CONILON
Coffee

3rd Edition
Updated and expanded




The Coffea canephora produced in Brazil

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Self-incompatibility and Sustainable Production of Conilon Coffee

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1 INTRODUCTION

Coffea canephora and the other diploid species known in the genus *Coffea* show self-incompatibility of the gametophytic type (CONAGIN, MENDES, 1961), unlike *Coffea arabica*, *C. anthonyi* and *C. heterocalyx*, which have the ability to self-pollination, that is, they are self-compatible (STOFFELEN et al., 2009; NOWAK et al., 2011).

Incompatibility is the name of the failure of certain crosses to produce offspring or the inability to self-fertilize (ALLARD, 1971). This physiological mechanism, with genetic basis, presents cell-to-cell interactions, between pollen and pistil, that prevent the pollen grain from germinating on the stigma of the same plant or another one with a similar genotypic constitution (LEWIS, 1954; GIRANTON et al., 1999; BRUCKNER et al., 2005).

Lundqvist (1964) and De Nettancourt (1977, 2000) define self-incompatibility as the inability of a hermaphrodite fertile plant to produce zygotes after self-pollination as a consequence of inhibition of pollen grain germination or pollen tube growth.

It is estimated that more than half of the angiosperm species present some type of self-incompatibility, including several of economic interest, such as plum, cocoa, coffee, sunflower, apple, passion fruit, brassica, crotalaria, some santalaceae, among others (RICHARDS, 1986). About 60% of the angiosperms are self-incompatible (DE NETTANCOURT, 2000; IGIC; LANDE; KOHN, 2008).

According to Conagin and Mendes (1961), the first reference to this phenomenon in plants of the genus *Coffea* was credited to Von Faber in 1910, who awoke to the accomplishment of different observations and subsequent studies. In *C. canephora*, self-incompatibility is of the gametophytic type, with monogenic inheritance, with expression controlled by multiple alleles of the S gene. The main consequences of this reproductive system characteristic are: formation of highly heterozygous populations with high genetic variability; absence of self-fertilization and depressive effects of endogamy; no fertilization between flowers of the same plant; and no development of fruits of related parents crosses (CONAGIN; MENDES, 1961). This system presents three main types of pollination: i) totally incompatible when both alleles are common; ii) partially compatible, where only one allele is common, then half of the pollen grains enter

the stigma and style, performing fertilization while the rest is inhibited, usually in the style; and iii) fully compatible, with all four different alleles (LEWIS, 1954).

As far as commercial production is concerned, the productive capacity of conilon coffee depends on the compatibility of the genotypes used in planting. In order to guarantee greater efficiency of pollination, it is of fundamental importance to use in the plantations a combination of different genotypes with great diversity of compatible alleles (FERRÃO et al., 2012).

This chapter discusses the theoretical knowledge of the self-incompatibility in its different aspects, in order to elucidate questions related to this system in the culture improvement and in the use of the recommended cultivars.

2 SELF-INCOMPATIBILITY

The plants reproduce sexually and asexually. It is understood by sexual reproduction the one in which occurs the union of the male gamete with the feminine in order to originate the zygote and produce viable descendants (KARASAWA et al., 2009).

The flower contains the reproductive structures. Of its component parts, sepals, petals, stamen and pistil, only the last two work producing gametes. Generally, the stamen consists of a filament, which supports the anther and contains the pollen grains (Figure 1). The pistil consists of an enlarged structure at the base, called the ovary, which contains the ovule, a thin tube-like extension called the style, and the stigma on which the pollen grains are deposited. In the ovary, the ovules are found, which give rise to the seeds after the fertilization process (ALLARD, 1971).

Male gametes formation occur by microsporogenesis, and female gametes by macrosporogenesis, (ALLARD, 1971). The formation of a viable zygote in angiosperms depends on pollination and fertilization (FU; YANG, 2014) and is a prime factor in producing seeds. Pollination consists of the transfer of pollen grains from the anther to the stigma and fertilization comprises the fusion of the male and female gametes in the ovary. The first key point of this complex fertilization process is the pollen-pistil interaction referring to a cellular and molecular interaction between haploid pollen and diploid stigma. The specific steps of this interaction are as follows: before effective contact, the still pollen is transferred by natural forces such as wind or insects. After the pollen grains deposition on the stigma surface,

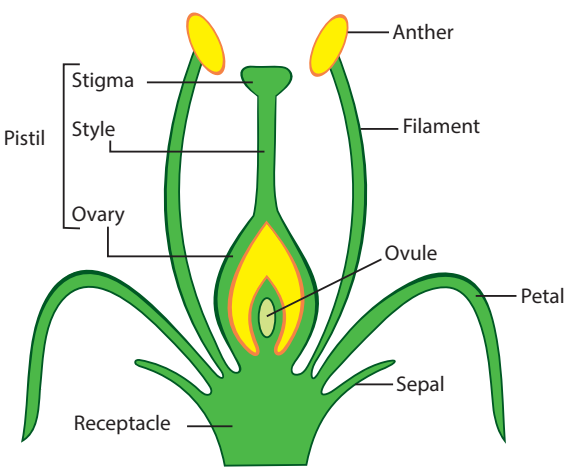

Figure 1. Basic structure of a true angiosperm flower, with anther, filament, stigma, style, ovary, pistil, petal, sepal and receptacle.

