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Revisiting pollen-pistil interaction and cross incompatibility in maize

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ABSTRACT

The review addressed aspects of plant fertilisation and the phenomenon of genetic cross-incompatibility in maize controlled by the *Gametophyte1* locus. This phenomenon determines the failure to accomplish successful fertilisation and a full seed set when pollen grains carrying the *ga1* allele pollinate female inflorescences carrying the *Ga1-strong* (*Ga-1s*) allele in the homozygous state (*Ga1-s/Ga1-s*). We divided the review work into several topics — first, the introduction of sexual plant reproduction. Second, pollen-pistil interactions in plants. Third, reproductive barriers during plant reproduction. Third, Incompatibility in plants. Fourth, fine mapping of the *Ga1* locus in maize. Fifth, recent researches on *Ga1*-related cross-incompatibility in maize.

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1. Introduction

In plants, the new generation derives from the union of the male gametes, carried by the pollen grain and the egg cell in the embryo sac. The process begins with the deposition of the pollen grain onto the female stigmatic tissue. If a positive pollen-stigma interaction occurs, the pollen grain hydrates and germinates a pollen tube. During this germination process, the pollen tube tip grows to establish the directional growth, and pollen tube elongation proceeds very fast (Krichevsky et al., 2007). In general, plant pollination following the four steps: 1) pollen recognition by stigma; 2) pollen germination within the stigma; 3) pollen tube growth in the pistil until reach the ovule through the micropyle; 4) pollen tube of sperm in the embryo capsular release and double fertilisation.

Female pistil provides guidance cues and essential nutrients to the appropriate pollen to support pollen tube growth but, at the same time, the pistil represents a physical barrier protecting the ovules from being fertilised by inappropriate pollen, especially pollen from other species. It is now clear that appropriate favourable interactions between the pollen tube and the female floral tissues need to be established for successful fertilisation to occur. In the past 20 years, a large number of gene products and chemical compounds involved in pollen tube growth and guidance have been identified in several model species (Higashiyama and Hamamura, 2008; Higashiyama, 2010; Lausser et al., 2010). However, the molecular still unknown. Mutant analysis and the detailed characterisation of their causal genes are crucial for the elucidation of this fundamental biological phenomenon. But not many mutants controlling pollen-pistil interaction well-known in crop species with the notable exception of those genes controlling self-incompatibility (Sanchez et al., 2004).

In a male, pollen coming from the microsporogenesis before pollination. After meiosis, microsporocytes generate a tetrad of four haploid microspores encased in a callose wall. Afterwards, the callose wall disappears, the microspores grow, exine and the outer pollen wall synthesised. Second, the microspore undergoes asymmetrical division to produce one vegetative cell and two of the generative cells. At this stage, pollen grain secretes intine, the cellulosic and pectic pollen inner wall. In many other plant species, such as petunia and tomato, the second mitosis takes place only after the germination of the pollen tube (Bedinger, 1992).

A large number of genes controlling pollen development has been identified in several plant species, mainly in maize, tobacco, and *Arabidopsis* (Xu et al., 2002; Engel et al., 2003; Honys and Twell, 2003). During microsporogenesis and pollen development, pollen encodes genes and stored as mRNA-protein complexes (mRNPs, messenger ribonucleoprotein particles) in mature pollen and their translation will postpone until pollen tube growth begins (Hafidh et al., 2011). Furthermore, pollen-specific genes encode for enzymes for cell wall metabolisms, such as pectate lyase, pectinesterase and cytoskeleton proteins, such as actin, profilin, and tubulin. An example of a late pollen gene in maize is *ZmC5*, a pectin methylesterase-like gene involved in pollen tube elongation (Wakeley et al., 1998).

Another class of genes, called "early", is detectable soon after the tetrad stage and its expression declines well before pollen maturity. In a higher plant, pollination and fertilisation involve complex physiological and biochemical networks. The molecular understanding during pollination process is well-described in model species, such as in *Arabidopsis*, *Nicotiana* (tobacco), *Lilium* (lily), and *Brassica oleracea* (broccoli) (Swanson et al., 2004). However, pollen-pistil interactions are still poorly characterised, and so it is essential to address the genetic and molecular mechanisms of the communication between pollen and pistil.

