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RESEARCH PROGRESS ON CALCIUM ION IN GAMETOPHYTIC SELF-INCOMPATIBILITY

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ABSTRACT

Calcium ions are involved in plant self-incompatibility response as important signaling substances in cells. In the sporophytic self-incompatibility response, Ca^{2+} enters the stigma papilla cells and plays a key role in inhibiting incompatible pollen tube growth. In the gametophytic self-incompatibility reaction of Papaveraceae, the female determinants in the style (*PrsS*) and the male determinants in the pollen (*PrpS*) recognize each other, promote extracellular Ca^{2+} influx into the incompatible pollen tube, destroy the calcium ion gradient at the tip of the pollen tube, and inhibit the pollen tube growth. In the S-RNase-based Rosaceae gametophytic self-incompatibility response, it is still unclear how the S-RNase interacts with the male determinant and how the S-RNase specifically degrades the RNA in the pollen tube. Therefore, we reviewed the research progress on the role of Ca^{2+} in self-incompatibility and, based on our research results, proposed a role model of Ca^{2+} as a signal substance in the gametophyte self-incompatibility response in Rosaceae.

Key words: calcium; pollen tube; gametophytic self-incompatibility; sporophytic self-incompatibility

ABP	actin binding protein
ACA13	autoinhibited Ca^{2+}-ATPase13
CaM	calmodulin
CBL	calcineurin B-like protein
CDPK	calcium-dependent protein kinase
CIPK	CBL-interacting protein kinase
CML	CaM-like
CRT	calreticulin gene
GLR	glutamate receptor-like channel
GSI	gametophytic self-incompatibility
MAPK	mitogen-activated protein kinase
PrpS	*Papaver rhoeas* stigma *S* determinant
PrsS	*Papaver rhoeas* stigma *S*
ROS	reactive oxygen species
SCR	S-locus cysteine-rich protein
SFB/SLF	S-haplotype-specific F-box/S-locus F-box
SI	self-incompatibility
SP11	S-locus protein 11
SRK	S-locus receptor kinase
SSI	sporophytic self-incompatibility

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INTRODUCTION

Self-incompatibility (SI) is a genetic mechanism to prevent the self-fertilization of flowering plants. SI response of flowering plants is a mechanism controlled by multiple alleles to avoid self-pollination (Lawrence et al. 1978). Conventionally, self-incompatibility was classified into two types, gametophytic self-incompatibility (GSI) and sporophytic self-incompatibility (SSI), based on modes of genetic control of pollen SI phenotype. In GSI, the haploid pollen itself determines S-specificity (e.g., in Papaveraceae and Solanaceae); by contrast, in SSI, the genotype of diploid donor tissues (e.g., anther tapetum in Brassicaceae) determines pollen S-specificity (Fujii et al. 2016).

GSI, as a common SI response system, is widely involved in the interaction between pollen and pistil cells (Franklin-Tong & Franklin 2003). The present research results on SI suggest two mechanisms. One is found in Papaveraceae, where the S protein secreted by the pistil (pistil *S*-determinant) works as a transmembrane receptor to interact with the incompatible pollen S protein (pollen *S*-determinant), triggering a Ca^{2+}-dependent signal network, which leads to the growth inhibition of homologous pollen tubes, changes in the cytoskeleton structure of the actin, and the programmed death of pollen tubes. In this case, the pollen tube cannot grow into the ovule to complete fertilization (Rudd et al. 2003; Jiang et al. 2014). The other GSI mechanism is in Solanaceae, Scrophulariaceae, and Rosaceae, where SI is controlled by pistil S-gene encoded S-RNase and pollen determinant S-haplotype-specific F-box/S-locus F-box (SFB/SLF) proteins. Incompatible and compatible pollen can be recognized by the interaction between the pistil determinant and the pollen determinant. In case of incompatibility, S-RNase would degrade pollen RNA to prevent self-fertilization (Wang et al. 2009; Eaves et al. 2014). Although many research results have been obtained on the male determinant SLF, it is not completely clear how S-RNase specificity is perceived on the pollen side (Bedinger et al. 2017). Therefore, some studies suggest that there may be other factors involved in SI (Bedinger et al. 2017). In the recent studies of the SI of gametophyte in *Pyrus pyrifolia*, the latest findings confirm that phospholipases C and D are also involved in the SI response (Qu et al. 2017; Chen et al. 2018). According to our results, calcium is also involved in the SI reaction process of pears (*Pyrus pyrifolia*). In both of these mechanisms, Ca^{2+} is indispensable in the signal transduction of pollen and pistil recognition as well as the growth of pollen tubes (McClure & Franklin-Tong 2006; Qu et al. 2016). Therefore, we review the role of calcium ions in the SI of gametophytes and hope to provide new research ideas for S-RNase-based GSI.