Source: Allard (1971).

recognition begins at molecular and cellular levels. Finding favorable environment condition the pollen germinates and then, the pollen tube grows and extends through the style to the ovule, and the male gametes are released for fusing with the female (FU; YANG, 2014).

However, in many plants, pollination is not always followed by fertilization, either because the pollen grains do not reach the stigma of the flower itself or other flowers or due to natural factors such as self-incompatibility, which is a mechanism that prevents the cross self-fertilization (SILVA; GORING, 2001), probably as a way of avoiding the deleterious effects of inbreeding (CASTRIC; VEKEMANS, 2004).

The self-incompatibility system provides the biochemical mechanisms necessary for plants to recognize and reject their own pollen, as well as the one with sufficiently similar genotype to initiate the self-incompatibility reaction. Plants need a pollen donor with a divergent genotype for successful fertilization (STEBBINS, 1970). This is not so unusual, as about 60% of the angiosperms are self-incompatible (DE NETTANCOURT, 2000; IGIC; LANDE; KOHN, 2008).

Classical genetic studies have established that the recognition of pollen grains compatible and incompatible by stigma is controlled in most species by an S gene with variable number of multiple alleles called S1, S2, S3, ..., Sn (TAKAYAMA; ISOGAI, 2005), responsible for the coding of at least two different components in pollen and pistil (ALLARD, 1971; WU et al., 2013). It is considered compatible the cross-breeding in which the pollen S allele is different from any allele present in the pistil (NEWBIGIN; ANDERSON; CLARKE, 1993; BRUCKNER et al., 2005).

The S *sterility* locus is a multiallelic complex, which segregates as a unit, and its variants are called S. haplotypes. The recognition of self-pollination occurs at the level of protein-protein interaction of the female and male determinants, and the incompatible response happens if the two determinants originate from the same S haplotype (TAKAYAMA; ISOGAI, 2005; NOWAK et al., 2011). The characterization of the S locus and the mechanisms underlying the acceptance or rejection of pollen are topics of great interest (FRANCESCHI; DONDINI; SANZOL, 2012).

Based on the constitution of the floral structures of the plants, two self-incompatible systems are known: homomorphic and heteromorphic, when there are no floral changes that follow the process, and when they exist respectively (ALLARD, 1971; REA; NASRALLAH, 2008; BRITO, 2010). The heteromorphic systems are characterized by morphological differences between two or three genotypes, associated with floral polymorphism, in other words, they differ basically in the style and anther (heterostyly) relative length. This difference implies a physical barrier to self-pollination, although cross-pollination may occur. Such incompatibility is present in 24 families and 164 genera (GANDERS, 1979), and the only compatible pollination in these species are those occurring between anthers and stigmas of the same height.

Differences in floral morphology act to enhance the ability of the pistil to discriminate between its own pollen or another similar. However, in most plants, the self-incompatibility is not followed by differences in floral morphology, and the pollination result can be predicted by means of tests of reciprocal pollination between individual plants (REA; NASRALLAH, 2008). This is the case in homomorphic systems, when incompatible genotypes can not be morphologically distinguished and the incompatible response depends, entirely, on physiological mechanisms (CASTRIC; VEKEMANS, 2004). In the case, there is no physical barrier, but the incompatible pollen

tube ceases its growth before fertilization of the ovule, due to the presence of the same allele S.

Among plants with homomorphic self-incompatibility, studies conducted in the 1950s identified two forms of distinct self-incompatibility systems, gametophytic and sporophytic (HISCOCK, 2002). It is believed that the gametophytic self-incompatibility system is the most common and, possibly, the most primitive (MOTA et al., 2010). The sporophytic system is similar to gametophytic because it presents monogenic control (S-gene) with multiple alleles, but differs in controlling the incompatibility reaction phenotype, that is, sporophytic. Alleles may show dominance, individual action or competitiveness in pollen and stigma according to the present combination.

The gametophytic and sporophytic systems can be distinguished by the pollen grain S phenotype. The pollen grain phenotype in the gametophytic system is determined by its own haploid genome, while in the sporophyte system, it is determined by the paternal diploid genome. In the gametophytic system, the S alleles can be co-dominantly expressed in the pistil, but in the sporophyte there may be complex dominance relations between the S alleles expressed in the pistil and also in the pollen grain, since the pollen phenotype is diploid. In this system, all individuals within the population will be heterozygous at the S locus, and a pollen grain will never fertilize a plant with the same S genotype. However, in a population with sporophytic system, with dominance allelic interactions at the S locus containing a mixture of heterozygous and homozygous for the S locus, it is theoretically possible that S genotype fertilization occur, since recessive S alleles may be "hidden" in pollen and/or stigma by a dominant S allele (DE NETTANCOURT, 1977; GIBBS, 1990).