2. Pollen-Pistil Interactions in Plants

The critical point in pollination is how male and female organs communicate with each other. Communication between male and female gametophytes is an intricate process involving physical, chemical, cellular and molecular mechanisms. Before releasing the sperm nuclei into the embryo sac, pollen tube needs to grow through stigma and style, in maize more specifically through the silk, which is an elongated stigma. To date, the pollen-pistil interaction mechanism is not entirely defined, and the causal genes at the bases of the molecular mechanism still unknown. It is not clear whether the signal to sustain the fertilisation process comes from ovules or pistils, and their actual function in the context of incompatibility process remains questioned. Its hypothesised either ovules or silks block the pollen tube growth from entering a female part, or the pollen has the self-degradation-machinery to interrupt the elongation process inside the silks. The intersection part between genetics and physiology is essential to reveal the incompatibility problems in plants.

Pollen-pistil interactions depend on the species, and they show many variations in terms of morphology and the presence of stigmatic exudates. There are two types of stigma: dry and wet, which differ by the presence or absence of a wet sticky secretion, the exudates. Female flowers with wet stigma surfaces, such as *Lilium*, have indiscriminate adhesion that relies only on liquid surface tension (Heslop-Harrison, 1979). In *Arabidopsis* and *Brassica*, the epidermis consists of large differentiated papillae cells which interact directly with the pollen surface. However, in *Brassica*, the impairment of papillae cells inhibit pollen tube growth, while *Arabidopsis* pollen tubes can germinate and grow even in nonfunctional stigmas. Selective pollen adhesion provides an essential opportunity for species-specificity.

In contrast, adhesion in a plant with dry stigmas showed different behaviours (Lord and Russell, 2002). Once released from the anther, the pollen grain contacts with the stigma where it adheres, hydrates and germinates. Selective cell-cell adhesion mediated by transmembrane proteins defined into four groups: selectins, integrins, immunoglobulin, and cadherins. In *Arabidopsis*, adhesion between pollen and stigma occurs within seconds after pollination and highly selective (Zinkl et al., 1999). The hydration of pollen grains in *Arabidopsis* is aided by the coat, which predominantly contains lipases and oleosins (Mayfield et al., 2001).

Moreover, the time between pollen capture and germination is less than 5 minutes in many grass species (Heslop-Harrison, 1979). Germination starts when the pollen grain extrudes a tube from an aperture or thin area in the wall. Afterwards, pollen tubes grow up to 1 cm/hour and deliver the sperm cells into the embryo sac (Lord and Russell, 2002). In general, pollen tube growth is influenced by several factors, such as chemotropic agents (Cheung et al., 1995, 2010), lipids, ions, proteins, and metabolites produced by the pistils (Lind et al., 1994; Wolters-Arts et al., 1998; Park et al., 2000; Holdaway-Clarke and Hepler, 2003; Palanivelu et al., 2003; Sanchez et al., 2004; Hepler et al., 2006). In summary, pollen-pistil interactions are an essential part of the selection process to eliminate undesirable pollen and to ensure that desirable pollen fertilises the female gametophytes.

Incompatible pollination also associated with the accumulation of intermediates of the phenylpropanoid pathway and showed in plant response to pathogens and stress (Elleman and Dickinson, 1999; Lantin et al., 1999; Dubitzky, 2013). In another study, pollination and wounding share a common signal transduction pathway (Lantin et al., 1999; Kim et al., 2003). Several studies addressed the regulation of pollen-pistil interaction, independently to incompatibility and several genes involved have been characterised. Receptor-like kinases (*RLK*) were reported to be involved in pollen tube growth and pollen-pistil interactions (Kim et al., 2003). Kinases, such as those belonging to the CaM-like domain protein kinase (*CDPK*) superfamily, are involved in the pollen signalling pathway, regulating pollen tube extension and growth polarity (Harmon et al., 1987, 2000; Yoon and Dowd, 2006; Escobar-Restrepo et al., 2007). Kinases directly activated by calmodulin/calcium (Yang et al., 2004; Thomas et al., 2008). Many signalling pathways have been identified during pollen germination and pollen tube growth (Bock et al., 2006; Krichevsky et al., 2007). Ca^{2+} (Cytosolic free calcium) is a critical element in the regulation of pollen tube growth and guidance. The calcium gradient marked by a high concentration at the tip, and a low concentration in the sub-apical and basal parts of the pollen tube. Ca^{2+}gradient disruption leads to the inhibition of pollen tube growth. Calmodulin is a Ca^{2+} sensor for stimulating pollen tube growth. Pollen tube growth was accelerated in styles when exogenous calmodulin was injected and inhibited when anti-calmodulin serum was injected (Harmon et al., 1987, 2000). Phosphoinositides and phospholipids are known to be involved in molecular signalling. D-myoinositol-1,4,5-trisphosphate (*IP3*) modulates Ca2+ levels, and Phosphatidylinositol-4,5-bisphosphate (*PIP2*) act in a common pathway with Rac GTPases. Both of *IP3* and *PIP2* can modify the pollen tube growth and reorientation of the axis growth (Franklin-Tong and Franklin, 2003; Malhó et al., 2006; Dresselhaus and Franklin-Tong, 2013). GTPases are small GTP-binding proteins belonging to the Ras superfamily. In plants, Rho GTPases annotated as *ROP* (Rho-related GTPase from plants) and crucial for pollen tube elongation (Zheng and Yang, 2000; Yang, 2002). In maize, *ROP2*, *ROP8*, and *ROP9* highly expressed in mature pollen. A reverse genetic approach concluded that *ROP2* protein has an essential role in male gametophytes function (Arthur et al., 2003).