Ca^{2+} is involved in pollen germination and directional growth of pollen tube

The effects of Ca^{2+} on pollen tube growth were investigated in a large number of studies, and it was found that Ca^{2+} was critical to pollen germination and pollen tube growth in vitro (Brewbaker & Kwack 1963; Steinhorst & Kudla 2013a). The Ca^{2+} gradient accumulation was observed for the first time in the tip of the lily (*Lilium longiflorum* 'Arai') pollen tube (Jaffe et al. 1975). It has been well proved that a typical Ca^{2+} gradient is present at the tip of all growing pollen tubes; otherwise, the pollen tube cannot grow normally (Feijó et al. 2001). The Ca^{2+} gradient in the cytoplasm of the pollen tube could not only affect the elongation of the pollen tube, but it also affects its growth direction and changes the orientation of the pollen tube tip to make it grow toward the part with high Ca^{2+} concentration (Malhó & Trewavas 1996). Cytosolic-free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) was present in the cytoplasm of the pollen tube, and the important messenger in the free Ca^{2+} signal network could regulate the directional elongation of the pollen tube (Guan et al. 2013). After the hydration of pollen grains, an obvious Ca^{2+} gradient was observed in the protruding part of the pollen tube (Iwano et al. 2004).

The presence of Ca^{2+} gradient was conducive to controlling the directional secretion, transporting and fusion of the Golgi vesicles, and constantly forming

new tube wall and plasmalemma. In the formation of plasmalemma, fracture points were caused in the cell wall, making the plasmalemma loose and realizing the opening of the Ca^{2+} channel, which in turn promoted the Ca^{2+} flow and vesicles fusion, beneficial to the continuous growth of pollen tube (Feijó et al. 2001; Gao et al. 2019). The inflow part of Ca^{2+} and the highest concentration part in the Ca^{2+} gradient determined the growth direction of the pollen tube (Malhó et al. 1995; Malhó & Trewavas 1996). Pollen tube growth was maintained through the deposition of the original cell wall materials at the apex. Once deposited on the tip, the wall would experience a hardening mature process, resulting in a viscosity/elasticity gradient between the growth tip and the nonelongating tube (Hepler et al. 2013; Cosgrove 2016).

Ca^{2+} is involved in pistil-stamen recognition and induces the growth of pollen tube

Successful pollination in angiosperms is achieved with the attachment of the pollen grains on the pistil stigma, then the germination of the pollen grains, the rapid elongation of the pollen tube in the style, and finally, the sperm cells are delivered to the ovule to complete the fertilization. Pollen tubes formed a tubular structure through polar growth (Qin & Yang 2011). Pollen germination was regulated by the interaction between pollen and stigma papilla cells. The pollen tube had a directional growth through the style, with Ca^{2+} involved in the whole process of growing into ovule and fertilization (Chen et al. 2015). A large amount of Ca^{2+} was observed at the attachment site for pollen grains on the surface of the stigma papilla cells, and a large amount of Ca^{2+} could be also observed in the style-transmitting tissues on the stigma surface, with the same direction as pollen germination and elongation of pollen tube tip (Iwano et al. 2004). The S protein secreted from the stigma of the Papaveraceae after pollination with incompatible pollen would interact with its homologous pollen's S receptor, causing a rapid increase in the cytoplasmic free Ca^{2+} ($[Ca^{2+}]_i$) in the pollen tube. The extracellular Ca^{2+} inflow disrupted the Ca^{2+} gradient at the tip of the pollen tube, so that the tip growth would be inhibited (Fig. 1A). However, for compatible pollen, no significant changes in free Ca^{2+} concentration were observed (Franklin-Tong et al. 1993; Takayama & Isogai 2005; McClure & Franklin-Tong 2006; Chen et al. 2015; Lin et al. 2015) (Fig. 1A).

