**Science Research Proposal for Review Board**

# Impact of Calcium on the Reaction Time of *Mimosa pudica*

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## Introduction

### I. Background

Cells communicate by transferring molecules from one to another. When a molecule is transferred it creates a reaction which then has an effect. Within multicellular organisms there is cell-to-cell communication. Direct communication between cells can occur in two ways. One of these is through cell junctions between adjacent cells. In animals this is called gap junction, in plants it is called plasmodesmata. Plasmodesmata(PD) are that act as intercellular cytoplasmic bridges that create an interconnected commune within adjacent cells and enable transport of materials between plant cells. The PD connect the symplastic space in plants. They are specialized channels that permit intercellular movement of water, various nutrients, and other molecules (including signaling molecules) (Epel, 1994).

Mechanoreception is the perception of mechanical stimuli in the environment by a living organism. This perception results in a process of rapid translation of a mechanical force into a biochemical or bioelectric message, also known as a signal molecule. When this process occurs within plant cells, the signal molecule trafficking would occur though the plasmodesmata. One

speculated way signal molecules traffic more specifically through the plasmodesmata is by an actin-myosin protein interaction, which is regulated by calcium ions.

The *Mimosa pudica* L. has a rapid response to touch, or a rapid movement in response to physical contact. When physically stimulated the *Mimosa pudica* L. responds with rapid folding of its leaflets and movement of the entire compound leaf .

## II. Question

In order to relate the nonmolecular reaction of the *Mimosa pudica* L. to the molecular process of an actin-myosin interaction within the plasmodesmata, I will regulate the amount of calcium within the plant. Calcium is essential for an actin-myosin interaction. My question is will the *Mimosa pudica* L. plants' response to touch become more rapid when there is a higher concentration of calcium within the plant? If my speculations are correct the plant cells will respond faster, this would allude to the presence of an actin-myosin interaction within the plasmodesmata.

## III. Significance

Plants are essential for life; they are the primary conduit for flow of energy between the sun and living organisms. 90% of Earth's biomass is made of plant life and is an essential source of food and medications. It is crucial for humans to understand the basic mechanisms of plants because it is the foundation for solving the more complicated problems related to agriculture, medicine and our environment. In order to live in a stable, healthy environment and to maintain

overall global sustainability we must understand the basic building blocks of these complex organisms.

## Literature Review

### I. Mechanoreception in plants

The perception of mechanical stimuli in the environment is crucial to the survival of all living organisms. A physical–mechanical stimuli is perceived by cells due to the differences in pressure and force. In plants a common stimulus is a touch or thigmo stimuli.Touch leads to thigmonastic movements or movements based on environmental stimuli (Jaffe et al.,2002). Some plants, such as plant traps, rapidly respond to a stimuli in order to obtain necessary nutrients (Brown, 1916). Other plants move in a defensive manner to protect themselves from predators, such as the *Mimosa pudica* L.,which closes its leaflets and "play dead."

Responses to thigmo stimuli result in a rapid translation of a mechanical force into a biochemical or bioelectric message. The first detection of a response is a change in action potentials and electric resistance (Jaffe, 1976). The second detection is shown by an increase of intercellular calcium, which has been documented in several plant systems (Toriyama and Jaffe, 1972; Knight et al., 1991, 1992;Trewavas and Knight, 1994;Legue´ et al., 1997;Pickard and Fujiki, 2005). The reason for this increase in calcium levels is still unknown (Knight, 2000).

Transport can occur in two forms: passive and active. Passive transport does not require energy and is simply the movement of molecules down the concentration gradient i.e. from high to low

concentration. Any soluble molecule can transport in this way. If a molecule is soluble in lipids it is thought to diffuse within the desmotubule(Roberts and  Oparka, 2003).

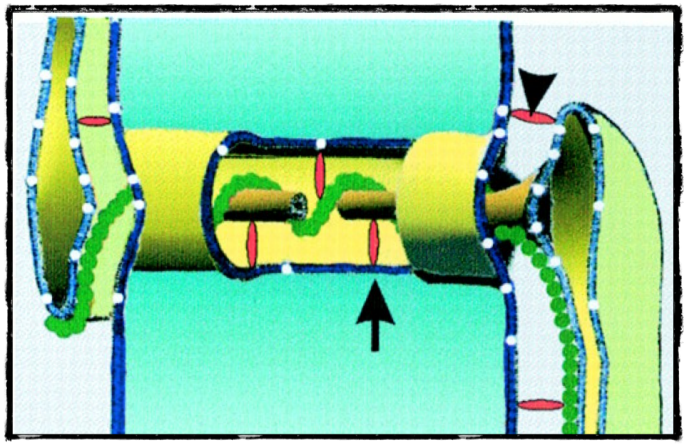
Active transport requires cellular energy. It uses ATP to pump molecules against the concentration gradient or from a low concentration to high concentration. Amino acids and other molecules can not diffuse or diffuse too slowly to survive and must be transported actively. There are two types of active transport: primary and secondary. In primary active transport, a group of ion channels exist and specialized trans-membrane proteins recognize the presence of a substance that needs to be transported, which then activate the channels to open and close causing change in concentration gradient. These pumps carry the desired molecules across with the assistance of ATP( Physiology). In secondary active transport, proteins force the molecules across using an electromagnetic gradient. Often, this energy is gained by simultaneously moving another substance down the concentration gradient(Cooper, 2009). Molecules that can not simply diffuse are shuttled through plasmodesmata via unidentified active transport mechanisms(Oparka, 2003).