Besides, phosphatidic acid and phosphatase protein, also known as lipid transport, are involved in lipid metabolism, while pyruvate kinase-like protein and putative α-L-arabinofuranosidase protein are involved in carbohydrates metabolism during pollination (Malhó et al., 2006). The results suggest that many genes up-regulated during pollination/fertilisation are also involved in defence responses. For examples, LTP (lipid transfer protein), thionin, S-like RNase protein precursors, isoflavone reductase-like proteins, salt-induced proteins, Pathogenesis-Related (PR) proteins. Proteomic approaches revealed that in rice, a PR class 10 protein (*OsPR-10*) which also salt-induced and found in the fungal-rice interactions. Arabinogalactan (AGPs) proteins in tobacco (Cheung et al., 1995) or γ-aminobutyric acid (GABA) in *Arabidopsis* are localised in pistils and guide the pollen tube to deliver the sperm cells into the embryo sac for fertilisation (Lord and Russell, 2002). In *Arabidopsis*, the pollen-pistil interaction 2 (*pop2*) mutant, is impaired in its capacity to grow at in vivo and in vitro when GABA is presence. *Pop2* encodes a transaminase involved in the degradation of GABA (Palanivelu et al., 2003).

3. Reproductive Barrier in Plants

The barrier determining reproductive isolation derives both from environmental and genetic factors. The effects of the environment are easier to understand, and it is not surprising that temperature and humidity play crucial roles. Again different species and different genotypes within the species respond differently to the environment, resulting in different overall fitness (Kakani et al., 2005; Zinn et al., 2010; Snider and Oosterhuis, 2011). In contrast, genetic reproductive barrier mainly concerns with incompatibility, which is a complex system. There are two kinds of the reproductive barrier, those that act before fertilisation (pre-zygotic barrier) and those that act after fertilisation (post-zygotic barrier). The pre-zygotic barrier appears to be somehow more complicated than a post-zygotic barrier, due to the complexity of the process behind the male-female communications and signalling before fertilisation occurs. An example of the pre-zygotic barrier was in *Arabidopsis* where the semi-sterile female gametophytic mutant, the so-called Feronia, showed disruption of pollen-pistil interactions: when the pollen tube reaches the synergid, the pollen tube tips are not able to release the sperm cells (Huck, 2003).

Moreover, in the *maa1* and *maa3 Arabidopsis* mutants, where female gametophytes development delayed, pollen tubes elongate in random directions and lost their way to the micropyle (Shimizu and Okada, 2000). Differently, although post-zygotic barrier prevents the new generation from developing, the fertilised ovule can be saved by using in vitro technology. An artificial method, such as embryo rescue, microdissection, and tissue culture techniques have been developed to overcome the post-zygotic barrier and rescue the imminent abortion from oat x maize and *Arabidopsis* (Riera-Lizarazu et al., 1996; Clarke et al., 2006).

Besides, the fertilisation process does not always succeed. In several species, growing in contact with related species, mechanisms are at play to avoid inter-specific fertilisation. Interspecific incompatibility acts in the pistils, where alien pollen grains rejected. Here, two main paradigms exist for explaining interspecific cross-incompatibility: incompatibility and incongruity (Hogenboom, 1975). Incompatibility happens from the inhibiting action of the incompatibility genes and makes the reproductive relationship nonfunctional. Differently, incongruity does not act by an active rejection of the alien pollen but is primarily a passive process in which non-functionality is due to a lack of genetic information from one of the partners. For example, the pollen-carrying a/- alleles cannot pollinate the *A/A* pistil, in this case, the relationship is incongruous. Incongruent between male and female exists when there is a specific barrier in gametophyte organs. Species that evolved new barrier mechanisms exert selection pressure on the male partners to contain new penetrative measures and evolve in isolation from one another. Barriers to interspecific crosses that are late-acting, those that do not inhibit pollen tube growth immediately, are more likely incongruity action as well. These mechanisms are common in crop species and overcoming them is a prerequisite for utilising wild species germplasms (Heslop-Harrison, 1979).

