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Enhanced calcium-mediated salicylic acid interaction in Pea (*Pisum sativum* L.)

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Abstract
It is a well-known fact that peas are used for a variety of purposes in day-to-day life. As a result of the experiment conducted by Gregor Mendel in 1863, the pea was crucial for the development of modern genetics. Pea is used for many purposes, so it needs to be able to meet the demand. As a result of their health benefits as well as their taste, there is an argument that the productivity of peas should be increased. As a part of a healthy diet, peas can be included in our regular meals as nutritious food. Additionally, peas can be found year-round due to their availability. It is known that regular consumption of a pea can have enormous health benefits. This can prevent severe diseases such as weight loss, diabetes control, and improved digestion as well. As a result of their high protein content (22-35%), essential amino acids, complex carbohydrates, and mineral content like iron, calcium, and potassium, peas are an excellent source of vitamins, minerals, and fiber. The pea is a delicious combination of taste and health that should not be ignored and one should work on increasing the consumption of this delicious fruit. Due to the fact that people are becoming more and more diet conscious these days, as well as their increasing demand for peas, it is vital that the production of peas increases in order to meet the growing demand. In this study, the effect of salicylic acid and calcium on the morphology, physiology, and yield of peas was examined. It has been observed in many scientific studies that the treatment of plants with salicylic acid and calcium has shown to have a positive effect on the growth of crops and fruits. In the present paper, we are looking at the growth of pea plants and pods after they have been treated with salicylic acid and calcium during the growth phase.

Keywords: Agriculture, biotic, calcium, hunger, poverty, pea, salicylic acid, sustainability

Introduction

As of the present day, peas have been used in day-to-day activities such as breakfast, cereals, health foods, pasta, processed meat, purees, and soups; they can also be processed to form pea flour, which is a source of protein. In the scientific community, the pea is known as *Pisum sativum* L., which is a cool-season legume that belongs to the Leguminosae family and is a diploid species. The fact that there are several pieces of evidence that indicate that pea has a new direction as a result of its experimental experience. In the middle of the nineteenth century, Johann Gregor Mendel used pea plants to test his inheritance theory (van Dijk, 2018) [25]. In his experiments on heredity, Mendel discovered that using the pea had many advantages. Pea plants are quick to reproduce and mature, have physically recognizable physical characteristics and can be easily fertilized artificially. As early as 1856, Mendel found that plants of the same species have different morphological appearances, including different colors, seed shapes, and heights (Edelson, 2001) [13]. Consequently, all of the parental characteristics would eventually blend together, resulting in a homogeneous amalgamation of all of the characters. In order to trace the heredity of physical characteristics through generations, Mendel needed to fertilize plants from one generation (F1) with plants from the same generation. It was Mendel who used controlled fertilization to breed generations of pea plants with the confidence that there would be little or no contamination from plants from previous generations (Andrei, 2013) [2]. Several reports suggest that pea seeds contain the compounds trypsin and chymotrypsin, which could be used as a contraceptive as well as an ecbolic fungistatic and spermicide (Duke, 2012; Duke, 1981) [11-12].

Morphology of Pea

Plants that grow as annual herbaceous plants and have 14 chromosomes are known as pea plants. It has a taproot that can penetrate the soil to a depth of 1.5 m. Its lateral roots grow in the top layer of the arable soil. The roots of peas are in complete symbiosis with nitrogen fixers, such as bacteria in the Rhizobium genus. Seeds come in a variety of shapes and sizes; they might be smooth or wrinkled, globular or angled. A variety of colors are available, including white, grey, green, and brown (Bryant, 2013) [6]. Unlike most angiosperms, mature seeds lack the typical endosperm, and the cotyledons serve as a source of nutrition for the germinating seed. In general, they are cylindrical, bulging as they ripen, bare, and have a short, curved rostellum. The seeds of peas are spherical in shape and can be smooth or wrinkled. Each seed is made up of an embryo and a testa. Two seed lobes are located beneath the testa and remain in the soil after seed germination (Yalçın, 2007) [26]. Flowers are produced on 1.5-3.5cm long axillary racemes of 1-3 flowers. They are available in white, pink, or purple corollas. The plant climbs by means of tendrils that are produced at the apex of its compound leaf (Burnham, 2013) [8]. The plant grows best in well-drained soils, such as sand, silt, and clay loam. It does not tolerate shade. The species prefers cool, humid climates, and escapees can generally be found on beaches or in fields (Teshome Gari, 2015) [24] (Figure 1 & 2)

