

for a few cycles without RBC, the culture at NCCS has been maintained for years now. It could be an ideal vaccine candidate but questions such as to whether it is really *P. falciparum* or a contaminant or whether the parasite requires some RBC or membrane fragments for replication, are still being investigated. It is, perhaps, worth a major initiative to address these questions once and for all and if an extracellular altered *P. falciparum* strain is

available, it could be an ideal candidate for a vaccine initiative.

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HYPOTHESIS

Possible molecular mechanism of mechanical responses in plants

Involvement of electrical phenomena in mechanical responses in plants, like leaf movements in Mimosa and Desmodium, had been proposed almost a century ago, subsequent studies established this fact. Recent evidences indicate that depolarization of pulvinar motor cells is involved in this process. In an attempt to look into the intracellular molecular mechanism associated with movement of leaves, here I hypothesize that downward movement of leaf is the consequence of sequential intracellular events in pulvinar cell – the transmembrane K⁺ efflux that results in depolarization, polymerization of intracellular actin and decrease in turgor pressure. Hyperpolarization, which is implicated with the cellular K⁺ influx, invokes opposite events in pulvinus, i.e. intracellular actin depolymerization, rise of turgor pressure and, as a result, the upward movement of leaf.

Plants respond to changes in the environment. About a century ago, J. C. Bose¹ first documented that many of these responses involve electrical phenomena^{1,2}. Since then, for several decades the studies on the concept of electrical activity in regulation of physiology of plants remained neglected. However, recent studies have established this concept^{3–7}. Plant action potential that resembles nerve action potential in many aspects^{2,3}, and being transmitted can regulate biochemical processes⁴. Bose noted that electrical activities are associated with mechanical responses like dipping of leaves in *Mimosa*, and rhythmic movement of leaflet in *Desmodium*. In *Mimosa*, strong electrical stimulation of pulvinus produces effects similar to those developed by a mechanical stimulus². In *Desmodium*, the excitatory electrical stimulus gives rise to downward contractile movement of standstill leaflet¹. Using an electrical probe, Bose demonstrated that the main pathway of transmission of the electrical phenomenon was the phloem². Sandlin *et al.*⁸ detected contraction of cell whenever an

action potential was produced after delivery of a pulse of current to the excitable tissue of *Nitella axillaris*.

Bose¹ further observed that leaf movement in these plants is influenced by environmental conditions. The frequency of leaf clapping rhythms in *Desmodium* is less at low temperatures and the pulsation is arrested at a temperature as low as 17°C; rise of temperature up to an optimum level of 43°C induces enhanced frequency and diminished amplitude of pulsation and beyond this optimal temperature there is a tendency of arrest of pulsation. In *Mimosa*, lowering of temperature induces a depression of the leaf, and rise of temperature results in erection of leaf. When water is withheld in *Desmodium*, the leaflet ceases to pulsate, and the activity was renewed on irrigation⁹. Increased hydrostatic pressure induces erection of leaflet¹. The arrested pulsation of detached leaflet may be revived by the application of internal hydrostatic pressure¹. In a cut specimen of *Desmodium* kept in appropriate condition in dark, the amplitude of rhythmic

pulsation of leaflet was gradually reduced and ultimately came to a total stop in 18 h. The pulsation was revived by application of a stimulus of electric shock. Application of strong light for 5 min gave rise to a single pulsation in such a dark exposed standstill leaflet⁹. The leaf movements, change of galvanometric polarity and turgor are interrelated. In *Mimosa*, the strong non-electrical mode of stimulation elicits electric response of galvanometric negativity similar to the result obtained in the case of excitation of animal nerve². The negative excitatory impulse generated by strong direct stimulus applied to the plants having motile leaves, e.g. in highly conducting petiole of *Mimosa* and sub-conducting petiole of *Averrhoa carambola* induces diminution of turgor, contraction and dipping of leaf². A stimulus of feeble intensity gives rise to opposite effects². In *Desmodium*, all modes of maximal stimulation induce diminution of turgor, contraction and decrease in pulsatile activity of leaflet⁹. The pulvinule of the leaflet exhibited periodic electric pulsations corresponding

to the mechanical pulsation. The upward movement due to sudden increase in turgor has an electric concomitant of galvanometric positivity; the opposite electric change of galvanometric negativity occurs during the phase of sudden diminution of turgor and fall of leaflet⁹. Recent studies by Antkowiak and Engelmann¹⁰ support these much earlier observations and show that gyration of leaflet in *Desmodium* is caused by rhythmic change in turgor pressure of pulvinal cells associated with periodic change in cell membrane potential difference (PD). They observed downward movement of leaflet when the cells were depolarized, contracted and lost turgor. The upward movement of leaflets, in contrast, is implicated with hyperpolarization, positive extracellular PD, expansion of cell volume and increase in turgor pressure^{10,11}. Antkowiak *et al.*¹¹ noted diminished period of oscillation with increase of temperature. However, to date, reports are not available explaining the molecular mechanism involved in the electromechanical process of leaf movement. The present work hypothesizes a microfilament-associated molecular mechanism to explain the fact.

In *Mimosa*, Bose observed that light caused erectile movement of leaf; however strong and prolonged exposure of light induced excitatory negative impulse resulting in folding and dipping of leaf². Subsequent studies show that, in *Chlorella pyrenoides*, light stimulates uptake of K⁺ into cells¹². Studies on stomatal guard cell contraction and relaxation mechanism exhibit similar facts. The inward K⁺ channel activity is required for the guard cell volume-increase^{6,13} that is associated with increase in turgor and stomatal opening^{14,15}. The actin microfilaments exist and play an important role in plant cells^{16,17}. These are involved in the volume change mecha-

nism in cells¹⁸. Cytochalasin-D, which induces hyperpolarization-dependent inward K⁺ channel facilitates actin depolymerization and promotes guard cell volume regulated stomatal opening; phalloidin blocks the inward K⁺ channel, induces polymerization and stability of actin filaments, and inhibits the stomatal opening¹⁹. Recent studies on plant action potential indicate that depolarization, which can be produced by external stimuli, results from an influx of Ca²⁺, followed by efflux of K⁺²⁰, and is coupled with efflux of water and loss of turgor⁷. The loss of turgor and contraction of pulvinal cells that result in dipping of leaf in *Mimosa*² and the downward movement of leaf in *Desmodium*¹⁰, therefore, may be associated with actin polymerization coupled with outward K⁺ channel activities. In these plants, the upward movement of leaf that is linked with the hyperpolarization, expansion of cell volume and increase in the turgor^{10,11}, is possibly the consequence of depolymerization of actin microfilament-induced by K⁺ influx. However, future works in this line will help further confirmation of this proposal.

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