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Short Communication

Application of Leaf Extract Causes Repetitive Action Potentials in *Biophytum sensitivum*

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A wound stimulus evoked a number of repetitive action potentials in the leaf of *Biophytum sensitivum*. When the cut end of a leaf was immersed in a leaf extract, the resulting repetitive action potentials continue for a long time. These repetitive action potentials disappeared immediately when the leaf extract, which contains a proposed stimulant, was removed and the cut end was washed with water.

Key words: *Biophytum sensitivum* — Leaf extract — Repetitive action potentials — Stimulant — Wound stimulus

Nearly 100 years ago, Haberlandt (1898) recognized that rapid downward closures of leaflets were repeated in response to a wound stimulus in the leaf of *Biophytum sensitivum* (Oxalidaceae). Bose (1907) found repetition of a number of deflections of a galvanometer connected to a leaf when the plant was stimulated by burning. Using this plant a series of action potentials in response to a wound stimulus in the leaf were recorded with a Lippmann's electrometer (Umrath 1928, 1929) and a pen recording oscillograph (Guhathakurta and Dutt 1963). In *B. dendroides*, repetitive action potentials were also observed (Sibaoka 1973), although the number of repetitions was less (3 to 5) than with *B. sensitivum*. However, stimulation with an electric pulse or with a drop of ice water only causes a single action potential in leaves of *B. dendroides* (Sibaoka 1973) and *B. sensitivum*. This study is aimed at finding the cause of the two different types of responses, i.e. elicitation of multiple and single action potentials.

Several young *Biophytum sensitivum* plants were transferred from the garden of Mr. K. Suzuki in Yokohama, to a greenhouse in our institute. A number of self-sown seedlings were used for our investigations. The experiments were performed in the laboratory with the intact potted plant or the detached leaf placed in an earthed wire-gauze cage and illuminated with a 100-watt incandescent lamp at a temperature of about 30 C.

A differential high input impedance ($>10^{10}\ \Omega$) electrometer (assembled with three operational amplifiers) and a chart recorder (Yokogawa 3056) were used for recording action potentials. A tapered polyethylene capillary (tip inside diameter, about 0.3 mm) was used as the recording electrode. The capillary was filled with a dilute saline solution so that a liquid connection was made through the tip opening between the plant surface and the Ag-AgCl wire inserted into the capillary. Another Ag-AgCl wire was positioned in a small glass vessel, in which the cut end of the detached leaf was positioned to serve as an electrode.

A typical pattern of repetitive action potentials evoked by a wound stimulus is shown in Fig. 1. Since a potted plant was used, one of the polyethylene capillary electodes was placed touching the stem surface to serve as the indifferent electrode. To simultaneously record the potential changes on two points of a pinna-rachis, two recording electrodes separated from each other by 19 mm

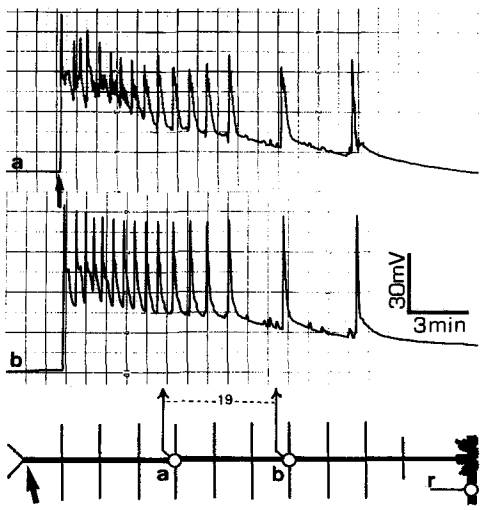

Fig. 1. Simultaneous records of repetitive action potentials of two different points in a pinna-rachis of *Biophytum sensitivum*. The tip of the rachis was stimulated by a slight touch with a burning joss stick. Two arrows indicate stimulated time (upper) and position (lower), respectively. The distance between electrode *a* and *b* was 19 mm. The time lag between the rising times of *a* and *b* are difficult to measure accurately, because of its small size on the figure. The indifferent electrode (r) is positioned in the stem. Sixteen action potentials are seen in each trace.

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were arranged on the rachis. Heat stimulation (slight touch with a burning joss stick) was applied on the most apical point of the leaf. The signal was transmitted basipetally. A series of 16 action potentials of the same shape and having a very short lag time were elicited at each measuring point over a period of 15 min. Intervals of the action potentials were about 0.5 min at the beginning, but were later gradually increased up to more than 3 min. Similar behaviour was observed in the peduncle.