For Cruciferae, the compatible pollen coat contained the substances that could promote the output of Ca^{2+} from the pistil papilla cells. The attachment of mastoid cells to pollen coat could induce a Ca^{2+} signal pathway. In the signal transduction process, the Ca^{2+} pump was used to maintain a moderate level of Ca^{2+} in the cytoplasm (Patergnani et al. 2011). In stigmas after compatible pollination and treated with compatible pollen coat, it was found that the transcription of autoinhibited Ca^{2+}-ATPase13 (ACA13) in the papilla cells was increased, and ACA13 was mainly located in the plasmalemma and vesicles and accumulated at the penetration site of the pollen tube. Then the ACA13 protein would output Ca^{2+} to the compatible pollen tube (Fig. 1B). If ACA13 expression at the stigma was affected and the output of pistil Ca^{2+} was decreased, the growth of compatible pollen tube would be also affected, failing to be fertilized normally (Iwano et al. 2014). A haplotype-specific interaction between S-locus protein 11/S-locus cysteine-rich protein (SP11/SCR) and S-locus receptor kinase (SRK) triggers the SSI response in the Brassicaceae that leads to incompatible pollen rejection (Sehgal & Singh 2018). Through signal transduction studies, self-pollination specifically induced an increase in cytoplasmic Ca^{2+} ($[Ca^{2+}]_{cyt}$) in papilla cells and suggested that Ca^{2+} influx mediated by a glutamate receptor-like channel (GLR) is the essential SI response leading to incompatible pollen rejection (Iwano et al. 2015) (Fig. 1B). Although the mechanism of inhibiting pollen tube growth is different between GSI and SSI, genetic boundaries between GSI and SSI may not be so rigid. Even so, there are convincing reports of the presence of a cryptic GSI system operating alongside the SSI system in the Brassicaceae and Asteraceae (Brennan et al. 2003). Therefore, we believe that calcium is involved in the recognition of pollen and stigma, which is necessary for S-RNase-based GSI response.