Pollen-pistil interactions control the plant sexual reproduction mechanism in angiosperms. To avoid unwanted fertilisation, plants build reproductive isolation with self-incompatibility or cross-incompatibility systems. In general, incompatibility comprises self-incompatibility (SI) and cross-incompatibility (CI) mechanisms. SI, which both mostly act as a pre-zygotic barrier, such as pollen tubes growth arrest or pollen tubes failure to penetrate the ovules. A post-zygotic barrier happens when ovules cannot develop after fertilisation, the endosperm growth-arrested, or seeds show reduced viability. CI prevents cross-fertilisation between different populations within the same species. However, the genetic basis behind cross-incompatibility remains questioned, particularly in the pre-zygotic barrier. However, considering physiological and biochemical aspects, the signatures where the pollen tubes growth arrests are clear in CI pre-zygotic barrier. Most pollen tubes never reach the base of style, showing severe abnormality in morphology, such as twisted tubes. Also, heavy callose deposition at the tip of pollen tubes growth is typical cellular signature during the CI process. Currently, the genetic and molecular basis behind the SI system well studied in the past decade (Franklin-Tong and Franklin, 2003; Chantha et al., 2013; Miao et al., 2013; Li and Chetelat, 2014). In contrast, the CI system remains questioned (Demerec, 1929; Kermicle, 1950, 2006; Kermicle and Evans, 2005; Kermicle et al., 2006; Lu et al., 2014).

4. Incompatibility in Plants

Self-incompatibility (SI) is common in plants and 30 out of 227 taxa show self-incompatibility (Igic and Kohn, 2006). SI is a genetically controlled process that results in the recognition and rejection of self, self-related pollen, and pollen tubes (De Nettancourt, 1997). In SI, pollen can reach the stigma of the same plant or another plant with the same incompatible allele, but pollen germination, pollen tube growth, fertilisation, or embryo development are blocked.

Based on the type of plant family (McCubbin and Kao, 2000), there are three models for SI events, such as Solanaceae, Papaveraceae, and Brassicaceae. In Solanaceae, *S*-haplotype specificity becomes the main player in SI response, while in Papaveraceae, *S*-gene controls female function during SI. In the case of Brassicaceae, *SLG, SRK, SCR/SP11* control the *S*-haplotype specificity.

In the SI system, there are two mechanisms involved in the incompatibility event. In sporophytic self-incompatibility (SSI), the pollen tube growth unable to penetrate the stigmatic surface and the compatibility determined by the S-haplotypes of the diploid sporophyte acting as the pollen parent.

In contrast, the gametophytic system of incompatibility (GSI) prevents pollen tube growth inside the style, which leads to pollen tube burst and the compatibility determined by the haploid genotype of the male gametophytes. Pollen rejected when its S-locus matches the S-alleles in the diploid pistils (Kumar and McClure, 2010). The molecular aspects of both SSI and GSI studied in several plant species (Franklin-Tong and Franklin, 2003; Kao and Tsukamoto, 2004). For example, a style-specific receptor-like kinase (SRK) involved in the recognition process of the *Brassica* SI system (Stein et al., 1991). In general, the pistil organ contains genes of mate selection that control gene flow during pollination (Bedinger et al., 2017).