Fig 1: Pea plant under open field condition

Fig 2: Pea pods after harvest

Salicylic Acid and their Impact

Salicylic acid, also known as salicylic acid, is an inorganic compound with the chemical formula $C_7H_6O_3$. It is a type of beta-hydroxy acid and phenolic acid. It's found in small amounts in a variety of plants and has been isolated from willow tree bark. It occurs naturally in plants as a growth hormone. A benzene ring in an ortho position is attached with a hydroxyl group (-OH group) to the carboxylic acid functional group (-COOH group). This compound has a molecular weight of 138.12 grams per molecular unit (Hayat, 2013) [17]. At room temperature, it appears as clear white or colorless needle-shaped crystals that are odorless. In terms of boiling and melting points, salicylic acid has a boiling point of 211°C and a melting point of 315°C. It has a flashpoint of 157°C, a molecule with two hydrogen bond donors and three hydrogen bond acceptors, as well as a flashpoint of 157 °C. Since this drug is lipophilic, it has low solubility in water, but it is soluble in organic solvents such as acetone, benzene, chloroform, ethanol, oil of turpentine, propanol, and toluene (Drugbank, 2022) [10]. In numerous studies, it has been demonstrated that salicylic acid plays an important role in the growth of the pea plant. It can be observed that salicylic acid has positive effects both quantitatively and qualitatively (Appu, 2014) [4] (Figure 3).

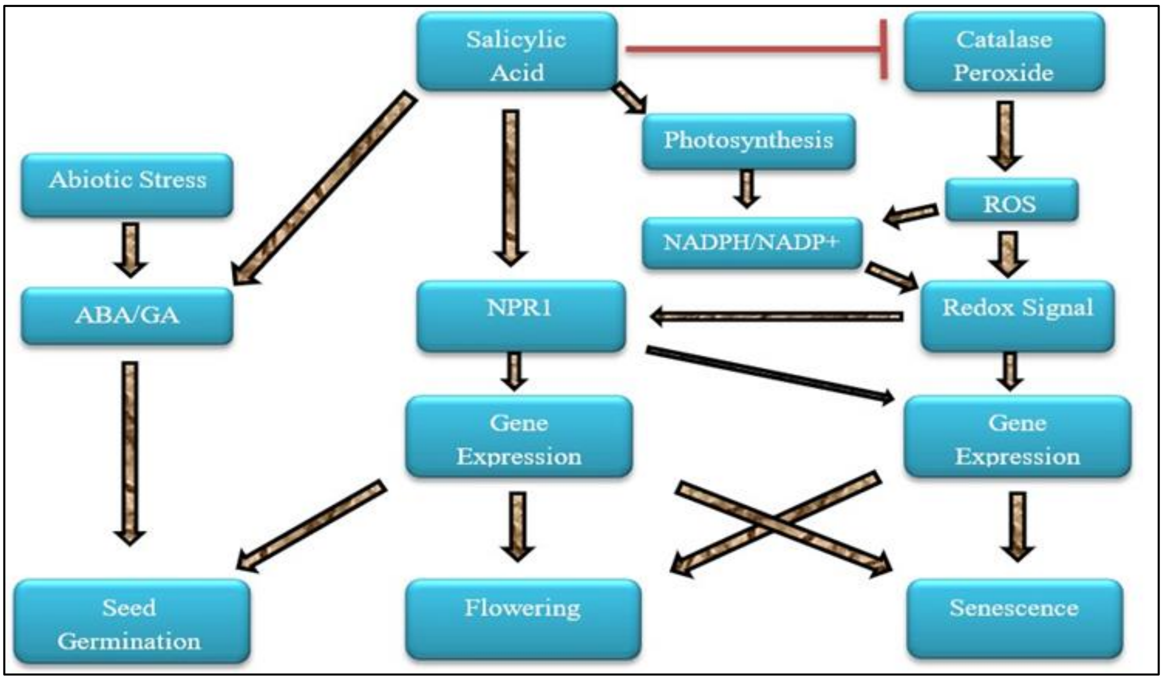

Fig 3: Descriptive model of salicylic acid function in plant growth and development (Rivas-San Vicente, 2011) [44].