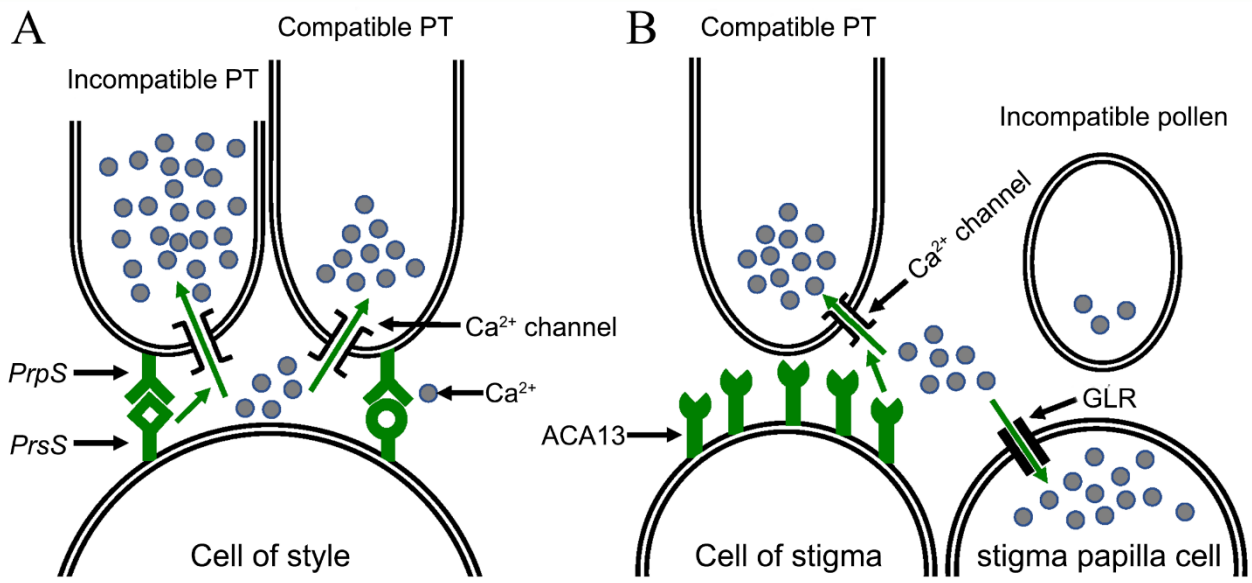


Figure 1. Schematic diagram of the role of calcium ions in self-incompatibility
(A) The role of Ca^{2+} in gametophytic self-incompatibility (GSI) in Papaveraceae. The incompatible pollen tube influxes a lot of extracellular Ca^{2+}, which destroys the calcium ion gradient at the tip of the pollen tube. However, the Ca^{2+} gradient at the tip of the compatible pollen tube was not affected. The green solid arrow is Ca^{2+} flow direction. PT – pollen tube; *PrpS* – *P. rhoeas* pollen *S* determinant; *PrsS* – *P. rhoeas* stigma *S*. (B) The role of Ca^{2+} in the sporophytic self-incompatibility (SSI) in the Brassicaceae. ACA13 promotes extracellular Ca^{2+} into the compatible pollen tube. When incompatible pollination occurs, GLR regulates extracellular Ca^{2+} into stigma papilla cells. ACA13 – autoinhibited Ca^{2+}-ATPase13; GLR – glutamate receptor-like channel

Ca^{2+} is involved in signal transduction in pollen tube growth

As a kind of ubiquitous second messenger, Ca^{2+} signals are involved in most signal processes and all aspects of cell life (Edel et al. 2017). It played a key role in the reproductive process of flowering plants. The interaction and recognition between pollen and pistils depended on the changes in Ca^{2+} concentration and distribution at the tip of the pollen tube (Steinhorst & Kudla 2013a; Gu et al. 2015). The calcium signal-sensing protein acted as a signal molecule to control the Ca^{2+} gradient and regulate the growth of the pollen tube. The intracellular Ca^{2+} signal is decoded and transmitted by a set of Ca^{2+} binding proteins that transmit this information to downstream reactions (Kudla et al. 2010; Bagur & Hajnóczky 2017). The complex structure of Ca^{2+}-sensing proteins provided binding sites for Ca^{2+} and provided a reliable mechanism for complex and flexible signal processes (Gu et al. 2015; Demidchik et al. 2018).

Various Ca^{2+}-related proteins play a crucial role in the growth of pollen tube tips. The calcium binding EF-hand superfamily was the most prominent among them (Konrad et al. 2011; Steinhorst & Kudla 2013b). Ca^{2+} sensory proteins regulating pollen tube growth included calmodulin (CaM), CaM-like (CML), calcium-dependent protein kinase (CDPK), and calcineurin B-like protein (CBL) (Zhou et al. 2009; Hashimoto & Kudla 2011; Steinhorst & Kudla 2013b). However, the detailed regulatory mechanism of Ca^{2+} sensory proteins is not yet clear. Ca^{2+} regulated the production of reactive oxygen species (ROS). CaM can promote Ca^{2+} inflow through the plasmalemma, increase the level of ROS at the tip, and stabilize actin filaments (Shang et al. 2005); CDPK phosphorylation and dephosphorylation could regulate the substrate activity (Cheng et al. 2002), and CBL often interacted with CBL-interacting protein kinases (CIPKs) to form a complex to regulate Ca signals (Weinl & Kudla 2009). During the SI response in apple, CBL may interact with S-RNase in the pollen tube cells to affect the combination of CBL and CIPK and destroy the signal transduction in the pollen tube, thus inhibiting the growth of the pollen tube. CBL was not only a signal response protein but also a feedback signal molecule that can regulate Ca^{2+} concentration (Gu et al. 2015).

In the SI response of *Papaver rhoeas*, the inflow of Ca^{2+} into the pollen tube was a key step in the early response. The cascade reaction of the calcium signal was helpful for Ca^{2+} to rapidly respond and achieve consistency with the SI response receptor and acts as a signal molecule for interaction with S proteins (Franklin-Tong et al. 2002). The S protein at an incompatible stigma interacted with the pollen S receptor in *Papaver rhoeas*, and then in some way, the pollen S receptor triggered the inflow of extracellular Ca^{2+} and the Ca^{2+} release in the cell compartment through the plasmalemma channel. Ca^{2+} inflow increased the concentration of free Ca^{2+} in the cytoplasm (Franklin-Tong et al. 1997). The increase in Ca^{2+}, as a second messenger, causes a series of downstream events, such as changes in cytoskeleton structure, ROS increase, and phosphorylation of mitogen-activated protein kinase (MAPK), so that the growth of the pollen tube was inhibited and was unable to complete normal fertilization (Eaves et al. 2014). Caruso et al. (2012) hypothesized that the proteins regulated by the Asp-rich encoding genes in *Citrus clementina* 'Comune' functioned as Ca^{2+}-trap elements, which could lead to a distinct decrease in Ca^{2+} availability. Even if the concentration of Ca^{2+} in the cytoplasm increased, the degree of the cascade reaction caused thereof decreased, and the Ca^{2+} concentration gradient required for the elongation of the pollen tube was changed (Caruso et al. 2012). In the SI reaction of the gametophyte of pear, self-S-RNase also can destroy the calcium gradient at the tip of the pollen tube, but inhibit the calcium influx, thereby arresting pollen tube growth (Qu et al. 2016).