Cross-incompatibility is one type of reproductive barrier that restricts gene flow between divergent populations within the same species. There are two types of CI mechanisms, the first occurs before fertilisation (pre-zygotic barrier) and the second mechanism takes place after fertilisation (post-zygotic barrier) when the development of the young embryo is stopping (Matsubara et al., 2003). *Gametophyte factors* (*Ga*), the presence of which has been reported in many species such as maize (Nelson, 1952, 1996; Jiménez and Nelson, 1965), lima bean (Allard, 1963), barley (Tabata, 1961), rice (Iwata, 1964), and tomato (Rick, 1971), are critical genetic factors shown to be involved in cross-incompatibility. The phenomenon of cross-incompatibility mainly studied in maize, because of its relevance in the production of hybrids. In fact, in maize, several different *Ga* loci and other loci have been identified among which the best characterised are *Gametophyte factors1* (*Ga1*), *Gametophyte factor2* (*Ga2*), and *Teosinte crossing barrier-1* (*Tcb1*) (Figure 1). All systems based on the presence of different alleles, whose interplay determines seed set failure and therefore classified as CI. The *Ga1* locus mainly studied because of the discovery of a dominant allele, named *Ga1-s*, which determines complete CI in female homozygous *Ga1-s/Ga1-s* when pollinated by ga1 pollen. CI based on *Ga1* was used in popcorn varieties to prevent pollination by nearby dent corn (Nelson, 1996). *Ga2* identified from maize genetic stocks based on transmission ratio distortion (Kermicle and Evans, 2010).

Similarly, *Teosinte crossing barrier1* (*Tcb1*) was identified in wild teosinte populations and prevented seed set from *tcb1* pollen, while allowing fertilisation by pollen carrying *Tcb1*. The study of CI in *Ga1* and *Tcb1* led to the hypothesis that the crossing barriers based on incongruity instead of active rejection (Kermicle and Evans, 2005). This hypothesis, therefore, invokes the incongruity model and not an *S*-locus system of active rejection for CI in maize. However, the crosses between different CI systems are not equivalent because the crosses between *Ga2* is not equivalent to *Ga1* or *Tcb1* system and vice versa because they do not have the identical genes (Evans and Kermicle, 2001; Kermicle and Evans, 2010).

The different growth responses of the pollen tube in *Ga1, Ga2,* and *Tcb1* during CI were characterised (Lu et al., 2014). In the *Ga1-s* system, pollen tubes showed misdirection of growth and intense, uneven callose deposition. In the *Ga2-s* system, a third defect was observed, with the majority of the pollen tubes showing a twisted callose plugin lateral positions. In the *Tcb1-s* system, pollen tubes had stronger callose staining, suggesting a thicker callose layer on the cell wall, but the pollen tube morphology was normal. In summary, the CI systems in maize suggest different biochemical pathways and indicate different mechanisms of the arrest of pollen tube growth.

The unilateral cross-incompatibility (UCI) system of *Gametophyte factors1* (*Ga1*) is probably the best studied in maize (Kermicle and Evans, 2010). The CI prevent sexual transmission of sperm cells by arresting pollen tube growth and therefore, it is an example of a pre-zygotic barrier (Dresselhaus et al., 2011). Briefly, in the *Ga1* locus, three allelic variants were discovered: *Gametophyte factor1-strong (Ga1-s), Gametophyte factor1-male (Ga1-m)*, and *ga1* alleles (Jiménez and Nelson, 1965). Specifically, the *Ga1-s* allele provides pollen grains with the characteristics of being universal pollinators, with the capacity to accomplish fertilisation independently on the allele(s) present in the female, whereas plants homozygous for the *ga1* allele (recessive) are unable to fertilise homozygous *Ga1-s* plants (Figure 1). In this system, females carrying *Ga1-s* in the homozygous state are selective against *ga1* pollen. On the contrary, females carrying the *ga1* allele at the homozygous state are universal acceptors, since a complete seed set is accomplished independently on the *Ga1* allele carried by the pollen. In heterozygous plant (*Ga1-s/ga1* or *ga1/Ga1-s*) pollen carrying the *Ga1-s* allele shows a competitive advantage over *ga1* pollen (Schwartz, 1950). Differently, *Ga1-m* behaves both as a universal pollen recipient and also as a universal pollinator (Nelson, 1952; House and Nelson, 1958). Uniquely, the way to distinguish between genotypes comes from crossing approach because of no significant biological appearance within genotypes. In addition to *Ga1* and *Ga2*, other gametophyte factors loci have been described and loosely mapped in maize. *Ga* loci have been reported on chromosome 1 (*Ga4* and *Ga6*), 3 (*Ga7*), 5 (*Ga2* and *Ga10*), 7 (*Ga3*) and 9 (*Ga8*) (Longley, 1961; Wang et al., 2012). The *Ga1* locus firstly mapped on chromosome 4, based on the observation of segregating distortion of the linked locus *sugary1*, in crosses between sweet (*ga1/ga1 su1/su1*) and popcorn (*Ga1-s/Ga1-s Su1/Su1*) maize (Mangelsdorf and Jones, 1926). *Ga1-s* alleles have been widely studied in the incompatibility systems in maize to identify the crossing barrier between the different alleles and used for improvement for a breeding program in many crops.