In the gametophytic system, the pollen is binucleated and the stigmatic surface is humid. This humidity facilitates the pollen hydration. In addition, the stigmatic surface ruptures at maturation. These facts favor rapid germination, so that the self-incompatibility reaction occurs with inhibition of pollen tube growth on stigma or style (NEWBIGIN; ANDERSON; CLARKE, 1993; BRUCKNER et al., 2005). Self-incompatibility occurs between the haploid tissue of the pollen grains and the maternal sporophyte tissue (GIBBS, 1990). In the case of sporophytic self-incompatibility, the rejection happens between the male sporophyte tissue carried by the pollen grain and the maternal sporophyte tissue, usually on the stigma surface. The pollen grain has no ability to germinate or penetrate the stigma (GIBBS, 1990). In both cases, the pollen tube does not reach the ovule (DE NETTANCOURT, 1977).

As pollen grains present independent expression and segregate 1: 1 (RICHARDS, 1986), in the gametophytic self-incompatible system, three possible situations can be verified in the pollination (Table 1): i) totally incompatible when both alleles are common; ii) partially compatible when only one allele is different; and iii) fully compatible, when the four alleles are different (KAUFMANN et al., 1992; SCHIFINO-WITTMANN; DALL'AGNOL, 2002; FERRÃO et al., 2012).

Table 1. Main differences between crosses in the gametophytic self-incompatibility system

Parents			**Gametophytic self-incompatibility**
Female		Male	Offspring
S1S2	X	S1S2	Pollen grains will be S1 and S2. Pollen tubes will not grow - there will be no progenies. *Totally incompatible - 0% offspring*
S1S2	X	S2S3	Pollen grains will be S2 and S3. Only S3 pollen tubes will grow - progenies will be S1S3 and S2S3. *Partially compatible - 50% offspring*
S2S3	X	S1S2	Pollen grains will be S1 and S2. Only S1 pollen tubes will grow - progenies will be S2S1 and S3S1. *Partially compatible - 50% offspring*
S1S2	X	S3S4	Pollen grains will be S3 and S4. All pollen tubes will grow - progenies will be S1S3, S1S4, S2S3 and S2S4 - *Fully compatible - 100% offspring*
Synthesis			Only pollen tube growth and fertilization happen when the allele, present in the pollen grain, is not present in the diploid tissue of the style.

Source: Adapted from Lewis (1954).

It is verified that in the *totally incompatible* crossing there is no progeny formation, since both alleles are common (Table 1). Progeny from the *partially compatible* crossing show two mutually compatible genotypes, both mutually compatible with the female parent, and only one of them exhibits compatibility with the male parent. The progeny originated from the *fully compatible* crossing present four mutually compatible genotypes, besides being compatible with the female and male parents (LEWIS, 1954).

The occurrence of high fertility in species with these mechanisms is due to the fact that the number of S alleles in different populations is very variable and may be very high in species that have a high fertility rate (SCHIFINO-WITTMANN; DALL'AGNOL, 2002). As an example, red and white clover (*Trifolium pratense*), with approximately 200 and 100 different alleles in the S locus, respectively (LAWRENCE, 1996) can be mentioned. This way, the fertility is not compromised, since the large number of different alleles present in the population ensures a sufficient number of compatible pollination (HESLOP-HARRISON, 1983).

In different families, distinct molecules are used to recognize the pollen itself (REA; NASRALLAH, 2008). In the case of gametophytic self-incompatibility, a female determinant, which is a glycoprotein called S-RNase (S of S-locus and RNase of ribonuclease), is believed to be involved. S-RNAs are expressed exclusively in the pistil, being located mainly in the upper part of the style. These proteins exert a cytotoxic role which, at incompatible crosses, is responsible for interrupting the pollen tube growth in the style. RNases act by inhibiting or degrading RNA from the pollen tube that shares the same pistil S-allele (SCHIFINO-WITTMANN; DALL'AGNOL, 2002; TAKAYAMA; ISOGAI, 2005; ZHANG; ZHAO; XUE, 2009; NOWAK et al. 2011; McLURE, CRUZ-GARCÍA, ROMERO, 2011; WU et al., 2013). Researches point that S-RNase glycoproteins are involved in the control of some self-incompatibility system of some Rubiaceae family species, including *C. canephora* (ASQUINI et al., 2011; NOWAK et al., 2011) and Solanaceae, Rosaceae and Scrophulariaceae (SCHIFINO-WITTMANN; DALL'AGNOL, 2002).

The S-locus of the pollen grains is the SFB gene (S-locus and F-Box), which encodes an

F-box protein that generally acts as a linking component of ubiquitin, involved in a protein degradation way (SASSA et al., 2007; ZHANG; ZHAO; XUE, 2009).

On the other hand, several studies have reported that some species that have gametophytic self-incompatibility present self-fertility restorative genes (IGIC; LANDE; KOHN, 2008; LI; CHETELAT, 2014). The polyploidization processes are also pointed out as possible causes of the gametophytic self-incompatibility system breakdown. The pistil shows capacity of recognizing and inhibit the growth of incompatible pollinic tubes. However, in the case of polyploid, it is believed that diploid pollen grains are unable to express the phenotype that causes their rejection in the pistil (DE NETTANCOURT, 1977). Mutations also act as a cause of fertility recovery, presumably driven by reproductive guarantee, under conditions where pollen from compatible partners is limiting (IGIC; LANDE; KOHN, 2008; LI; CHETELAT, 2014). In diploid species, deletions and insertions may be associated with reduced S-RNase protein activity (STONE, 2002).