Calcium and their Impact

Calcium is a mineral that is essential for both plants and animals alike. This is a soft, alkaline earth metal with a gleaming silver surface that doesn't exist as a pure element in nature. The fifth element in the periodic table, calcium, is the third most abundant metal in the earth's crust, with an atomic number of 20. In plants, calcium plays an essential role in the formation of plant tissues and the growth of the plant. Calcium is responsible for the formation of cell walls in plants. This enzyme is also important for the activation of enzymes and the transmission of signals that coordinate the activities of the cells (Harvey, 2016) [16]. In the past few years, scientists have observed that calcium stimulates photosynthesis and increases the size of saleable plant parts by increasing the absorption of ammonia, potassium, and phosphorus. In addition, it improves nitrogen utilization efficiency, lowers production costs, and reduces nitrogen pollution in the environment (Feagley, 1998) [14]. The walls of the plant's cells are held together by calcium, which is in the form of calcium pectate. When calcium levels are low, new tissues, such as root tips, young leaves, and shoot tips, often exhibit distorted growth as a result of improper formation of the cell wall. In addition to being used as an ion, calcium is also used to activate enzymes and to send signals which help cells coordinate their activities (Buechel, 1998) [7]. There is no doubt that signaling is a vital part of a plant's life cycle, and it plays a vital role in fulfilling its life cycle in plants that are at the top of the food chain. The process of signal transduction can be broken down into a number of steps: first, there is the perception of the developmental and environmental signals, then is communication between cells, and finally, there is the physiological and morphological response. There is increasing evidence that ions, molecules, genes, proteins, biological processes, and biochemical reactions have an important role to play in the signaling cascades that take place in the cell (e.g., guard cell ABA signaling) [1]. Recent biochemical, molecular, and genetic studies have demonstrated that plant signaling is crucial for even simple physiological functions carried out by specific cells, such as communication between guard cells during fertilization and communication between guard cells during pollination. This review will examine the significance of how Ca2+ and actin cytoskeleton contributes to the vital role that guard cells and pollen play in a physiological and biochemical process. The interactions between the Ca2+ and actin cytoskeleton in guard cells and pollen will be discussed in this review. Calcium plays a vital role in both the structure and signaling of the cell, and it also plays a role in the development and growth of plants. In light of these findings, a growing body of research suggests that calcium is an essential nutrient for plants. A recent scientific study also suggests that cytosolic free Ca2+ ([Ca2+] cyt) could act as an intracellular second messenger that facilitates physiological and biochemical reactions in plants when exposed to environmental stimuli. Several studies have been conducted on the latter topic as to how Ca2+ plays a significant role in plant signaling, ranging from whole-plants down to individual proteins. Plant cells are maintained at a Ca2+ concentration lower than 100 nM at rest, while Ca2+ resides at millimole levels both extracellularly and organellar. Depending on the stimulus, the concentration of free Ca2+ in the cytosol can elevate or oscillate, leading to the [Ca2+]cyt signal that occurs as a consequence of an influx from extracellular and release from extracellular organelles (e.g., endoplasmic reticulum and vacuole) through Ca2+-permeable channels on the plasma membrane and endomembrane, respectively. The Ca2+ signaling pathway has been studied by many research groups in an attempt to discover its upstream components and downstream effectors. It is known that actin dynamics play an important role in many cellular signaling pathways in plants. Filamentous actin is found in almost all eukaryotic cells, and monomeric actin or globular actin is found in almost all eukaryotic cells. A number of signaling pathways have been identified involving F-actin filaments (with diameters between 5 and 7 nm), including shaping, dividing, growth, vesicle and organelle movements, and cell death induced by biotic and abiotic stresses. As the G-actin polymerizes into F-actin, which has a characteristic architecture and polarity in the cytosol and forms bundles and cables of actin filaments, the cytoskeleton behaves as homeostasis within the bundles. Actin dynamics is capable of regulating actin function in a variety of different ways. For instance, actin dynamics could regulate the actin polymerization and depolymerization between G-actin and F-actin in response to developmental signals and environmental abiotic factors. The presence of sufficient amounts of G-actin bundles accompanied by visible F-actin bundles is essential for the dynamics of actin. Moreover, there is evidence that the G-actin activity is highly pronounced in Arabidopsis pollen, and is at about 50 mM, whereas only a very small portion of each plant's G-actin can be polymerized to form F-actin.

