The Influence of Plasmodesmata Number and Opening State on Molecular Transports in Plants

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Abstract—Molecular Communication (MC) studies the transport of information encoded in signaling molecules. To date, its application field is mainly restrained to health-related uses. However, MC in plants has been gaining increasing interest. The primary transport route in plant cell-to-cell communication are Plasmodesmata (PDs), pore-like structures dotting the plant cell wall. PDs opening state is influenced by several environmental damaging factors (i.e., plant viruses), and plant cells try to restore homeostasis through defense mechanisms. In this letter, we seek to depict the complexity of plant-based communication, and we propose a simple model that proves the influence of the PDs number and opening state in the transport of information in plants.

Index Terms—Molecular communication, plasmodesmata, plant modeling

I. In-plant transport of information

First used in 2005, the term Molecular Communication (MC) describes "a new and interdisciplinary research area that spans the nanotechnology, biotechnology, and communication technology" [1]–[3]. Indeed, MC is a communication paradigm aiming to understand, describe, and model communication systems that use molecules as information carriers [4]. Although MC has been historically envisioned as a tool for human health-related applications, it has recently found novel applications in information transmission in plants. On the other hand, several new challenges have yet to be addressed [5]–[9].

Plants are multicellular organisms and, as such, comprise many cells of different types. These cooperate to implement various communication strategies [5]. Such a distinctive feature increases the complexity level of the communication system, so that we urgently need to develop new tools and methods to build models suitable to describe information transmission in complex organisms. For instance, in [7], [10] Awan *et al.* modeled electrical signaling by a voxel-based setting representing the interior of the plant, while Huges *et al.* in [9] modeled plant cell-to-cell communication as a narrow escape problem. Therefore, MC research in plants is scientifically relevant to i) improve our understanding of plant biology by providing new insights into plant physiology and the mechanisms leading plants to respond to external stimuli, ii) build a plant-based environmental monitoring system, and iii) develop new mathematical tools to model complex multicellular systems.

In this context, this work aims to give a critical hint on the complexity of the transmission of information in plants

focusing on the bio-metabolic effects triggered by pathogens (i.e., plant viruses) and proposing a simple but exploitative model describing the effects of metabolic changes on the transports in plants.

The paper is structured as follows. Section II describes the plant cell-to-cell transports and the defensive metabolic response to pathogens. Section III outlines the implemented model and the results. Section IV explains the main take-home message of the work.

II. In plant cell-to cell transports and its defensive mechanism

A. *The plant cell-to-cell communication*

Plants respond to stimuli by taking advantage of a communication system that moves information from the simplest unit to the most complex system, which are the cell and many plants, respectively. The higher the communication level, the more complex the description of the process, as any lower level is involved. In this analysis, with the term cell-to-cell (i.e., intercellular) communication we refer to the communication that occurs between neighboring cells (i.e., intercellularly). In higher plants, this process happens through Plasmodesmata (PDs) (Fig. 1) [11].