Gibbs (1990) points out the existence of another self-incompatibility system, called late-acting self-incompatibility, which manifests itself after fertilization. The self-incompatibility mechanism occurs in the ovary, before fertilization or as a result of abortion or ovules inhibition.

The pollen tube development and its presence in the ovary indicates that there is no self-incompatibility classic system that prevents the pollen grains germination or the pollen tube growth, suggesting late-acting self-incompatibility, which may be due to inbreeding depression or for being related to post-zygotic responses of an indefinite genetic nature (LIPOW; WYATT, 1999; POUND et al., 2003; SANTOS et al., 2007). Late-acting self-incompatibility was observed among perennial species such as Eucalyptus (POUND et al., 2003), Myrtaceae (SANTOS et al., 2007) and plants of the Caesalpinioideae family (GIBBS, 1990), among others.

There is also the situation when species pollen or self-compatible populations is usually rejected in related species or populations pistils, while at reciprocal crossings (self-compatible plants pollinated by self-incompatible plants) pollen rejection does not occur. This pattern is recognized as unilateral incompatibility (LEWIS; CROWE, 1958, LI; CHETELAT, 2014).

In this approach, from the molecular and physiological analysis of several species reproductive system, it was verified that the self-incompatibility system transformed itself many times during the angiosperms evolution (REA; NASRALLAH, 2008). According to the authors, there are different biochemical routes acting to interrupt the pollen tube development, which is complex, variable and dependent on the environmental conditions and the constitution of the stigma maternal tissue (LOSADA; HERRERO, 2014). In *Oenothera* sp., the incompatibility reaction is accelerated by the increase in temperature, whereas in *Petunia* sp., this fact does not happen (LEWIS; CROWE, 1958).

Studies on the pollen tube development are important not only to determine the presence of self-incompatibility, but also to identify the different types and control of the self-incompatibility reaction. By means of cytological analyzes, the pollen tube location can be observed. The sporophytic system can be differentiated from gametophytic and late-acting self-incompatibility by the fact that the pollen tube development is paralyzed on the stigma surface, which does not happen in the last two cases. When the growth is interrupted inside the style, before it enters in the ovary, there is gametophytic self-incompatibility, and, on the

contrary, when the pollen tube reaches the ovary, there is late-acting self-incompatibility (SCALONE; ALBACH, 2014).

Characteristics associated with the pollen tubes germination and their growth rates can also be studied by microscopy techniques, making it possible to analyze hybridization in breeding programs, without being influenced by environmental factors that may act on pollination carried out in field conditions (COMPANY et al., 2013).

With the new techniques in Proteomics, the interaction between pollen and pistil can be studied more effectively and under new aspects. Proteomics approaches may help in the in-depth knowledge of a number of previously unidentified mechanisms required for self-incompatibility response. A complete protein map can be constructed to discover their biological function in pollination. Although there has been no complete understanding of what happens during the interaction between the pollen tube and the pistil, some key regulators and receptors have already been identified by multidisciplinary approaches including biochemistry, molecular genetics and functional genomics (FU; YANG, 2014).

Within this theoretical approach, important research works have been published covering morphological, physiological, molecular and genetic aspects related to the factors and mechanisms that determine the self-incompatibility reaction in the different plant species (BREWBAKER, 1957; DE NETTANCOURT, 1997; CASTRIC; VEKEMANS, 2004; SANTOS et al., 2007; REA; NASRALLAH, 2008; MOTA et al., 2010; ASQUINI et al., 2011; FRANCESCHI; DONDINI; SANZOL, 2012; SANKARANARAYANAN; JAMSHED; SAMUEL, 2013; FU; YANG, 2014; SCALONE; ALBACH, 2014; LOSADA; HERRERO, 2014). However, the answers to the different existing interactions and doubts are not yet completely elucidated and in need of continuous studies.

3 SELF-INCOMPATIBILITY IN PLANT IMPROVEMENT

For the genetic improvement of plants, the knowledge about the reproductive system of the species is very important, since the mode of reproduction is largely responsible for the genetic structure of the population (STEBBINS, 1957). In addition, breeding methods are determined and vary according to the reproductive system. With the knowledge of the reproductive system, the understanding of the particularities of the species pollination contributes to the definition of the type of cultivar to be commercialized or made available to the farmers.

The populations and allogamous species reproduce by cross-fertilization and are characterized by great heterogeneity, each individual being highly heterozygous and distinct from the others. Several mechanisms may favor allogamy, contributing to the creation of genetic diversity among populations, which will increase the probability that at least one individual in the population will survive changes in environmental conditions (REA; NASRALLAH, 2008), involving biotic and abiotic factors. Among these mechanisms, genetic self-incompatibility is mentioned, which is an important reproductive characteristic present in most angiosperms, favoring the maintenance of diversity within the species (ZHANG; ZHAO; XUE, 2009).