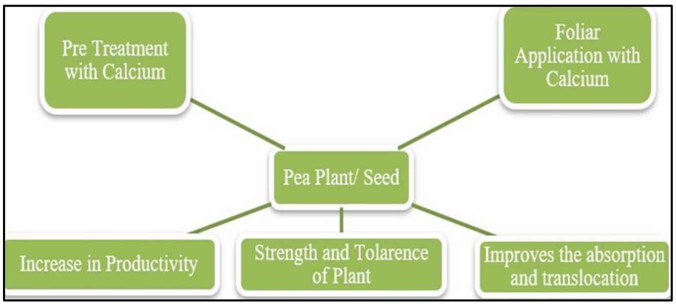

Fig 4: Impact of calcium on plant

Interaction of Stomata and Calcium

Furthermore, cells are not only the basic building blocks for all organisms, but they also act as sensors for internal and external stimuli, both of which they respond to in a variety of ways. So this is why it is important to study signaling networks both at the cellular and molecular levels. It is a known fact that plants contain many types of specialized cells such as guard cells and pollen. It is believed that a specific cell's characteristic is regulated by the expression of a specific set of genes, while the rest of the genes present in the cell are repressed, and epigenetic regulation plays a critical role in their differentiation processes during the development of a cell. A large-scale transcriptomic and proteomic analysis was used to identify the difference between guard cells and pollens and the hypothesis has been confirmed by several studies. As result of these findings, suggests that the signaling pathways might differ between different plant cell types or even between different developmental stages of the same cell type. In this way, comparing the signaling networks of different specialized plant cells with the signaling networks of cells at different stages of their development will be very helpful to understand how different signaling mechanisms respond to developmental and environmental stimuli in plants and animals. Plant leaves play a significant role in the growth and development of the plant's vegetative body, as they are the main photosynthetic organ. The leaves, along with the roots, are also important for transpiration, and also they play an important role in responding to biotic and abiotic stresses, enabling plants to cope with the environment and survive. Stomata, which are microscopic pores on the surface of leaves that are responsible for the uptake of atmospheric carbon dioxide (CO2) (as a substrate for carbohydrate biosynthesis through photosynthetic reaction) and the loss of water vapor through transpiration, play a significant role in controlling both water vapor absorption and loss (Engineer, 2016) [3]. Stomata are made up of paired guard cells and are composed of a functional complex, which is separated from the epidermal cells to which they are attached by no plasmodesmata (Xu, 2016). Pathogens can also enter the body through the stomata, which is a major entry point. As a result of the opening and closing of the stoma, plants can exchange gases as well as cope with environmental conditions and thrive in response to stresses caused by phytohormones and environmental stressors (Melotto, 2008) [35]. As a result of these and a number of other factors, the stomatal aperture is well known to be regulated by both internal and external factors (biotics and abiotic), including hormones (e.g., ABA, auxin, and ethylene), blue and red light, water status, CO2 concentration, circadian clock, temperature, and pathogens (Gudesblat,2009) [36]. This turgor alteration is what controls the stomatal movements, and the volume change in the guard cells is the result of the volume change in guard cells (Chen, 2013). The increased osmolality induced by the accumulation of solutes such as potassium, anions, and malate during the stomatal opening, in turn, facilitates water uptake and swelling of guard cells, resulting in the opening of stomata (Outlaw, 1983) [38]. A major contributor to the process of stomatal closure is the efflux of osmotically active solute by guard cells from the stomatal periphery, and subsequently, malate metabolizes into osmotically inactive starch, which facilitates the shrinking of guard cells and closing of the stomata (Mano, 2021) [39]. The physiological significance of guard cells has led to them being well studied at the whole plant, whole leaf, cellular, subcellular, and molecular levels by using various investigation tools. Therefore, guard cells have been developed as the first model system for plant cell signaling because they play such an important role in plant development (Araújo, 2011) [40]. There is evidence that both Ca2+ dynamics, as well as the dynamics of actin, may play a role in guard cell signaling networks. Though there is still a lot to be learned about the development of reproduction in flowering plants, despite the transition from vegetative to reproductive growth having taken place. In order for female fruit and seeds to reproduce, the main function of the processes is fertilization, which occurs when mature pollen drops on the top of a suitable pistil. In this process, the pollen is hydrated, germination occurs, the pollen tube grows, and the female gametophyte is fertilized. The process of pollen germination involves a number of regulatory networks that include several factors such as calcium ions, potassium ions, hydrogen ions, chloride ions, NO, ROS, and cytoskeletal components. It has been suggested that one of these factors plays a crucial role in pollen signaling via Ca2+. In addition, there has been evidence that the function of actin, as well as its configuration, plays an important role in cellular signaling as well as in the structure of cells. In light of the fact that pollens and pollen tubes can serve as models for investigating haploid and polarized growth of plant cells, as well as their role in double fertilization, pollens, and pollen tubes, have been seen as very important systems for reproductive and cellular signaling research throughout history. Guard cells, as well as pollens, are well-established model systems for investigating plant signaling networks, therefore understanding the similarities and differences between them will allow us to determine the specific signaling characteristics of plant cells, and the function and signaling of each cell. About 20 years ago, it was discovered that the elevation of [Ca2+]cyt in pollen tubes triggered the fragmentation of actin bundles and the reorganization of actin in guard cells.