Structurally, PDs are complex tunnels in the cell wall that generate cytoplasmic and plasma-membrane continuity between neighboring cells and enable the exchange of informational molecules such as non-cell-autonomous transcription factors, small RNAs, and pathogenic and some non-pathogenic RNAs [11], [12]. The Plasmodesma's (PD) structure was first spotted in early electron micrograph studies that led to a simple consensus model describing PDs as wall-embedded plasma membrane-lined unbranched cylinders containing a central axial component generally termed as Desmotubule (DT) [11], [13], [14]. It is believed that the DT derives from and is continuous with the Endoplasmic Reticulum (ER) (i.e., a membranous cell organelle) of neighboring cells, and the DT and the PD's plasma membrane are connected through spoke-like filamentous strands linked through the globular proteins embedded on both surfaces (Fig. 1). The cylindrical space between the DT and the plasma membrane is referred to as the cytoplasmic annulus or sleeve [11], [13], [14]. The cell-to-cell transfer of information molecules is strictly regulated by DT; indeed, according to the molecular specie, the DT i) allows for the flux of molecules through its internal
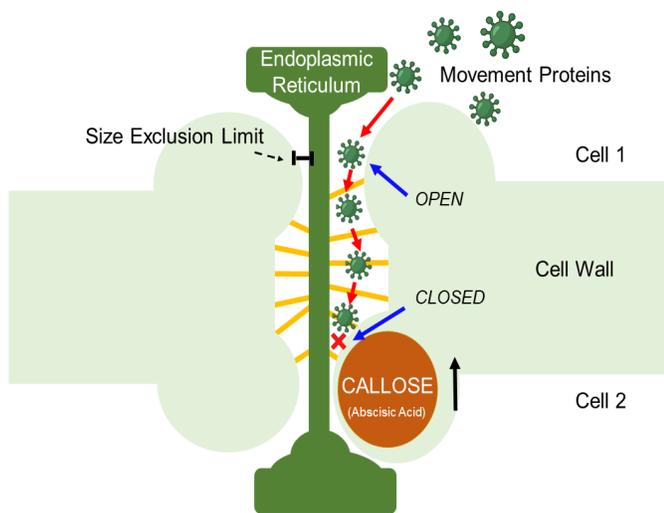
Fig. 1. Scheme of a Plasmodesma (PD), and representation of the regulation mechanisms. The PD's lumen contains an extroflection of the Endoplasmic Reticulum (ER) (in dark green) that regulates the passage of molecules between neighboring cells through spoke-like filamentous strands (dark yellow lines). The Size Exclusion Limit (SEL) changes according to molecular signals. For instance, Movement Proteins (MP) produced by pathogens increase the SEL and OPEN the PD, while the production of phytohormones, such as Abscisic Acid (ABA) in response to stressors, reduces the SEL through the deposition of callose (in brown).

lumen, ii) their diffusion along its membrane, or iii) attaches them to its cytoplasmic side and performs an active transport of the molecules through the PDs' cytoplasmic sleeve [11]. Moreover, the annulus often appears constricted at each end of the PD: these constrictions are regulated to control the flux of molecules through the deposition of callose, a cell wall-related polysaccharide [15]. The regulation of PDs opening depends strictly on the plant response to environmental stimuli as described in Sec. II-B.

B. PDs opening regulation

PDs regulate the passage of molecules in their lumen according to the presence of other molecular species [16]. Indeed, the Size Exclusion Limit (SEL) (Fig. 1) of PDs (i.e., the maximum dimension of particles that can freely move through PD) in resting condition is around 2.5 nm in diameter, although it has been demonstrated that larger molecules and particles are transported cell-to-cell [11], [13], [14], [16]. This phenomenon is possible due to some molecular signals that increase the PD's SEL [16]. Among these, there are some phloematic proteins such as KNOTTED 1 and Movement Proteins (MPs). The latter are specialized viral proteins that increase the SEL allowing the viral genome transport [16]–[18]. Briefly, plant viruses initially infect plant cells through mechanical damage, and spread short-distance through PDs that enlarge because of the viral MPs [19].

Since they use PDs to reach every cell compartment, plants activate defense mechanisms to hamper the viral spreading to other cells. Most plant defense mechanisms are mediated by phytohormones, small organic molecules that respond to environmental stimuli [20]. Among them, Abscisic Acid

R (nm)	D (m^2/s)	**Biological meaning**
10	10^{-11}	Viruses [23]
1	10^{-10}	Proteins, mRNA, siRNA etc. [24], [25]
0.1	10^{-9}	Small solutes

(ABA) is a key endogenous messenger produced in response to biotic and abiotic stressors, such as viruses. High ABA levels regulate the expression of PD proteins involved in callose synthesis/degradation, such as callose synthases (CALS) (i.e., a PD located protein like kinase (PDLP)), and b-1, 3 glucanases (BG). Callose accumulation at PD sides leads to the formation of sphincters that restrict cell-to-cell transports, and thus the passage of MPs and growth promoters. Interestingly, the number of PDs per cell is regulated by the ABA concentration: the long-term effects of high ABA concentration on plant cells is the reduction of the PDs number [21], [22].