In genetic breeding programs, in order to achieve success with plant selection, it is a basic condition to have genetic diversity in the population to be improved to select individuals or families with a higher frequency of favorable alleles (RAMALHO; ABREU; SANTOS, 2001). However, self-incompatibility may also be a limitation in breeding programs (BANDEIRA et al., 2011), since it does not allow self-fertilization of plants. From an agronomic point of view, the mechanism of self-incompatibility may also be undesirable for those species that are highly dependent on a successful fertilization process and the formation of seeds for their production (FRANCESCHI; DONDINI; SANZOL, 2012).

Bruckner et al. (2005) emphasized that the genetic diversity between plants should be sufficient in relation to the self-incompatibility so that the pollination is efficient, favoring the production. Due to the existence of self-incompatibility mechanisms in the crop, it is necessary to adopt genotypes with genetic diversity, aiming to increase pollination efficiency and guaranteeing production (BRITO, 2010).

By favoring cross-fertilization, the self-incompatibility system increases genetic variability within and between populations, avoiding inbreeding depression and the consequent expression of deleterious recessive genes.

In most cases, the incompatibility is prezygotic, manifesting itself as failure of the pollen grain itself to germinate in the stigma (genetic control of spore) or as the interruption of growth of the pollen tube in the stigma or style after the germination of the pollen itself (gametophytic genetic control). In some cases, however, the incompatibility reaction is postzygotic (late or ovarian action), in which the pollen tube develops to the ovary and the gametic fusion occurs leading to fruit development initiation. Incomplete fruit formation following the post zygotic incompatibility reaction can be confused with inbreeding depression. However, the abscission of immature fruits resulting from inbreeding is not restricted to a single stage, as observed in late-acting self-incompatibility. The fruit formation failure due to inbreeding depression might occur at any stage of fruiting (PANG; SAUNDERS, 2014).

The identification and characterization of the S alleles, as well as the understanding of the reproductive self-incompatibility of each cultivar, through controlled pollination tests, can generate useful information in the planning of breeding strategies, controlled hybridization and crop composition, ensuring suitable (SANTOS et al., 2007; BRITO, 2010; MOTA et al., 2010; CONTI et al., 2013) and genetically divergent genotypes, increasing productive efficiency (BRITO, 2010). For example, molecular markers can be used to identify and evaluate the diversity of S alleles (KHADIVI-KHUB, 2014).

Methods that predict the genetic compatibility of S alleles between cultivars are of great interest. They allow early selection of fully compatible cultivars from a genetic-reproductive point of view (MOTA; OLIVEIRA, 2005). In the case of Japanese plum cultivars, compatibility between plants has been evaluated by conventional pollination methods and pollen tube growth tests. From the molecular identification of the S alleles, it was possible to propose adequacy in the indication of pollinators for the studied cultivars, mainly for use in controlled hybridization in the breeding process, associated with the validation in field conditions.

S-allele analysis based on the Polymerase Chain Reaction (PCR) is a method with great

application potential to identify groups of self-incompatible cultivars. Both the knowledge of the S alleles, responsible for the gametophytic incompatibility, and the identification of one that can extinguish this incompatibility, will allow the controlled transfer of these alleles between cultivars with flowering synchronization, which will increase the efficiency of fertilization, fruiting and management within the breeding program (MOTA et al., 2010).

Basic and applied studies on self-incompatibility systems are fundamental in research programs. Genomic related technologies have enabled researchers to identify and characterize genes more efficiently (FERNANDEZ-POZO et al., 2015) and may also contribute to the understanding of the interaction mechanisms involved in self-incompatible systems, helping the planning and success of plant breeding programs (DEREEPER et al., 2015).

In plant breeding, it is important to differentiate the self-incompatibility system from other forms of sterility also existing, where there is no viable seed formation due to chromosomal abnormalities or some form of major functional alteration that affects the formation of gametes or the development of embryo, for example, male sterility, in which gametes are generally infeasible, whereas in self-incompatibility, they are fertile.

4 SELF-INCOMPATIBILITY IN *Coffea canephora*

The genus *Coffea* belongs to the Rubiaceae family and comprises 124 species (DAVIS et al., 2011). However, only two, *C. arabica* L. and *C. canephora*, Pierre ex A. Froehner, are commercially cultivated.

C. canephora and the other species of this genus are diploids (22 chromosomes). Differently from the previous one *C. arabica* is a polyploid, with 44 chromosomes (KRUG; CARVALHO, 1951; BERTHAUD, 1980; CHARRIER; BERTHAUD, 1985; N'DIAYE et al., 2005). According to Lashermes et al. (1999), *C. arabica* is a segmental amphidiploid formed by the natural crossing between *C. eugenioides* and *C. canephora*.

In most *Coffea* species, the self-incompatibility response is of the gametophytic type. Nevertheless, three species are self-compatible (*C. arabica, C. anthonyi* Stoff. & F. Anthony and *C. heterocalyx* Stoff.), that is, they have the ability to self-pollination (STOFFELEN et al., 2009; NOWAK et al., 2011). Self-compatibility in *C. arabica* is not surprising due to the strong association between polyploidy and self-incompatibility break.