In guard cells, pollen, and other plant cells, the dynamics of calcium citrate and actin networks play an imperative role in development and physiological response to external stimuli (Jezek, 2017) [41]. Our aim in this paper is to describe both the individual roles of each messenger and to compare their interaction between two different types of growing plant cells: guard cells, a reversible model cell, and pollen, a polar model cell. It is becoming increasingly clear that [Ca2+] cyt and actin often act together in these plant cells and are essential to their normal function. On the other hand, while the fundamental mechanisms by which these two signaling components interact are still poorly understood, recent evidence suggests that the regulation of actin dynamics by [Ca2+]cyt is largely dependent upon the downstream Ca2+ sensor (Tuteja,2007) [31]. However, it is still not clear at this point in time how the activation of [Ca2+]cyt, a cytoskeletal protein, is regulated by actin via Ca2+-permeable channels at the molecular level. Could this be due to a decrease in the amount of G-actin in the cytosol or the ratio of G-actin to F-actin? In human actin, gesolin is linked to the regulation of Ca2+ channels. The root-associated proteins gelsolin, myosin XI, and villin/gelsolin/fragmin are also found in plants, and their roles in actin dynamics are still largely unknown, but they have been found to play a role in activation of Ca2+ channels and generation of [Ca2+] cyts in plants (Qian, 2019). There is no doubt that signaling is a vital part of a plant's life cycle, and it plays a vital role in fulfilling its life cycle in plants that are at the top of the food chain. The process of signal transduction can be broken down into a number of steps: first, there is the perception of the developmental and environmental signals, then is communication between cells, and finally, there is the physiological and morphological response. There is increasing evidence that ions, molecules, genes, proteins, biological processes, and biochemical reactions have an important role to play in the signaling cascades that take place in the cell (e.g., guard cell ABA signaling) (Alberts,2002) [29]. Recent biochemical, molecular, and genetic studies have demonstrated that plant signaling is crucial for even simple physiological functions carried out by specific cells, such as communication between guard cells during fertilization and communication between guard cells during pollination. This review will examine the significance of how Ca2+ and actin cytoskeleton contributes to the vital role that guard cells and pollen play in a physiological and biochemical process. 