## II. Plasmodesmata

Eduard Tangl in 1897 noticed the presence of the PD within the symplasm. In 1907, Strasburger termed these microchannels PD. The introduction of the electron microscope allowed closer study of the PD; in the 1980's scientists began to study the movement of molecules through the PD using fluorescent probes (Oparka, 2001).

**Figure 1**

Schematic model (Overall and Blackman, 1996) of a plasmodesma showing the modified ER element, in the form of the desmotubule (brown rod), which traverses the cell wall (pale blue), associates with actin (green pearls), and is interlinked with the plasma membrane (dark blue line) via myosin VIII (red spokes, arrow).



Baluška F, Cvrčková F, Kendrick-Jones J, Volkmann D. *Sink plasmodesmata as gateways for phloem unloading. Myosin VIII and calreticulin as molecular determinants of sink strength? Plant Physiology 2001a;126:39-46*

The figure above is a visual model of the PD. Plant cells are surrounded by cell wall which separates neighboring cells. PD are located in areas in cell walls called primary pit fields. There is up to one million pit fields per square millimeter, this makes up one percent of the entire area of the cell wall (Salisbury and Ross, 1992).

There are two forms of PD, primary and secondary. Primary PD originate during cell division and remain in growing cells. Secondary PD are formed in mature plant cells.

PD vary in formation. They can be simple, twinned, or branched. A simple PD is a single linear channel. A simple PD is the starting point to generate two side-by-side PD, called twinned PD, via intermediate structures that can be V, X, Y or H shaped. Branched PD are complex and have a common central cavity that connects the "branches". Branched PD are more commonly secondary PD, however simple secondary PD exist as well (Roberts and Oparka, 2003).

The plasma membrane is continuous between cells, the inner leaflet contiguous with the plasmodesmal pore and the outer leaflet contiguous with the cell wall (Overall and Blackman, 1996; Oparka, 1993). A modified strand of endoplasmic reticulum (ER) termed the desmotubule runs the length of the PD (Hepler, 1982).Thus, the desmotubule is essentially a tube within a larger tube surrounded by the plasma membrane which is bordered by the cell wall. Since the desmotubule is linked to the ER in adjacent cells a dynamic endomembrane continuum is formed. The ER can serve as a pathway for transporting various lipids between plant cells (Grabski, et al., 1993).

The space between the plasma membrane and the desmotubule is called the cytoplasmic sleeve or cytoplasmic annulus. Plasmodesmal transport is theorized to occur both through the lipid portions of the desmotubule and the cytoplasmic sleeve. The neck region at each end of the plasmodesmal channel is where the plasma membrane closely associates with the central desmotubule. The neck region is proposed to contain proteins that regulate the passage of materials through the PD, much like gap junctions in animal cells (Roberts and Oparka, 2003). The accumulation of polysaccharide callose around the neck region of PD forms a collar, reducing the diameter and thereby controlling permeability of substances in the cytoplasmic sleeve (Robards, 1976).

There is an actin-myosin interaction network within the PD (White, et al.,1994). Overall and Blackman (1996) suggest the actin and myosin wrap around the central desmotubule and help stabilize the desmotubule. Various macromolecules require assistance to traffic through the PD. Actin has been thought to help alter the size exclusion limit of the neck region to allow for the transport of larger molecules (Overall and Blackman, 1996). In addition, myosin may also act as

a cytoskeletal motor in plasmodesmal transport. Myosin has been found in several species of plants (Avisar, et al. 2008; Radford, et al.1998). It is an ATP-dependent protein that generates directional movement by converting chemical energy into kinetic energy through hydrolysis. High ATPase activity has been demonstrated in PD (Fleurat-Lessard, et al. 1995). This alludes to the presence of myosin activity within the PD.

III. Myosin-actin interaction

Myosin is a mechanochemical enzyme or a type of enzyme that converts chemical energy (in this case ATP) into mechanical energy. Myosin is also an ATPase that traffics along the actin filaments by converting ATP via hydrolysis into conformational changes. Myosin is the motor, actin filaments are the tracks to which the myosin moves and ATP is the fuel that powers the interaction. Calcium too plays a key role in this interaction (Cheney and Mooseker, 1992). Myosin is composed of three main domains. The head domain contains actin and ATP-binding sites and is responsible for generating the force. The head domains among different classes of myosin generally are conserved. Next is the neck region, which regulates the activity of the head domain. Finally the tail domain contains binding sites determine the function of that given myosin.That being said, the tail domain's structure is the main way of characterizing a myosin (Oliver et al. 1999).