In contrast to the repetition of action potentials evoked by a single wounding stimulation in *Biophytum*, in leaves of *Mimosa pudica*, although a wound stimulus (cutting or burning) evoked both a single action potential and a slow potential fluctuation, no repetition of action potentials was observed. The slow fluctuation is perhaps due to the stimulant, released as a result of the stimulation, being carried along with the water current through the vessels (Houwink 1935, Sibaoka 1953). Such a slow fluctuation was not seen with a wound stimulus in *Biophytum*; instead a very slow negative drift of the base line was observed (Fig. 1). Thus, the slow potentials fluctuation in *Mimosa* seems to be replaced by repetition of an action potential in *Biophytum*. When the leaf extract of *M. pudica* is applied at the basal cut end of the petiole of this plant, slow potential fluctuation, in addition to the action potential, was recorded in the petiole (Sibaoka 1953). The extract would stimulate the excitable cells near the cut end, eliciting the action potential, at the same time being sucked up, from the cut ends, through the vessels causing slow fluctuation to occur.

Similar trials were carried out with the leaves of *B. sensitivum*. The leaf extract was prepared as follows (Fitting 1930): 1 g of freshly chopped leaves were extracted with 20 ml of distilled water at 100 C for 10 min, and the filtrate collected. Leaves of *B. sensitivum* were detached at the basal end floated on water for 2-3 hr to recover. When the leaf extract was applied to the cut end of the detached leaf, repetition (8 to 10 times) of the action potentials took place. Intervals between each action potential were irregular (3-8 in). The repetition lasted for more than 40 min. The effect due to the leaf extract was weaker than a wound stimulus, since the repetition frequency was lower than that in case of a wound stimulation of an intact plant.

Therefore an extract was used which was five times stronger. Five grams of chopped fresh leaves were extracted with 20 ml of distilled water at 100 C for 10 min. Figure 2 shows a representative result. A pair of electrodes were positioned in the petiole and the uppermost leaflet pair of a detached leaf. When the cut end was immersed in the extract, a series of acropetally transmitted action potentials appeared over a period of 30 min. Intervals of the action potentials were about 0.5 min in the beginning and gradually increased to longer than 2 min. The repetition was immediately stopped when the extract was removed and the cut end was washed with water. The same results were obtained when the tip of a leaf was cut and immersed in the extract showing that the signal

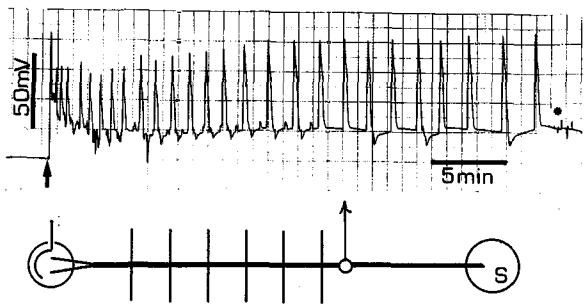

Fig. 2. Repetitive action potentials evoked by the application of leaf extract. Vessel (S) in which the cut end of the petile was based, was filled with leaf extract at time zero (arrow). Action potentials are picked up in diphasic manner, i.e. petiole and uppermost leaflets in a leaf. Some monophasic action potentials seen in the later half are presumably due to the transmission fading out. The asterisk indicates removing the extract and washing the cut end of the petiole with water, at which time the repetition stops.

was bidirectionally transmittable.

The time course and kinetic pattern of repetition produced by the leaf extract is very similar to that induced by wound stimulation. However, in *Biophytum* unlike *Mimosa* the proposed stimulant seems not to move from the affected part, since no significant slow voltage fluctuation was detected in *Biophytum*. Since the extract must contain stimulant(s), the extract applied to the cut end of the rachis may continuously stimulate the cells near the cut end, without penetrating further into proximal tissues, resulting in the generation of repeated action potentials. The same may occur at the site of a wound stimulation.

Time intervals between the repeated action potentials may depend on the refractory period of the generation of action potential. Umrath (1935) reported the absolute refractory period for the transmission of action potential as 8-11 sec in *B. sensitivum*. This is much shorter than that in *M. pudica*, where more than 100 sec was observed (Sibaoka 1950). Changing the time interval between the two stimuli (touches of ice cold water on the leaf surface), the refractory period in *B. sensitivum* was found to be 35-42 sec. The reason for the change from 8-11 sec to 35-42 sec is still not known. The time intervals of the repeated action potentials shown in Figs. 1 and 2 can be explained by the refractory period and fatigue of the apparatus generating action potentials. An electric or a cold stimulus that does not kill cells evokes a single action potential, whereas a wound stimulus (cutting or burning) produces stimulant(s) for a long time, and this eventually generates the repetitive action potentials.

The *Biophytum* leaf extract effectively generates repetitive action potentials only in this plant. The *Mimosa* (Legminosae) extract was not effective when used with a *Biophytum* (Oxalidaceae) leaf, and vice versa. Although this clearly suggests that the proposed water-soluble stimulant(s) acts in species-specific manner, the chemical

nature is still not known.

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