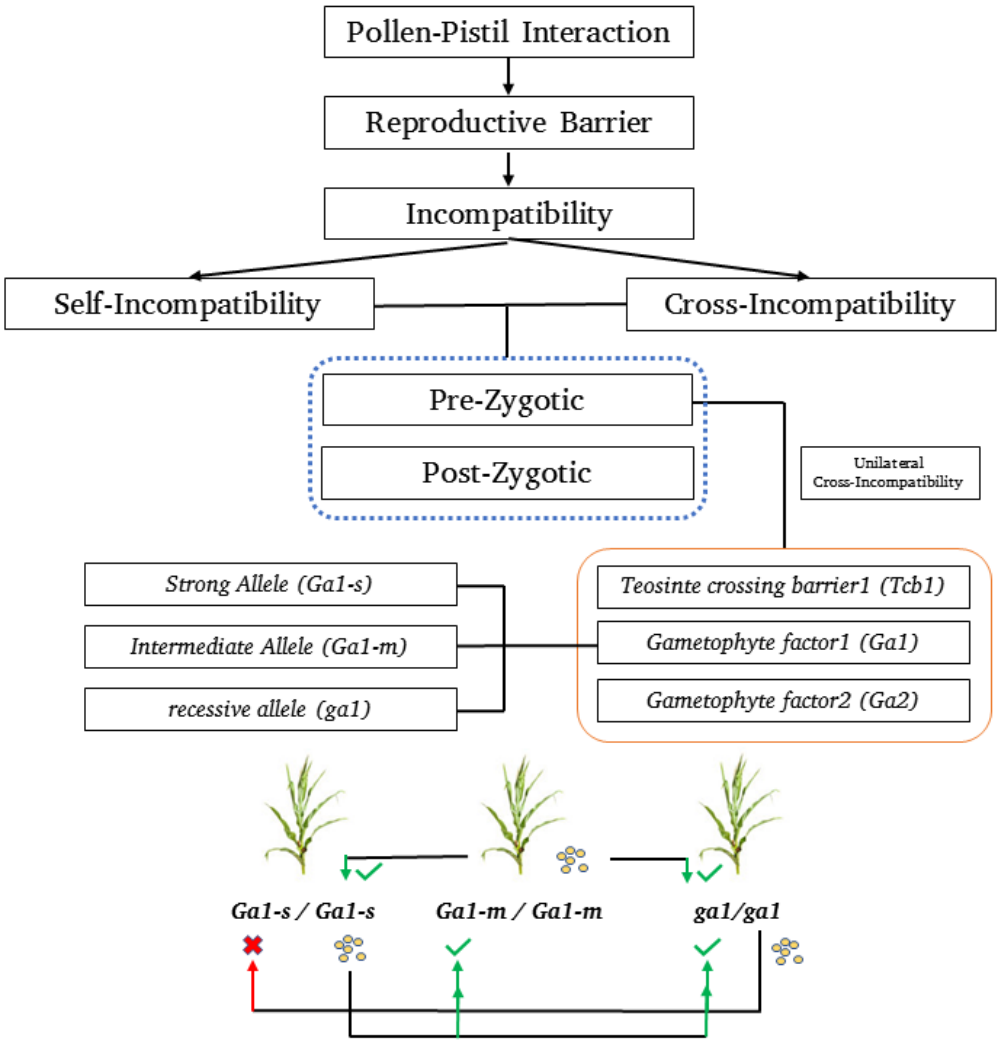

Fig. 1. Illustration of Cross-incompatibility Mechanism in Maize. The *ga1* allele as a recessive allele cannot pollinate the *Ga1-s* allele.

Many popcorn inbred lines carrying the *Ga1-s* alleles can fertilise dent and flint maize lines (*ga1*). However, the reciprocal crosses are unsuccessful. The *Ga1* trait can be used in the future as a reproductive barrier among different maize lines to prevent the maize plants from unwanted pollination, such as Genetic Modified (GM) plants (Scott et al., 2019).