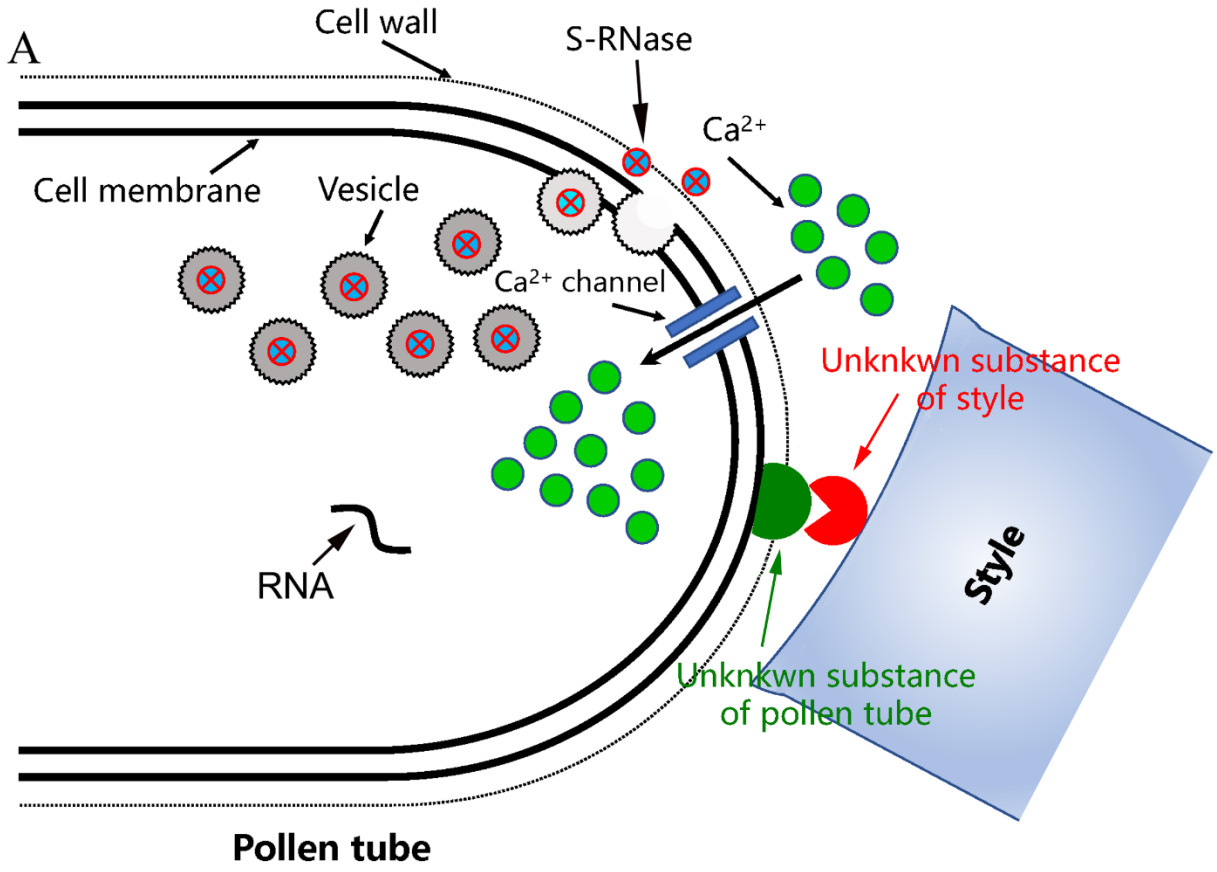
Ca^{2+} is involved in the stabilization of the Ca^{2+} gradient at the tip of the pollen tube