The coffee tree exhibits gregarious flowering, that is, all the plants of a region flourish simultaneously, with variable number of flowering, from a few to several throughout the year, in the equatorial regions (ALVIM, 1973). Its flowers are hermaphrodites with stamens attached to the corolla tube. In *C. canephora*, the flowers blooming concentrates in a few days, and the flowers usually bloom in the morning, in the early hours, and their corolla starts withering on the second day. The flowering, in natural conditions, is caused by the first rains of the season, after a period of drought. In this allogame species, in which self-incompatibility occurs, cross-fertilization takes place after the flowers blooming, and pollination is performed with the aid of wind and insects.

According to Conagin and Mendes (1961), self-incompatibility is a common feature in *Coffea* species and they mention that the first reference to this phenomenon in plants of this genus was credited to Von Faber in 1910, who, studying the floral biology of coffee trees, observed that penetration of the pollen tube into the flower's own styles was much slower than in other plants flowers. The said author assumed that chemical similarities or differences would be responsible for the slow or rapid growth of the pollen tubes, respectively, in the cases of self-pollination or cross.

For Mendes (1942, 1949), the observations about the self-incompatibility in the coffee diploid species began in Java, when it was verified that some 'batches' containing a large number of plants of the same clone of *C. canephora* constituted a true failure in the production. Based on this fact, subsequent studies were carried out referring to the failure of artificial self-pollination in robust coffee. In 1943, in the Cytology section of the Instituto Agronômico de Campinas - IAC (Agronomic Institute of Campinas), cytological and genetic studies were started with the purpose of knowing and detailing the form of self-incompatibility present in the coffee species. The self-pollinated coffee trees were self-sterile and did not form seeds. Of the crosses made, about 50% were compatible. Cytological studies showed that pollen tube growth was normal in compatible crosses, whereas in self-pollination, after germination, the pollen tube growth was paralyzed (CONAGIN; MENDES, 1961).

Devreux et al. (1959), Conagin and Mendes (1961), Berthaud (1980) and Lashermes et al. (1996), using genetic and molecular markers, demonstrated that the self-incompatibility in *C. canephora* is of the gametophytic type, with monogenic inheritance, controlled by the S gene consisting of about three alleles (S1, S2 and S3). The locus S was located in the linking group 9 (LASHERMES et al., 1996). Asquini et al. (2011) and Nowak et al. (2011) believe that the mechanism of self-incompatibility in Rubiaceae, especially in *C. canephora*, is related to protein S-RNAses.

Devreux et al. (1959) described that, after self-pollination, the growth of the pollen tube on the stigma of *C. canephora* became corrupted and its penetration was blocked. Thus, gametophytic type self-incompatibility occurs when a certain S allele of a series of multiple alleles is common to the pollen grain and stigma, generally determining inhibition of the pollen tube development. When the S factor of pollen is different from the two S factors of the style, the pollen tube grows normally, reaching the ovary, where fertilization (compatibility) happens. Figure 2 illustrates the example of three plant crosses with combinations of four different alleles and progeny.

Evidence has shown that the mechanism of gametophytic self-incompatibility in *Coffea* is homologous to that self-incompatibility mediated by S-RNases (ASQUINI et al., 2011; NOWAK et al., 2011). According to Charlesworth et al. (2005), the self-compatible plants in species with S-RNase systems arise by tetraploidy or duplication of the S locus. For the *C. heterocalyx* and *C. anthonyi*, diploid species, the self-compatibility mechanism is not well understood (STOFFELEN et al. 2009; NOWAK et al., 2011). Coulibaly et al. (2002) argued that it would be reasonable to associate self-compatibility in *C. heterocalyx* with reduction or interruption of S-RNAs protein activity, with no interference in the pollen tube development.