5. Fine Mapping of *Ga1* locus

Classical genetic mapping allowed to place *Ga1* on the genetic linkage map on chromosome 4 in loose linkage with *su1* (Mangelsdorf and Jones, 1926). More recently (Woriedh et al., 2013; Lu et al., 2019; Ma et al., 2019), the position of *Ga1* refined using molecular markers. *Ga1* gene was found to have homology with a gene coding for a pectinesterase (Pectin Methylesterase/*PME*). This observation reinforces the hypothesis of this being *Ga1* because in the literature PME was shown to be involved in pollen tube growth in other species, such as *Arabidopsis*, tobacco (*Nicotiana tabacum*), and lily (*Lilium*) (Bosch and Hepler, 2005; Bosch et al., 2005; Tian et al., 2006).

The genomic localisation of *Ga1* locus improved by combining the results coming from different experiments. One experiment refers to the analysis of segregation distortion and the identification of a quantitative trait locus (QTL) based on B73xHP301 recombinant-inbred lines (RIL) (Bloom and Holland, 2012). *Ga1* locus was shown to overlap to a 2.2 Mbp interval on chromosome 4, based on the maize B73 RefGen_v2 reference, containing 13 predicted genes and many genes of unknown homology (Bloom and Holland, 2012). Also, based on BC1F1 populations, created from SDGa25 popcorn lines, and by using map-based cloning, the *Ga1* locus was positioned between the SD3 and SD12 markers identifying, spanning approximately 2 Mbp on the maize B73 RefGen_v2 reference (Zhang et al., 2012). Liu et al. (2014) used a Chinese popcorn inbred line carrying either *Ga1-s* or the *Ga1-m* alleles (Liu, Unpublished data), the ones used to mapped *Ga1* between markers SD3 and SD12 in previous work (Zhang et al., 2012). The authors identified a physical distance of about 2 Mbp based on the B73 RefGen_v2 sequence (Schnable et al., 2009). As for *Ga1-s*, the locus was mapped in a 100 kb region between markers dCS1 and ID7, identifying three candidate genes *GRMZM2G027021*, *AC204382.3_FG010*, and *GRMZM2G039983*. The *Ga1-m* region was fine mapped using these newly developed markers based between SNP markers 13-4 and 25-5. The physical distance was about 246 Kbp based, resulting in three candidate genes *GRMZM2G419836*, *GRMZM2G027021* and

GRMZM2G039983, two of which the same as for *Ga1-s* (Liu et al., 2014). *GRMZM2G419836*'s product is a member of the thioredoxin superfamily. *GRMZM2G027021* is a GTP-binding protein involved in pollen tube growth. *GRMZM2G039983* has homology with WDL1 of *Arabidopsis*, which is a microtubule-associated protein. All three genes might be involved in pollen-pistil interactions. In order to isolate the *Ga1-m* gene, an SDG25 BAC library was constructed and screened. The primers used in screening the BAC library were primers for the fine mapping. The positive recombinant BAC clones covering the whole mapping interval were identified and sequenced. However, this candidate gene was obtained based on the sequence derived from B73, which carries only the *ga1* allele. In another study (Emery, 2015), six predicted genes and two transposable elements absent in *Ga1-m* haplotype but found in B73 (*ga1*) genotype.

From the above, it is clear why the characterisation of both pectinesterase/*PMEs* is of great interest to the unravelling of the *Ga1*-mediated CI phenomenon (Woriedh et al., 2013; Lu et al., 2019; Ma et al., 2019). *PME*s belong to a large and highly evolutionary conserved gene family, and the corresponding enzymes produced on bacteria, fungi and plants (Markovic and Janecek, 2004; Pelloux et al., 2007). In plants, pectinesterases are involved in both vegetative and reproductive phases, such as root, fruit, and pollen development (Wen et al., 1999; Tian et al., 2006). In *Arabidopsis* (Bosch and Hepler, 2005; Tian et al., 2006), *PME* was shown to be involved in pollen tube growth and was expressed in female gametophytes in maize (Wakeley et al., 1998; Woriedh et al., 2013; Lauter et al., 2017; Lu et al., 2019; Ma et al., 2019).

Moreover, *PME* expressed as a stress-specific response gene. Several defence responses induced after recognition of biotic or abiotic stresses via various signal transduction mechanisms and *PME*s translocated to the plant cell wall, where they de-methylesterify homogalacturonan (HGA, here pectin). *PME* is pH-sensitive and modulated by medium alkalinization, as responses in some plant-pathogen interactions or by acidification, as a result of increases in levels of H30+, a secondary product of PME activity (Pelloux et al., 2007).