A tip-focused calcium gradient is an essential requirement for pollen tube growth. Both Ca^{2+} channel inhibitors and Ca^{2+} chelating agents could inhibit the normal growth of the pollen tube (Qu et al. 2007). Too high or too low concentration showed adverse effects on pollen germination and pollen tube growth, indicating that suitable Ca^{2+} concentration in the cytoplasm shall be maintained for the normal growth of the pollen tube (Guan et al. 2013; Gao et al. 2019). The free Ca^{2+} was distributed in a gradient manner at the tip of the pollen tube, and the factors leading to the disappearance of the Ca^{2+} gradient would cause the pollen tube to stop growing (Wu et al. 2011). A large number of Ca^{2+} influx channels may be present in the cells at the tip of a polar growing plant cell. The electrophysiological study on the plasmalemma of the pollen tube showed that hyperpolarization could activate the Ca^{2+} influx channel. With the increase of hyperpolarization activity of the plant's plasmalemma, these hyperpolarized Ca^{2+} channels would play an important role in Ca^{2+} transport (Qu et al. 2007). The Ca^{2+} in plant cells was maintained at a very low level; otherwise, it was easy to interact with plasmalemma and karyolemma to form phosphorylated precipitates. Ca^{2+} was constantly pumped out of cells or pumped into the cell compartment, maintaining the plasmalemma-specific Ca^{2+} concentration gradient or forming transient Ca^{2+} changes (Chen et al. 2015).

Extracellular Ca^{2+} inflow was essential for maintaining the polar growth of pollen tubes. The high gradient of plasmalemma was inseparable from an efficient Ca^{2+} transport system and effective signaling mechanism (Iwano et al. 2009; Qu et al. 2012). Activation of the Ca^{2+} influx channel on the cell surface or in organelle could promote Ca^{2+} inflow and rapidly increase intracellular Ca^{2+} concentration. Even minor changes in Ca^{2+} concentration were enough to make the related enzymes sense the response regulation of downstream receptors, thus regulating a series of cellular responses (Chen et al. 2015). Intracellular calcium also played a key role in regulating actin-binding proteins (ABPs) and pollen tube growth (Staiger et al. 2010; Qu et al. 2015). The cytoplasmic Ca^{2+} gradient at the tip of pollen tube was closely related to the growth rate and morphology of elongated pollen tubes (Steinhorst & Kudla 2013b). Although ABPs were evenly distributed in the whole pollen tube cytoplasm, their activity still shall be regulated by the concentration of Ca^{2+} in the cell, and the concentration difference would affect the assembly of actin in each area of the pollen tube. Therefore, the appearance of a Ca^{2+} steady state was important for maintaining the structure and function of actin; otherwise, the tip of the pollen tube could not continue to elongate.

The expression of the calreticulin gene (CRT) was necessary for maintaining the Ca^{2+} gradient as well as proper actin cytoskeleton structure and functions (Suwińska et al. 2017). However, ABP was involved in the functional mechanism of CRT in the pollen tube growth, which was required to form actin filaments at the pollen tube stem and actin kinetics at the tube tip. In addition, most of the ABP was regulated by Ca^{2+} (Fu 2010; Staiger et al. 2010; Qu et al. 2015; Hepler & Winship 2015).

In conclusion, it is believed from the existing research results that the mechanism of Ca^{2+} in SI for fruit trees among the genus Rosaceae is as follows: pollen and stigma recognize each other after pollination (Li et al. 2020). In the case of compatible pollen, the activity of the Ca^{2+} channel at the tip of the pollen tube is not affected, and a normal Ca^{2+} concentration gradient is maintained at the tip, where the vesicles are fused with the plasmalemma to form more plasmalemma. The release of precursors for the cell wall could lead to the elongation of the pollen tube (de Win et al. 1999), and the S-RNase enveloped by the vesicles would be discharged out of the cells (Fig. 2A). However, for the incompatible pollen, the pollen tube germinates on the stigma, but some unknown substance in the style, which may be S-RNase, reduces the activity of the calcium channel at the tip of the pollen tube, inhibits the inflow of extracellular Ca^{2+}, destroys the Ca^{2+} concentration gradient, and ruptures the vesicles in the pollen tube. S-RNase is released from vesicles and degrades RNA as a cytotoxin when accumulated above a threshold concentration in the pollen tube (Qu et al. 2017) (Fig. 2B). Related studies have shown that Ca^{2+} in the incompatible pollen tube is also involved in mediating other downstream events, such as programmed cell death in cells at the pollen tube tip (Franklin-Tong & Franklin 2003; Wang et al. 2010). In recent years, great progress has been made in the understanding of Ca^{2+} in pollen, with great achievements in research on SI. However, the specific regulatory mechanism of Ca^{2+} in SI is still unclear. Further studies on Ca^{2+} channel regulation and Ca^{2+} signal regulation network would help to further understand the mechanism of SI.



