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Action potential – A possible signal in root to shoot communication caused by water deficit around roots of sunflower seedling

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Exposure of sunflower roots to polyethylene glycol (PEG-6000) induced osmotic stress caused a rapid depolarization of surface electrical potential at the shoot apex within 25 seconds. It was found that while the leaf water potential and the osmotic potential in control seedlings showed no variation during the entire period of experimentation, the leaf water potential in polyethylene glycol treated seedlings started decreasing after 2 min of treatment without any marked change in the osmotic potential. Similarly, the stomatal resistance in control seedlings remained constant during the course of experimentation but the polyethylene glycol treatment affected the stomatal resistance within 30 seconds and it continued to increase 3 min after the treatment. All these events occur in a time scale of seconds to minutes and in a sequence. It is concluded that the action potential acts as a signal in root-to-shoot communication under osmotic stress.

PLANTS regulate their metabolic processes and morphology when growing in drying soil by reducing the size of the plant, leaves, etc. However, with the exception of extreme drought, the annuals do complete their life cycle. In wheat cultivars and *Triticum* species grown across a line source irrigation system on a deep alluvium with a charged moisture profile at planting, very small differences in water potential (ranging -0.2 to -0.3 MPa) were observed between the maximum irrigated and non-irrigated plants, though differences in growth among them were enormous[1]. On the basis of various experiments, Gowing *et al.*[2], Passioura[3], and Passioura and Munns[4] suggested the occurrence of a root signal which regulates initiation of leaf growth, stomatal resistance, etc. despite no change in water potential. Based on several experiments, it was envisaged that the effects of soil drying on signalling between root and shoot must be primarily of a positive nature, i.e. an increase in the supply of some physiologically active substance[5]. Analysis of the composition of xylem sap from unwatered plants showed a decline in the concentrations of most components except the concentration of ABA, which increases substantially following soil drying[6]. However, the central role of ABA as a root sourced signal has been challenged[7], and an unknown substance was implicated in root-to-shoot communication under soil water deficit. We demonstrate here that in the sunflower seedling an action potential acts as a signal in root-to-shoot communication during PEG induced osmotic stress.

Sunflower (*Helianthus annus* L. cv. RHA 274) seedlings were grown in sand culture in a net house at the ambient sunlight, temperature and humidity for one

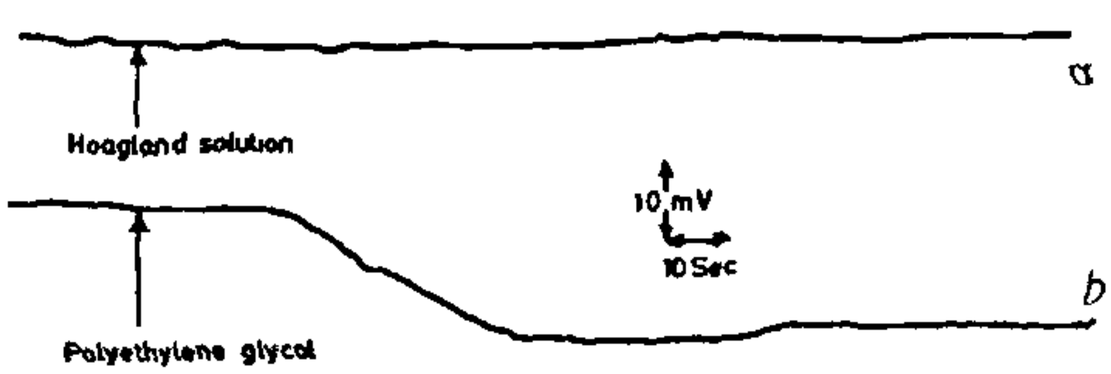


Figure 1. Effect of osmotic stress on surface electrical potential at the shoot apex of sunflower seedling Methods to create osmotic stress and to measure surface electrical potential have been described in the text *a* = pattern of electrical potential in control, *b* = pattern of electrical potential in response to osmotic stress