III. MODELING THE EFFECTS OF PLASMODESMATA OPENING IN MOLECULES PASSAGE

Sec. II highlights the complexity of the phenomena underpinning the intercellular transport mechanisms mediated by PDs and their regulation. These are summarized as follows:

- PDs are a sort of membrane pores whose lumen, and the SEL, is regulated by different molecules;
- viruses and other pathogens transport molecular factors (i.e., MPs) that enlarge the PDs lumen. This allows them spread cell-to-cell;
- ABA counteracts the viral spread by tightening the PDs' lumen and decreasing their number.

Unfortunately, these mechanisms and their quantitative dynamic relation in time are still unclear in biology. Therefore, in this letter we model the whole effect of these phenomena on plant cell-to-cell transport mechanisms focusing on classes of molecules of different size. Specifically, we assigned a given dimension to the different classes of molecules (Tab. I) that undergo cell-to-cell transport, as described in Sec. II. Moreover, we consider changes in the dimension of PDs' lumen, and in the number of PDs to define the effects of viruses (i.e., the increase of the SEL) and the activation of the defense system (i.e., the decrese of the SEL and the reduced number of PDs). Although these phenomena are time-variant, in this paper we do not take into account time changes, as they are not fully characterized in plant biology. In this letter, we do not refer to a specific molecule type, but the aim is to give a hint about the behavior of different species. For this reason, variations in the diffusion coefficient of particles are also considered to determine how species of different sizes are differently affected by the same phenomenon.

A. The model

The model we propose considers a cell-to-cell movement of molecules by free diffusion. The choice of such simple modeling tool relies upon two main reasons. First, the complexity of the cell *milieu* due to the presence of organelles, cell cytoskeleton, vesicles, etc., is usually simplified in literature through pure diffusion because of the inability to simulate it in a different way. Second, pure diffusion is one of the main transport mechanisms through PDs, as reported in Sec. II-A [11]. In this simple scenario, the dependency of molecules size was approximated in the diffusion coefficient D using the equation (see Tab. I)

$$D = \frac{k_B T}{6\pi\eta R}, \qquad (1)$$

where $k_B = 1.38 \times 10^{-23}$ J K^{-1} is the Boltzman constant, T is the temperature of the cell environment in K, η is the dynamic viscosity of the fluid that is for cell *milieau* 2×10^{-3} kg m s^{-1}, and R is the cell radius in m [26], [27]. Of note, η is obtained from literature by experimental quantification in eukaryotic (mammalian) cells [28]

The simulation of diffusion phenomenon was implemented through particle-based stochastic simulations. A 3-Dimensional (3D) system composed of four cells (i.e., Transmitter (Cell 1), Receiver 1 (Cell 2), Receiver 2 (Cell 3), and
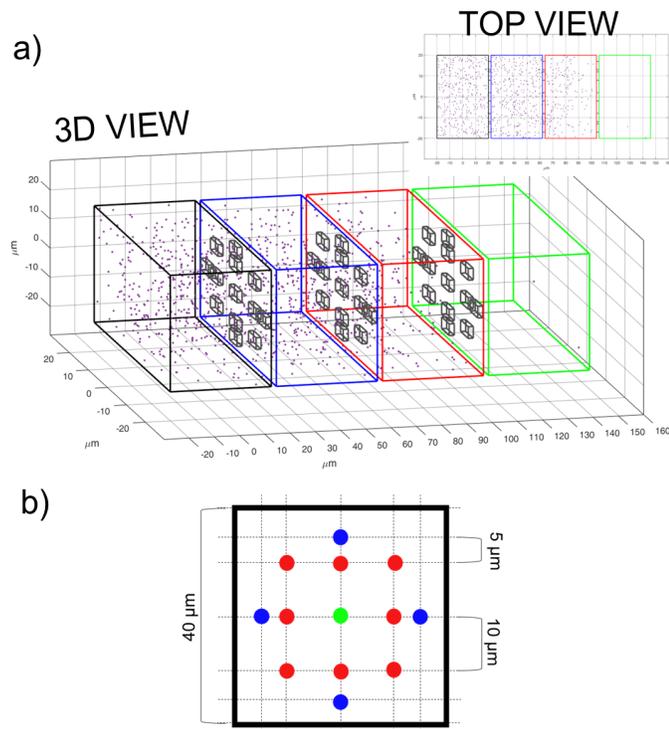