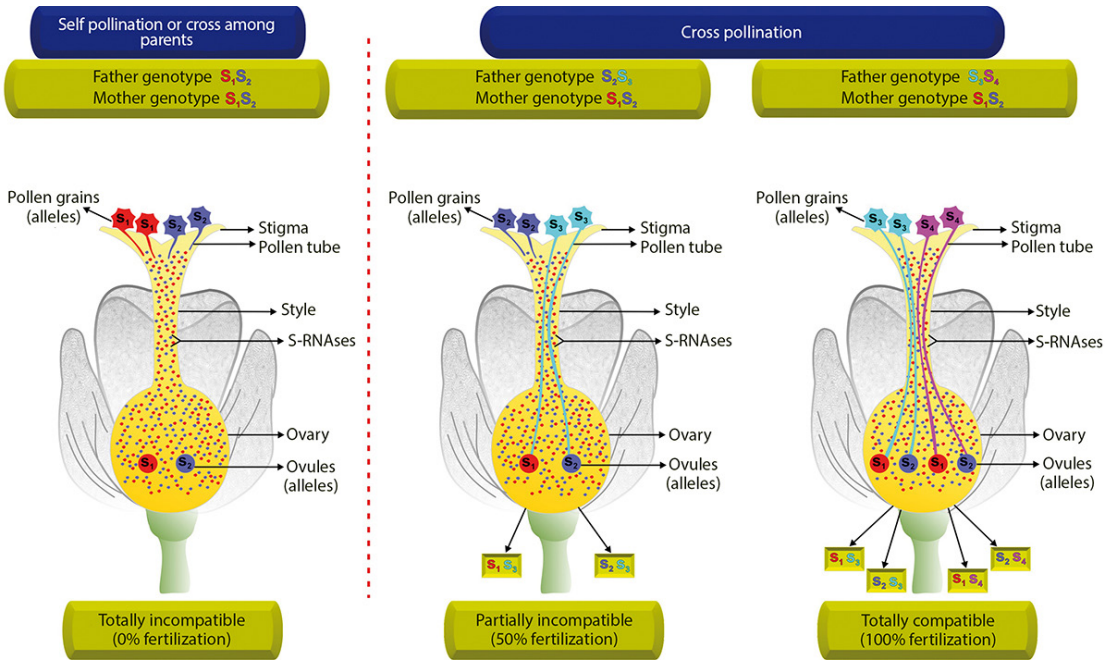

Figure 2. Illlustration of the gametophytic type self-incompatibility system.
Source: Adapted from Devreux et al. (1959).

In *C. canephora*, tests to identify compatible varieties are carried out under field conditions, through artificial pollination and observation of production (ALEKCEVETCH, 2013). This same author affirms that, through DNA-based studies, it would be possible to identify the most recurrent individuals in the offspring, regardless of the reason (greater pollen production, higher fertilization rate, greater genetic compatibility, among other factors). Thus, they could estimate which plants or clones are best suited to be used in clonal cultivation due to the observed frequency of paternal and maternal genotypes, suggesting the use of molecular markers of the microsatellite type, from the data crossing obtained in a work of descendants and ascendants genotyping.

The possibility of self-compatibility introgression of the *C. heterocalyx* species in *C. canephora* was evaluated along with inheritance studies, S locus location impact of the self-incompatibility on the fruiting and potential of the molecular marker assisted selection use (COULIBALY et al., 2002). Asquini et al. (2011) argued that breeding assisted by genetic markers could seek to break the self-incompatibility system in *C. canephora* commercial strains.

Studies on genetic divergence have shown that *C. canephora* possesses great natural variability, promoted by the occurrence of natural interbreeding between and within populations (IVOGLO et al., 2008; SOUZA, 2005), including highly heterozygous individuals (CONAGIN; MENDES, 1961; BERTHAUD, 1980). The genetic diversity of the species presents priceless value for breeding programs, since it comprises an important source of genes that can be used to create new genotypes (IVOGLO et al., 2008). The transfer of desirable characteristics of *C. canephora* to *C. arabica* should also be considered in these programs (HERRERA et al., 2002), reinforcing the importance of studying the reproductive system, involving the mechanism of self-incompatibility present in *C. canephora* to assist in the

crosses between plants of these species, among other factors.

Although much has already been done, it is still necessary to obtain information for a broader understanding of the mechanisms of self-incompatibility action found in *C. canephora*, *a*nd how this knowledge can be used to contribute to the improvement of the quality and yield of the coffee crops.

5 SELF-INCOMPATIBILITY AND SUSTAINABILITY OF CONILON COFFEE

C. canephora is the second most cultivated species in the world, accounting for about 40% of total coffee production. Espírito Santo stands out as the largest Brazilian producer of this culture, known in the State as conilon coffee.

The propagation of the conilon coffee can be done sexually, through seeds, and asexually, especially by means of cutting (FONSECA, 1996; FERRÃO et al., 2007; IVOGLO et al., 2008), always having to pay attention to the problems of incompatibility within the progenies and their consequences on productivity and genetic variability of offspring. For the authors, genetic self-incompatibility in *C. canephora* promotes the formation of highly heterozygous populations with high genetic variability, absence of self-fertilization, non-fertilization between flowers of the same plant and deficiency in crosses, when cultivating related genotypes.

The seed propagation system is undoubtedly the simplest and the one that guarantees the natural variability of the species. It is the main strategy to generate hybrids, recombinant populations and highly heterozygous offspring. However, for coffee growers, crop heterogeneity is undesirable because it hinders cultural practices (FONSECA, 1996; FERRÃO et al., 2007). In order to reduce this unevenness, it is indicated the planting of clonal cultivars, because, under this aspect, asexual reproduction is an important propagation system, especially when superior individuals are found for the target characteristics. Cloning maintains the characteristics throughout the generations with the selected genotypes multiplication, which is important in the constitution of clonal varieties for the commercial crops formation.

In breeding conilon coffee, systems of seminal and cloning propagation are used concomitantly considering the genetic particularity of the self-incompatibility when cultivating related genotypes (FERRÃO et al., 2012).

It is worth mentioning that for the fruit production in a crop, it is necessary that it be formed by genetically compatible clones, that is, with different plants. Consequently, crops consisting of only one clone will not bear fruit. If the crop is formed by two or even a few genetically similar clones, there will also be fertilization failures and, consequently, small fruit production (Figure 3).

