sites, by placing ice cubes for 15–20 s, gradually reduced the intensity of the stimulation induced response...
reported. However, there may be explanations for observed faster conduction than has been earlier study response up to 10 m was recorded and we been reported in different plants [22,23]. In this organs of the plants. Faster conduction (2–8 cm tissue while the response was picked up in the seedlings. Since the stimulus was given to the root way for conduction of a fast signal in shoot region, it is likely that there exists a mechanism for communication between the two main tissues, hence the same was studied for S. bicolor. In order to plot a S–D curve, the duration of the stimulus was kept constant and the current strength was slowly increased till the response appeared i.e. the minimum current strength required to get the response was noted. The duration of the stimulus pulse was gradually increased in steps of 25 μs and at each position the minimum current required to produce the response was noted. S. bicolor followed a classical S–D curve as shown in Fig. 4.

The S–D curve was plotted for different plants of the same age and morphology, and in each case the plots were almost identical to each other. As shown in Fig. 4, the response was not observed
Fig. 4. Strength–duration curve of *S. bicolor* seedling. As seen from the plot, the response was not obtained unless a minimum strength of 100 µA and a minimum duration of 150 µs was delivered to the plant tissue. The plot shows mean (± SD) of recordings from five seedlings. R — Rheobase; C — Chronaxie.

unless a minimum duration of 150 µs was delivered to the plant tissue. The rheobase (threshold stimulus intensity) was 100 µA and chronaxie (minimum duration to get the response at an intensity twice that of the rheobase) was 370 µs.

Based on the observations in this work it can be concluded that *S. bicolor* tissue has excitable properties. The seedlings when stimulated electrically either in the root or epicotyl region produced a characteristic response, which could be recorded further up from the stimulating region. The response produced by the electric stimulus (pulse) had a threshold, followed the all-or-none law and exhibited a characteristic *S–D* relationship. Moreover, it could be modulated by properties of the biological conducting tissue.

3.6. Electrical stimulus can replace the requirement of light

We have earlier observed that exogenous addition of calcium and/or other ions could partially replace the requirement of light for primary leaf formation [24]. In this paper we show that in addition to chemically induced leaf formation, etiolated leaves could be obtained in the absence of light by stimulating the 5 day dark grown seedlings with an electric pulse of low intensity (Table 2). The electric pulse was provided to a group of 20–25 seedlings by placing the electrodes in the root medium, such that they did not come in direct contact with the seedlings. The data obtained indicated that leaf formation induced by electrical stimulation, showed a qualitative dependence on the current strength, as an optimum response was obtained on providing 5 mC current (0.5 mA for 10 s or 1.0 mA for 5 s). With a further increase in current there was a decline in the percentage of leaf formation obtained. It is possible that the electrical pulse directly or indirectly triggers a secondary pathway, involving a second messenger system, which in turn after a required period of 12–24 h, would lead to the desired response i.e. emergence of the leaf from coleoptile sheath and its expansion.

Since the experiments were conducted in a group of seedlings (not individual seedlings) and also in the dark, precise placement of the electrodes (stimulating electrode as well as recording electrode) was not possible. The electrical current was delivered to the medium where only part of the roots were exposed and the other parts of the seedlings were unexposed to the medium and, since the current had to pass from the medium to the root of the seedling, a much higher intensity of current (c.f. direct stimulation of individual seedling as in Fig. 3) was required to induce the response. Although this data suggests that the electrical current induced leaf opening was likely to have been mediated through the root, direct experimental evidence is needed.

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<tr>
<th>Intensity of electric pulse (mA)</th>
<th>Duration of electric pulse (s)</th>
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<tr>
<td></td>
<td>1 s</td>
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<tr>
<td>0.5</td>
<td>34.7±1.9</td>
</tr>
<tr>
<td>1.0</td>
<td>42.3±2.1</td>
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Table 2
Effect of an electric pulse on primary leaf formation. Etiolated leaves were obtained by stimulating the 5 d dark grown seedlings with an electric pulse. The percentage of leaf formation obtained after 48 h is shown.
4. Conclusion

The existence of an electrical signaling mechanism in higher plants has been reported by measuring action potentials in insectivorous plants [31] and during leaf movements in *Mimosa pudica* [10,11]. Experiments have been done on several higher plants using intra-cellular microelectrodes and surface-contact electrodes [32]. Frachisse et al. [33] have observed a correlation between electrical events and plant development by studying shoot-apex formation in *Bidens pilosus*. Wildon et al. [18] have shown that wounding induced the propagation of electrical transients in tomato seedlings. These have shown that wounding induced the propagation of electrical transients in tomato seedlings. These studies were performed on older plants (16–22 days), which exhibited high excitability levels (requiring a minimum stimulus of 2 V, 1 s). Plants of similar size, shape and age, grown under identical conditions, exhibited high variability in the degree of excitation.

In this study we observed that electrical stimulus can replace the requirement of light for primary leaf formation in *S. bicolor*. It was also seen that root–shoot interaction plays an important role during the early stages of growth and the establishment of seedlings. The rapid transmission of the message/signal seems to involve the generation and transduction of electrical impulses which, in turn, can manifest a response either directly or through a second messenger pathway. The young seedlings (5–7 day) were highly excitable and showed a consistency and repeatability in the response (Fig. 4), but with an increase in age the tissues became less excitable and lost the repeatability of response. The generation of a fast response due to an electric stimulus and the existence of a pathway for the conduction of this response, suggests that this signaling process could form the basis of rapid communication between different tissues and/or organs in *Sorghum*, in addition to the known chemical signaling mechanisms.

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References