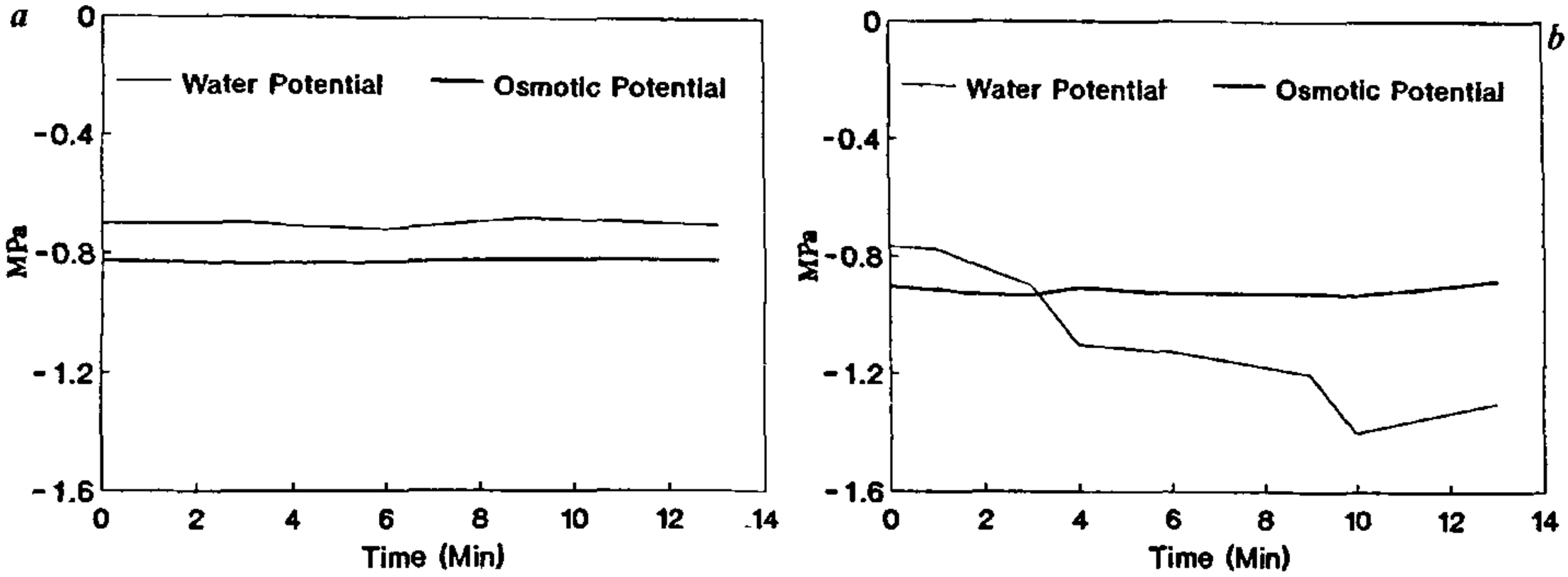


Figure 2. Water potential and osmotic potential in (*a*) control and (*b*) PEG (25% w/v in Hoagland solution) treated sunflower seedlings at different time intervals.

week. They were daily supplied with one tenth strength of Hoagland solution. Later the seedlings were transferred to plastic containers to grow them hydroponically in an aerated half strength Hoagland nutrient medium. Experiments were carried out on 25-day-old seedlings.

Surface electrical potential was measured following the method of Malone and Stankovic[8], with slight modifications as follows. A pelleted silver/silver chloride electrode was inserted in a plastic cone fitted with disposable polypropylene tips (250 mm^3 capacity) for Gilson type pipettor. The plastic cone and the tip was filled with cool (approximately 30°C), but still liquid, 0.5% agar containing 3M KCl. The reference electrode was connected at shoot apex surface and the measuring electrode was connected 25 mm below the reference electrode. A drop of 10 mM KCl was placed between the plant surface and the electrodes to have a proper contact. The output from the electrodes was recorded by a pen recorder connected to an electrometer amplifier having an input impedance of 10^{12} Ohms.

Seedlings were exposed to osmotic stress by pouring PEG-6000 (25% w/v in Hoagland solution) after siphoning out the Hoagland solution from the container using a plastic tube without disturbing the seedlings. In control Hoagland solution was used instead of PEG-6000. Electrical potential was continuously monitored throughout the experimental period.