6. Recent Reports on *Ga1*-related CI

Li et al. (2014) performed transcriptomics studies in maize pistils of an inbred line carrying the *Ga1-s* allele by using Digital Gene Expression-tag profiling. The study showed 1,378 differentially expressed genes (DEGs) of which 737 up-regulated, and 641 down-regulated when comparing maize Ga125 line (*Ga1-s/Ga1-s*) + W22 line (*ga1*♂) and W22 (*ga1/ga1*♀) + Ga25 (*Ga1-s*♂). DEGs classified in the catabolic process, translations, carbohydrate metabolic, and structural molecule activity and significantly enriched during pollination at 5 hours after pollination (HAP). Based on this study, it is suggested that the inhibition of the remodelling structure of the transmitting tract and the pollen tube tip is the *Ga1-s* style way to prevent *ga1* pollen from reaching the ovules (Li et al., 2014).

Moreover, a similar study based on proteomics of *Ga1* in maize near-isogenic line (NIL) of W22 carrying either the *Ga1-s* or the *ga1* allele found that the proteins involved in hydrolase activity, nucleic acid binding and nucleotide-binding (Yu et al., 2014). The identified proteins are related to stress-specific responses as well as plant defence responses, suggesting the activation of plant defence mechanisms during *Ga1*-mediated CI. By using functional classification of fragments expressed, about 31% contains genes encoding of unknown proteins, 21% stress-response factors related, and 20% are genes which involved in RNA processing, modification, translational, ribosomal structure, and biogenesis. In summary, defence-related genes, such as genes coding for ATP binding proteins, heat shock proteins, and proteins with chaperone activity, are activated during plant-pathogen interactions as well as in response to both compatible and incompatible pollination. In another study, Huffman (2017) found that *Ga1-s* pollen expressing fewer proteins and haplotype-specific proteins than *ga1* and 24 proteins also in concordance with Yu et al. (2014), but only five of them showed identical expression patterns.

In QTL study using Recombinant Inbred Lines (RILs) from (B73xKy21) and (B73xM162w) carried by Shrestha (2016), *Ga1-s* behave differently on rejecting the *ga1* pollen with a combination of B73 allele but not for Ky21 allele, while with the latter RILs populations, *Ga1-s* showed the effectivity of *ga1* pollen rejection. Another study in W22 populations carrying *Ga1-s* and *ga1* allele (Lauter et al., 2017), *ZmPME3* was identified as a silk-specific gene which lacks in *ga1* genotypes. In *Ga2-s* case from two genotypes (Wang et al., 2018), 511L (*Ga2-s*) and B73 (*ga2*) lines showed the consistent result with other studies that reveal pollen tube elongation disorder during pollination. Also, *PME* genes were differentially expressed between two genotypes both in silks and pollen.

Revilla et al. (2018) showed that *Ga1-s* allele could protect another allele (*sh2* - *shrunken2*) from pollen contamination on sweet corn populations (EP2013-09, EP2013-11, EP2013-12, EP2013-13, EP2013-18). However, the agronomic performance of hybrids needs to be improved. Hurst et al. (2019) described the GWAS research on popcorn populations carrying *Ga1-s* and *ga1* alleles. As a result, some SNPs and genes associated with *PME* and Calcium-binding proteins (*GRMZM2G157241*) found in *Ga1* locus on chromosome 4 in B73 RefGenV3. In another study about *Tcb1* in W22 and B73 backcrossing populations (Lu et al., 2019), a pistil-expressed *PME* homolog gene which is a probable female barrier gene (*Tcb1-female*). These findings found quite similar results that *PME* genes could be a key factor during the pollination.

7. Conclusion

The molecular mechanism controlling pollen-pistil interactions is a crucial phase of sexual plant reproduction with critical outcomes in terms of plant fitness in general, and plant success as a crop, since most of the crop products are the direct or indirect results of successful sexual reproduction. In this scenario, mutants affecting pollen-pistil interaction are not only of interest per se but may play a fundamental role as genetic tools to produce knowledge and models of action. The cross-incompatibility phenomenon in maize and the genes controlling it has all these features, and a better understanding of it might have a significant impact

on plant scientist to comprehend and control both successful fertilisation and incompatible ones. Here, we described the summary of researches regarding *Ga1* locus in maize and showed that many studies related to cross incompatibility are in a similar agreement, which there are a probable pistil or pollen barrier genes that control and become a selective pressure from one population to another in maize populations. To date, the exploration of *PME* genes in maize populations carrying *Ga1* locus become more attractive due to similar findings on *Ga1* research in maize.

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Conflicts of interests

The authors declare that this research has no conflict of interests.

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