Fig. 2. a) Visual representation of the simulated scenario; molecules (orange dots) move from the transmitter cells (black cell) to the receivers cells (blue, red, and green cells) through PDs (yellow boxes). b) Schematic representation of the position of the PDs in the cell wall. For $PD_{number} = 1$, only the green PD is present. For $PD_{number} = 9$, the green and the blue PDs are present. For $PD_{number} = 13$, all the PDs are present.

TABLE II
VALUE FOR SYSTEM PARAMETERS

Variable	Definition	Value
Q	Number of Molecules	10^3
T	Simulation Time	10 s
dt	Time discretization	10^{-4} s
D	Diffusion Coefficient	$10^{-9,-10,-11}$ m^2 s^{-1}
D_{side}	Cell edge length	4×10^{-6} m
$D_{thickness}$	Cell-to-cell distance	2×10^{-6} m
PD_{side}	PD side length	$4 \times 10^{-6,-7,-8,-9}$ m
PD_{number}	Number of PDs	$1, 9, 13$

Receiver 3 (Cell 4), see Fig. 2a) with cubic sections and edge length D_{side} is considered. Cells are separated by the cell wall with thickness equal to $D_{thickness}$. At $t = 0$, a number Q of molecules is instantaneously released at the center of the Transmitter. Molecules positions evolve in time through purely random Brownian motion according to the equation

$$x(t + \Delta t) = x(t) + \delta(\Delta t), \qquad (2)$$

where x represents one of the 3D space variables, t is the time instant, Δt is the time discretization, and $\delta(\Delta t) = \mathcal{N}(0, 2D\Delta t)$ [26]. Molecules freely move inside the cells whose boundaries (i.e., the cell wall) act as a reflecting surface except in correspondence of PDs. PDs centers are modelled as cubes with an edge equal to PD_{side} located as in Fig. 2b. The PDs disposition and number changes according to PD_{number}. Every simulation is run for 10 times for $T = 10$ s and the number of molecules per time step is mediated. The simulation parameters are collected in Tab. II.