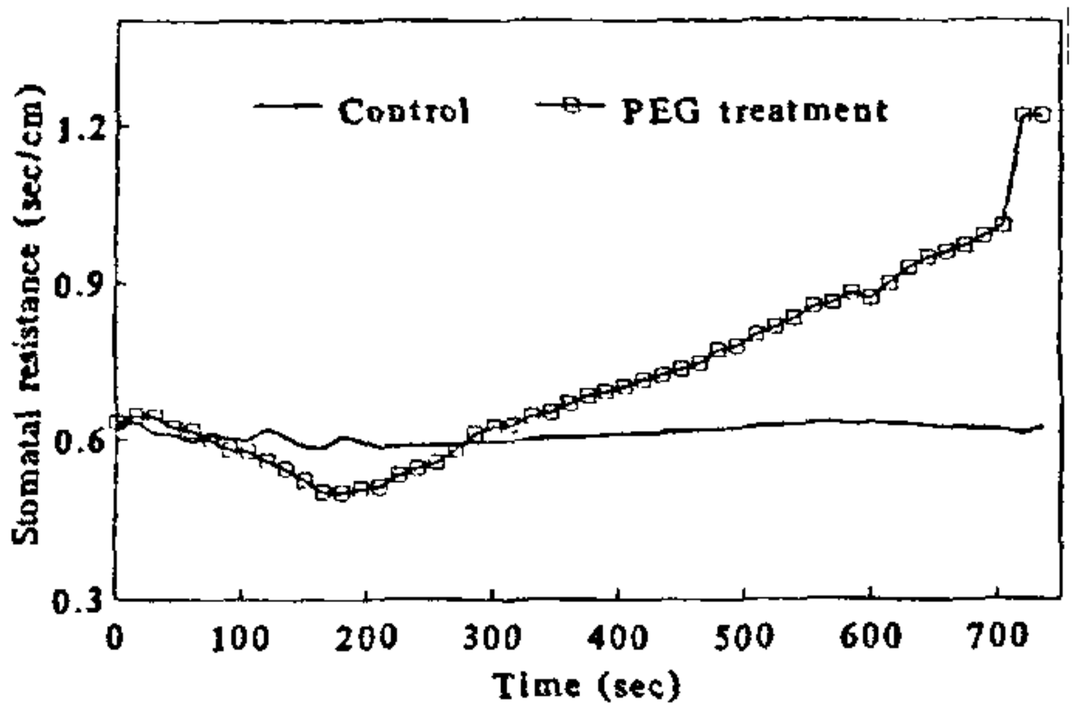
The fully opened topmost leaf was used to monitor stomatal resistance in control and osmotically stressed seedlings. Stomatal resistance was measured continuously throughout the experimental period using an infra-red gas analyser (Licor 6200).

The fully opened topmost leaf was used for water potential and osmotic potential measurement. Leaf water potential was measured using a pressure chamber (Soil Moisture Equipment, USA) at different time intervals in control and stressed seedlings. The same leaf was used to determine osmotic potential using vapour pressure osmometer (Wescor, model 5500).

The surface electrical potential at the shoot apex remained around 18 ± 4 mV in control seedlings. Addition of 25% PEG caused a rapid change in electrical potential (action potential) and depolarization was observed in 25 seconds. There was a rapid negative shift of approximately 14 mV. The electrical potential stabilized to 4 mV after 70 seconds of the polyethylene glycol treatment (Figure 1).

Water potential and osmotic potential remained constant throughout the experiment in control seedlings (Figure 2 *a*). Interestingly, water potential in stressed plants dropped down from −0.76 MPa to −0.84 MPa after 2 min and reached a value of −1.4 MPa in 10 min with no further change. Osmotic potential, however, showed no change (Figure 2 *b*). Stomatal resistance in control seedlings maintained a constant rate during the entire period of experimentation. When Hoagland solution was replaced with PEG solution, it decreased from 0.57 s cm^{-1} to 0.52 s cm^{-1} in 1 min and after 3 min it started increasing and doubled by 12 min (Figure 3).

Our experiments have shown that the sunflower seedlings do have electrical activity (resting potential) under normal conditions and that electrical activity propagates (action potential) from the root to shoot in response to osmotic stress, which we call the electrical signal. Electrical signals moved with a velocity of 7.2 mm/s (a change in the surface potential at the shoot apex, 18 cm away from the roots, was observed within 25 s) in response to water deficit around roots which



PEG= Polyethylene glycol

Figure 3. Changes in stomatal resistance in control and PEG (25% w/v in Hoagland solution) treated sunflower seedlings with time.

precedes changes in water potential or stomatal resistance. Such a value of the rate of action potential movement is quite encouraging since Bose[9] obtained these values falling in the range of 4 to 14 mm s^{-1} for *Mimosa* in response to mechanical stimuli. In spite of interesting results, Bose's work on electrophysiology did not receive due attention.

An early decrease and further increase in stomatal resistance could be due to a change in membrane permeability caused by electric potential[10]. We have also found that the stomatal resistance increases by flowing external electric field through the leaves (data not shown). The depolarization of the membrane causes stomatal closure[11].

These events, i.e. changes in electrical potential and stomatal resistance, occur sequentially and in a time scale of seconds to minutes. The transmission speed of electric potential is quite high compared to the transmission speed of a chemical in the phloem[12] or that of ABA, either in the phloem or xylem[13]. More importantly, the stomata closed immediately after the arrival of the action potential[13]. We, therefore, conclude that earliest signal arising from roots under osmotic stress is action potential and which in turn closes stomata. In fact, earlier reports have also shown that the action potential can propagate through plasmodesmata and is capable of triggering a number of physiological and biochemical responses[14, 15]. In field-grown plants of wheat with and without irrigation, significant differences in resting potential were recorded (data not shown) indicating the possibility of occurrence of this phenomenon under natural environment.

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Methane budget from paddy fields in India

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