Thus, genetic compatibility studies are of fundamental importance in the definition of the clones that should be grouped for the formation of clonal varieties. The number of clones should be associated with the safety, sustainability and longevity of crops.

To obtain superior cultivars, it is necessary that the selected clones have favorable characteristics and genetic compatibility. To do so, they must be previously tested and evaluated (FERRÃO et al., 2012).

Figure 3. Illustration of problem arising from the self-incompatibility in clonal conilon coffee crop (A) Production of clonal crop implanted with all 13 clones components of the cultivar Vitória Incaper 8142 (correct implantation[1]); (B) Implemented crop production with few clones with failure in pollination/fertilization and consequently in fruit production (incorrect implantation).

[1]**Source**: Fonseca et al. (2004).

The Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural - Incaper (Capixaba Institute of Research, Technical Assistance and Rural Extension) genetic breeding program for conilon coffee has made available, so far, eight clonal cultivars and one of seed multiplication, therefore contributing to the sustainable development of its productive chain. It should be emphasized that the clustering of the clones that are part of each clonal cultivar developed and recommended by Incaper was carried out based on the genetic compatibility between them and in the set of relevant agronomic characteristics, such as high productivity and stability, disease and drought tolerance, uniformity of fruit maturation, distinct maturation season, plants architecture and vigor, yield in the processing, among others.

The cultivars developed by Incaper were Emcapa 8111, Emcapa 8121 and Emcapa 8131, composed of 9, 14 and 9 clones, respectively (BRAGANÇA et al., 2001); 8141 Robustão Capixaba, formed by 10 clones (FERRÃO et al., 2000); Emcaper 8151 Robusta Tropical, propagated via seeds (FERRÃO et al., 2000); Vitória Incaper 8142, with 13 clones (FONSECA et al., 2004); Diamante ES8112, ES8122 - Jequitibá and Centenária ES8132, each composed of nine clones; Marilândia ES8143, with twelve clones (FERRÃO et al., 2015a, 2015b; 2015c; 2017).

These clonal cultivars together present great genetic variability with about 75 different

genotypes, which guarantee the expression of the species productive potential, stability, longevity and greater genetic base.

It is crucial that producers do not exclude from an improved cultivar clones that are deemed inferior and, therefore, changing its characteristics. This behavior compromises the stability of *C. canephora* crops in Espírito Santo and Brazil (FONSECA et al., 2004; FERRÃO et al., 2007; FERRÃO et al., 2012). It must maintain the genetic constitution of each clonal cultivar, since each clone has a reason and a defined role within it. It is important for the activity perpetuation that there is genetic variability, since it allows to find individuals with the desired characteristics and genes of interest to be incorporated into the breeding.

Cultivation of crops with a limited number of clones, especially when dealing with nearby genotypes in relation to their genetic constitutions, generates, over time, erosion or genetic vulnerability with which most geneticists and plant breeders of the different species cultivated worldwide are highly concerned (FONSECA, 1996; FONSECA et al., 2004; FERRÃO et al., 2007; FERRÃO et al., 2010; FERRÃO et al., 2012; PINTO, 2012; SOUZA; SILVA-MANN; MELO, 2014; SILVA et al., 2015).

In the cultivation of Incaper clonal cultivars available up to now, 9 to 14 clones are used to guarantee pollination, fertilization, fruit production and the activity sustainability. All clones are genetically distinct, which is important for the production stability in case of a new pest or disease or even in an adverse climatic condition such as water stress and ambient temperature increase.

Conilon coffee, of cross-pollination and with gametophytic self-incompatibility, requires, for commercial production, the planting of compatible, genetically divergent, seedling or clonal cultivars with flowering synchronism. Problems related to pollination and fertilization can reduce both yield and fruit quality by decreasing the effective fruiting and number of fruits per rosette and per branch. High yields with the crop can only be obtained if the conditions for pollination and fertilization are favorable.

6 FINAL CONSIDERATIONS

The mechanisms of self-incompatibility are diverse and complex in their physiological, morphological, biochemical and genetic aspects. Therefore, there is a need to expand the studies on these systems in order to approximate the theoretical knowledge of the practical application in plant breeding. The understanding of the self-incompatibility system can generate useful information in the planning of breeding strategies, controlled hybridization, cultivar composition and crop formation, which will guarantee an adequate pollination with the use of compatible genotypes.

As conilon coffee is cross-fertilized and presents genetic peculiarities in its reproduction mode, such as genetic self-incompatibility, it is possible to affirm that, in order to produce fruit in a crop, it is necessary that it be formed with genetically different modified plants. Crops consisting of only one incompatible clone will not produce fruits and those formed by two or

more highly similar clones may also fail in fertilization and small fruit production.

The conduction of clonal crops with a reduced number of clones could lead to disastrous results for the producer and, in the future, for conilon coffee culture, by the genetic base reduction, which is the raw material for the advance in the cultivars improvement and development with characteristics of interest to society. Conilon crops should be implanted with high genetic diversity cultivars in relation to the self-incompatibility so that there is efficiency in the pollination, with high fructification.

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