B. Simulation results

As mentioned in Sec. III-A, particle simulations were run to determine the influence of i) PDs' diameter, ii) PDs' number, and iii) D on their cell-to-cell movement. Fig. 2a shows a representative visualization of the simulation results (Simulation parameters: $D = 10^{-9}$ m^2 s^{-1}, $PD_{number} = 13$, $PD_{side} = 4$ μm). Molecules move from the first cell (i.e., the transmitter) to the others through the PDs (i.e., the cubic holes). As depicted, the presence of the cell wall hinders the free-movement of molecules, such that there are only two molecules that reach the last receiving cell in the short term simulation. This finding supports the protective role of the cell wall against pathogens' passage. To substantiate this finding (see Fig. 3a), simulations were run 10 times (see Sec. III-A), and the mean number of molecules per time step in each cell was obtained i) in the absence, and ii) in the presence of cell wall and wide PDs. The absence of the cell wall results in an equal number of molecules per cell in all four cells, while the presence of the cell wall hinders the molecule passage, such that only between the transmitter and the first receiver cell there is a balance of the quantity of molecules. Of note, since we i) used a pure diffusive model, ii) considered
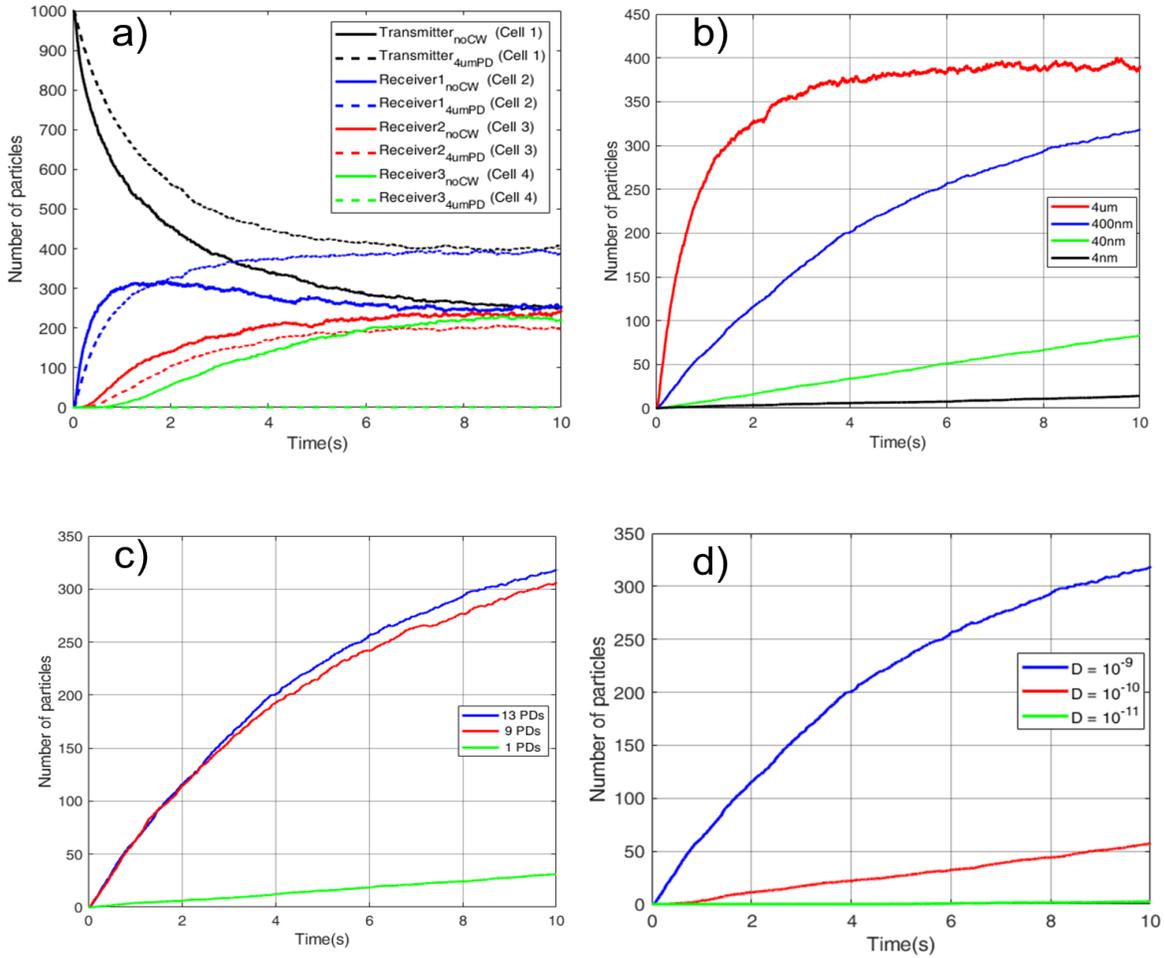
Fig. 3. a) Variation of the number of molecules per cells (i.e., transmitter and receivers) in full permeable conditions (i.e., no presence of cell wall, solid line), vs $PD_{\text{number}} = 13$ (i.e., dashed line) with $PD_{\text{side}} = 4$ μm, $D = 10^{-9}$ m^2 s^{-1}, $Q = 1000$, $t = 10$ s. b) Effects of PDs diameter on molecules diffusion. $D = 10^{-9}$ m^2 s^{-1} , $PD_{\text{number}} = 13$, $t = 10$ s, $Q = 1000$, receiver cell 1. c) Effects of PDs number on molecules diffusion. $D = 10^{-9}$ m^2 s^{-1} , $PD_{\text{side}} = 400$ nm, $t = 10$ s, $Q = 1000$, receiver cell 1 . d) Effects of the diffusion coefficient on molecules diffusion. $PD_{\text{side}} = 400$ nm, $PD_{\text{number}} = 13$, $t = 10$ s, $Q = 1000$, receiver cell 1 .