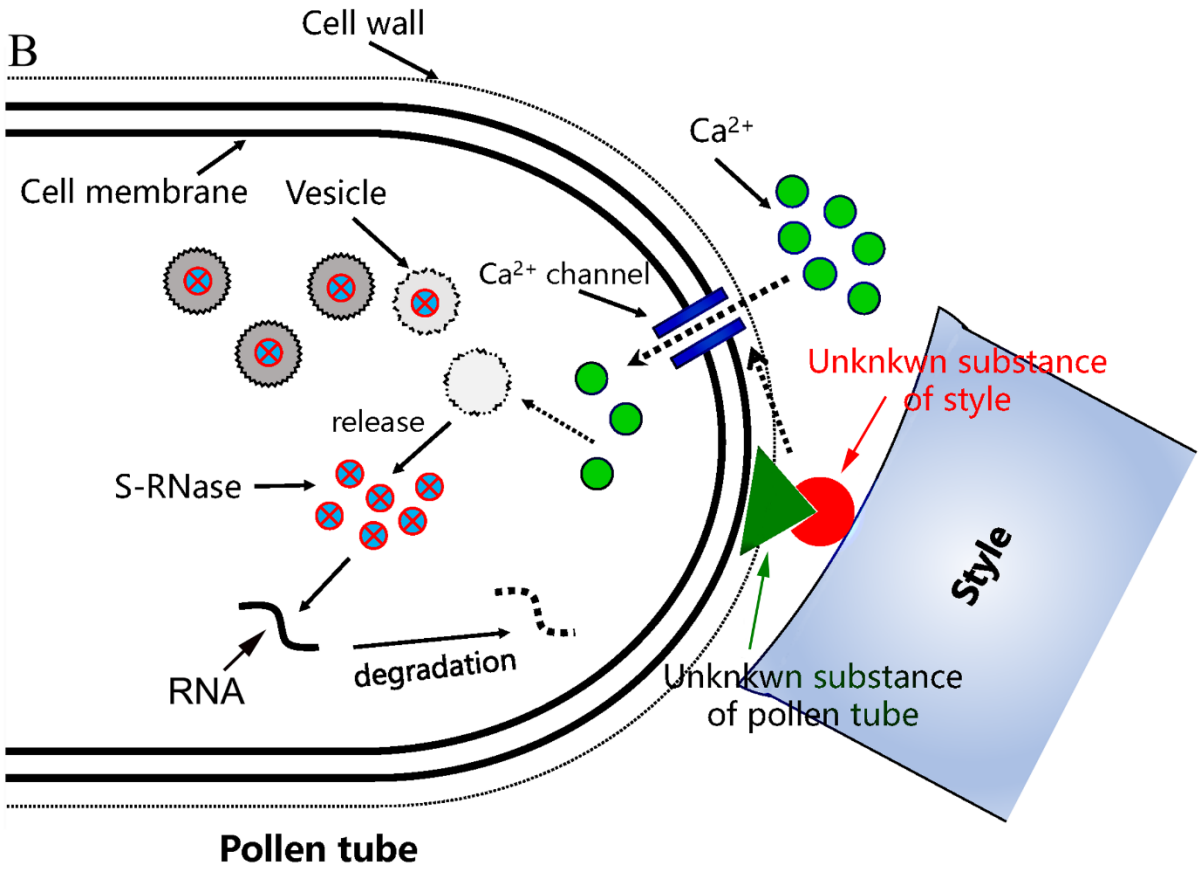


Figure 2. Schematic diagram of calcium ion signal transduction process in S-RNase-based gametophytic self-incompatibility response (A) When compatible pollination occurs, the Ca^{2+} gradient in the pollen tube is not affected and promotes the transport of the vesicles to the tip. These vesicles contain S-RNase, and when the vesicles are fused with the tip cell membrane, the S-RNase is discharged outside the pollen tube so that the RNA cannot be degraded. (B) When incompatible pollination occurs, the activity of the tip Ca^{2+} channel in the pollen tube is inhibited and the tip Ca^{2+} gradient is destroyed. When the Ca^{2+} gradient is disrupted, the vesicles cannot be transported to the tip and rupture within the pollen tube, releasing S-RNase, which degrades the RNA when the concentration of S-RNase accumulates in the pollen tube to a certain level

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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