The first step of myosin ATPase activity is ATP binds to the myosin head which results in the myosin head releasing from the actin. Next ATP is converted into ADP+ a phosphate via hydrolysis. This bends the myosin protein into a high energy conformation. In the high energy state the phosphate is released, which causes the myosin to push onto the actin. Finally the ADP

is released which locks the myosin head in place and the cycle begins again (Tyska and Warshaw, 2002).

Myosin ATPase activity will not occur unless it is associated with actin. Therefore conformational changes of the actin results in the regulation of myosin movement. Two proteins that assist with regulation of myosin are tropomyosin and troponin. Tropomyosin coils around the actin protein and blocks myosin binding sites on the actin. Troponin attaches the tropomyosin to the actin. In its unmodified conformation these two proteins prevent myosin from moving. The only way to reactivate the myosin activity is to have a conformational change of the tropomyosin and troponin. Calcium ions bind to the troponin which then changes the configuration of the tropomyosin, revealing the myosin binding sites. When there is a high concentration of calcium the myosin is able to move. When there is a low concentration the tropomyosin and troponin go back to their blocking states (Robertson et al, 2010).

*IV. Mimosa pudica*

The response of *Mimosa pudica* to a stimulus is immediate and has the tendency to capture the attention of anyone observing it. Charles Darwin was intrigued enough to devote time to describing the leaf-closing response of this plant to external stimuli (1880).

*Mimosa pudica* is a short-lived sub-shrub that is native to Brazil however in recent years has become pan-tropical. It has prickly stems that can grow to a spread and height of about one meter. *Mimosa pudica* can grow in most well-drained soils with high or low nutrient availability but is not shade tolerant. Being a member of family Fabaceae, the roots of *Mimosa* contain nitrogen-fixing nodules. In cultivation the plant will produce pink fluffy flowers from which

viable seeds may be collected. A rapid movement of the leaves occurs when it is stimulated by touch, vibration and change in heating. There are very slow, periodical movement of the leaves called nyctinastic movement at night. These movements are controlled by the plants biological clock. The leaves of the plant can adapt their response so that they remain closed until it has adapted to the given stimuli. The more intense the stimuli, the longer it takes the plant to adapt and reopen its leaves (Ueda et al., 1999).

## Research Question

I. Putting it all together: molecular on a non-molecular level

The hardest part of this process is not the learning and becoming literate in a subject, but the actual experimental design. Taking the knowledge you know and applying it to something a high school student could experiment. I have come up with a way of researching a molecular occurrence on a macro scale using low tech methodology.

Here is what we know: Plant communication of signal molecules could occur via PD through an actin-myosin interaction. When a *Mimosa pudica* L. is stimulated or touched its leaflets react by closing. This reaction occurs through signal molecules which alert the cell of a stimulant. Actin-myosin interactions are regulated by calcium. Therefore an influx of calcium would mean an increase in the trafficking of molecules. Ergo if the *Mimosa pudica* L. reacts faster with an increase of calcium, it would allude to the possibility that actin-myosin interaction is occurring within PD. I will be increasing the amount of calcium within the plant and timing how fast the leaflets react to a stimulus.

# Methodology

## I. Overview of approach

My experiment will consist of 3 groups of *Mimosa pudica*, with the upwards of 100 plants per group. These plants will be hydroponically grown from seeds. This means they will be grown without soil. This way, I will have more control over the nutrients exposed to each plant. The control group will have a total of .73 grams per liter of water in its nutrient solution. Experimental group A will have a decrease in the level of calcium, going down to approximately .31 grams per liter of water. Finally experimental group B will have an increase of calcium levels, 1.16 grams per liter of water.

The plants in the experimental and control groups will be stimulated (stroked in one swift movement) and recorded using HD video recording via my personal SLR camera. From the video I can slow it down and find a precise reaction time.

### Data Collection( timeline)

For sufficient amount of data I will do an upward of 20 tests over approximately 2 months. Variables including temperature and humidity will be recorded for each test as well.

## II. Data Analysis

The videos of each *Mimosa pudica L*. reactions will be slowed down. A reaction is measured from the moment the leaflets are stimulated to when the leaflets stop closing in. Findings will be recorded and then analysed for patterns in reaction times.

As my independent variable is calcium within a given *Mimosa pudica* plant, I will vary the amount of calcium to find the "perfect" amount of calcium that will increase intercellular trafficking without giving the plants too much of it.

III. Interpretation(problems that could occur)

One of the main potential issues is that certain plants may have a impaired growth due to the different levels of calcium. I will add to my experiment observations on how the plants health seems and try to keep this in consideration with my results.

IV. Expected Results

The influx of calcium within the *Mimosa pudica* will cause it to react more rapidly to a physical stimuli alluding to the presence of a possible Ca2+ driven transport mechanism, such as an actin-myosin interaction within the PD. This would add to the body of knowledge of the basic mechanisms of how plants react to their environment. This would open new research on crucial environmental and global problems. All plants react to their environment, but only a few have visible rapid physical response. By using the response of the *Mimosa pudica* to study the way signal molecules are trafficked, we could open the door to see how every plant trafficks molecules and interacts with the environment.

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