a constant molecules amount, and iii) did not account for dynamic changes in PDs lumen in time, while simulating $t \to \infty$ means the achievement of an equilibrium in which all the cells contain the same quantity of molecule. This means that the proposed scenario represents well short time intervals (~ 10 s), that correspond to the time of invariance of i) the PDs size, and ii) the concentration of molecules.

We investigated the role of PDs' opening state as well. In Fig. 3b the dependency of molecules transport on the PD_{side} is reported. The graph shows that for $PD_{\text{side}} = 4$ μm or 400 nm, the passage of molecules is favored, while for lower length it is hindered. In nature, PDs have a maximum diameter of 40 nm that is related to the presence of MPs or phloematic protein, while in the resting condition and in the presence of activation of defense mechanisms the PDs diameter is in the order of nm (see Sec. II-B). From the data, we can speculate that the cell-to-cell flow of molecules takes longer than the simulated time. Nevertheless, the intracellular movement of molecules may be mediated by active transport.

These findings are consistent with the existing literature where the movement of molecules through PDs relies on different transport mechanisms including diffusion, active transports, and flow [11], [16]–[18], [29].

In addition to the PDs' opening state, also the number of PDs influences the passage of molecules (see Fig. 3c). From the data, it is apparent that the higher the number of PDs, the faster the molecular flow. It is worth noting that the reliability of the data can be improved by simulating the exact number and frequency of PDs according to [30], [31].

Finally, we explored the dependency of D on molecular intercellular communication. In Fig. 3d it is reported how molecules with a $D = 10^{-11}$ m^2 s^{-1} are not transported in the simulation times. This means that the transport of bigger molecules is even further hindered by the presence of PDs, and that the dynamic of transport takes longer than the simulated time. This finding points out the inherent limitation of particle-based simulation, which is the high computational cost. Of note, to obtain reliable results, model parallelization is needed.

IV. CONCLUDING REMARKS

This letter aimed to show the incredible complexity of nature and how a simple attempt to model it could provide interesting parallelism with nature, but also the sharp difference with the actual phenomena. Indeed, the lack of experimental data hampered us from quantitatively comparing our results with the real scenario. Nevertheless, we believe that one of the most important preliminary steps to be taken before a simulation is to acquire the best possible knowledge of the biological phenomenon, either physiological or pathological, and establish the limits of one's own model.

In the case examined in this work, the opening state of PDs and their number rely on several environmental factors and plant mechanisms. Unfortunately, their modeling is only carried out through extreme simplification. Nevertheless, the numerical results herein discussed show the role of PDs in hindering/favoring the cell-to-cell transport of molecules. Taking advantage of a simple computational model, this work tackles the influence of several parameters on the transport of molecules in plants and gives biological significance to computational parameters.

Future work will address the intrinsic limitation of particle-based simulations (i.e., the computational costs, and their relation with the short simulation time), and increase the reliability of the model. In this context, i) other transport mechanisms along the PDs could be taken into account [29], ii) a more reliable and complex shape of the PDs will be considered, and iii) an effective diffusion constant [9] should be used to mimic the presence of PDs as a narrow escape problem in an analytical model.

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