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A number of signaling pathways have been identified involving F-actin filaments (with diameters between 5 and 7 nm), including shaping, dividing, growth, vesicle and organelle movements, and cell death induced by biotic and abiotic stresses (Cooper,2000) [27]. As the G-actin polymerizes into F-actin, which has a characteristic architecture and polarity in the cytosol and forms bundles and cables of actin filaments, the cytoskeleton behaves as homeostasis within the bundles. Actin dynamics is capable of regulating actin function in a variety of different ways. For instance, actin dynamics could regulate the actin polymerization and depolymerization between G-actin and F-actin in response to developmental signals and environmental abiotic factors. The presence of sufficient amounts of G-actin bundles accompanied by visible F-actin bundles is essential for the dynamics of actin. 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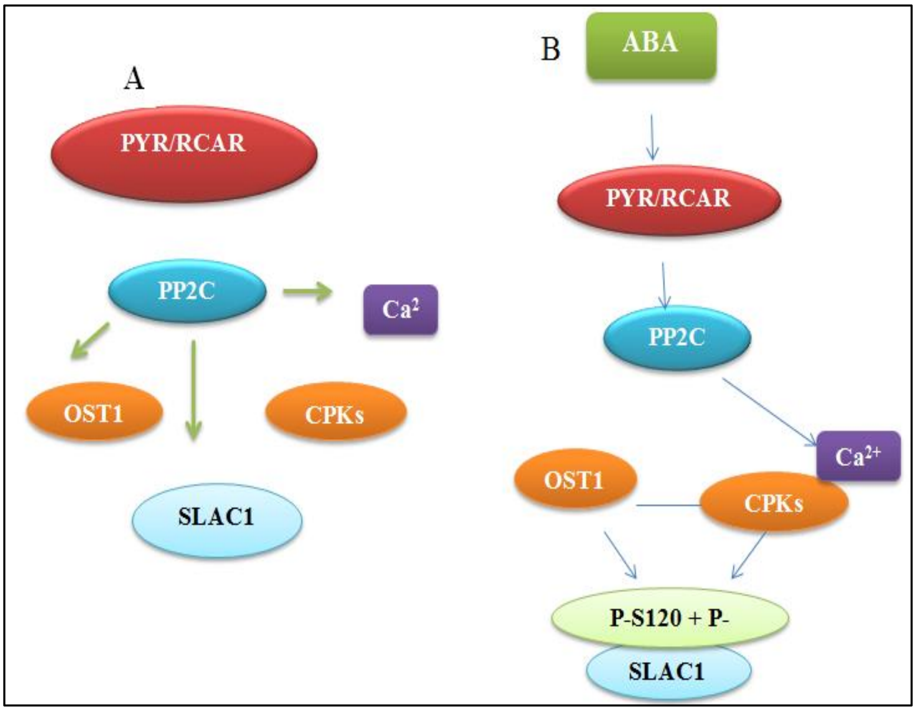

Fig 5: Ca^{2+} specificity mechanism within ABA dependent SLAC1 Activation in guard cells (Brandt, 2015) [45].

Conclusion

A number of studies have been carried out using a combination of salicylic acid and calcium combined with other materials such as silicon, spermidine, and ascorbic acid for the pre-treatment of seeds and treatment during the growth phase. Several studies have shown that salicylic acid has adverse effects on the growth of pea plants. It has been shown that using salicylic acid for the treatment increases the yield of the pea. Additionally, it increases the quality of the flowers and pods as well. Furthermore, in numerous research studies, it has been demonstrated that calcium is an important nutrient that assists in the structure of cell walls and membranes, as well as fruit growth and development and overall fruit quality, as well as providing strength, to the plant which increases its ability to withstand environmental factors.

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