

CONILON

Coffee

3rd Edition

Updated and expanded

The Coffea canephora produced in Brazil

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ISBN: 978-85-89274-32-6

Editor: Incaper

Format: digital/printed

May 2019

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International Cataloging Data in Publication (ICP)

C129 Conilon Coffee / technical editors, Romário Gava Ferrão ... [et al.]; english translation Marcelle Gualda Pasolini. - 3 edition updated and expanded. - Vitória, ES : Incaper, 2019. 974p.: il. color.

Translated from: Café Conilon, 2017 - Incaper.

System Required: Adobe Reader

Access mode: <https://bibliotecaruitendingha.incaper.es.gov.br/>

ISBN: 978-85-89274-32-6

1. Brazil. 2. Espírito Santo (State). 3. Coffee Cultivation. 4. Conilon Coffee I. FERRÃO, Romário Gava (Ed.). II. FONSECA, Aymbiré Francisco Almeida da (Ed.). III. FERRÃO, Maria Amélia Gava (Ed.). IV. DE MUNER, Lúcio Herzog (Ed.). V. Capixaba Institute for Research, Technical Assistance and Rural Extension. VI. Title.

CDD: 633.73

Elaborated by Merielelem Frasson da Silva - CRB-6 ES/675





Coffea canephora Breeding

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1 INTRODUCTION

The genus *Coffea* is composed of 124 species (DAVIS et al., 2011), but practically all coffee produced, marketed and consumed in the world is processed only with *Coffea arabica* and *Coffea canephora* species. These two species are very different in aspects of breeding and propagation systems, chromosome number, genetic background, origin, plant size and cycle, type and size of cherries and beans, nutritional requirement, drought tolerance, pests, diseases and nematodes, beans biochemical constitution, among others.

The *C. arabica* species represents around 62% of the world's coffee and produces the most appreciated coffee for consumption in traditional forms of preparation, but is highly susceptible to pests, diseases and nematodes. The *C. canephora* species has been specially used in the production of soluble coffee and also very consumed in the form of mixtures with the arabica coffee (*blends*), participating with a little more than 50% in the composition, without interfering negatively in the quality of the drink, provided the robusta and arabica coffees are of good quality. The species *C. canephora* has been widely used in *espresso* coffees, contributing with "body" and density. It has also been used as a valuable source of favorable alleles for resistance to pests, diseases and nematodes in breeding programs using both species.

In a contemporary agriculture, to increase productivity, there is a need for special attention, for the use of superior cultivars obtained by genetic breeding, together with the improvement of planting conditions and crop management. In several cultures, the additive relationship between these conditions has been quantified (PALLET; SALE, 2006).

Genetic breeding of plants has been understood as a science that aims to manipulate plants in the direction of the social, economic and environmental interests of mankind. In order to be successful in this research area, especially in the context of quantitative genetics, it is necessary to have available information about the species to pass through breeding, the genetic variability of the germplasm, the breeding methods to be used and the domain of genetic- biometric methodologies of analysis; in addition, to verify if there are suitable physical, financial and personnel structures to carry out the peculiar activities of the program.

The breeding has effectively participated in the generation of fundamental knowledge

regarding different characteristics of the genus *Coffea* plants. Therefore, it has provided an increase in the productive capacity of the plants; reduction in the size and improvement of plants architecture, aiming to allow the densification and make the harvesting operations easier; incorporation of alleles that confer resistance to pests and diseases; development of more adapted and stable genetic materials to the different cultivation environments and improvement of the agronomic characteristics, such as maturity uniformity and fruit size. Thus, the genetic modifications have the purpose of obtaining cultivars with better quality of drink, type and chemical composition of the beans to meet the consumer demands and guarantee the activity sustainability, as well as to promote greater socioeconomic return for coffee and for society as a whole.

The main applied result of a breeding program is the development of superior cultivars. For this, the establishment of adequate planning is fundamental in this context. There are a number of factors involved in obtaining this technology, among them: development and knowledge of the population to be bred, use of appropriate methodologies for evaluation and selection of individuals, since most of the characters of economic value is polygenic in nature.

Greater genetic gains through the breeding of *C. canephora* will be possible through technical, political and structural actions that prioritize the researches with the species, mainly with germplasm that represents the great social and economic importance in the world, as of conilon. By means of works involving multidisciplinary teams from different institutions and the use of the knowledge of several areas, such as cytology, classical breeding associated with biotechnology, phytopathology, statistics, biometrics and other correlates, results will be obtained faster, with lower and more applicable costs.

In this chapter, the genetic breeding of *C. canephora* involving aspects of cytology, breeding systems, character inheritance, program objectives and breeding strategies, biometric analysis methods and the major scientific advances achieved by breeding the species worldwide will be approached.

2 CYTOLOGY AND REPRODUCTIVE SYSTEMS

Studies on the number of chromosomes in coffee have been carried out since 1930, with the publication of Sybenga (1960) works. The basic number, $x = 11$ chromosomes, is typical for most species of the genus *Coffea*. In it, all species, including *C. canephora*, are diploids with $2n = 2x = 22$ chromosomes. The exception is *C. arabica* which is tetraploid and has $2n = 4x = 44$ chromosomes.

It is possible, through the artificial treatment of seeds with colchicine in germination, to double the number of chromosomes of *C. canephora* and to transform it into self-tetraploid (MENDES, 1939). This procedure is of fundamental importance in the accomplishment of the interspecific breeding involving the cross between *C. arabica* x *C. canephora*.

All species of the genus *Coffea*, with the exception of *C. arabica*, are allogamous; therefore, they are sexually reproduced by cross-fertilization, by the genetic self-incompatibility in them.

Conagin and Mendes (1961), Monaco and Carvalho (1972) and Berthaud (1980) analyzed the self-incompatibility in diploid species of the genus *Coffea*. According to Conagin and Mendes (1961), it was Von Faber, in 1910, the first to describe self-incompatibility in *C. canephora*.

2.1 GENETIC SELF-INCOMPATIBILITY

Self-incompatibility is the inability of the hermaphrodite plant to produce zygotes by self-pollination. As a consequence, the gene flow and the frequency of heterozygosis in the total *loci* within the plant populations of the species is increased. In terms of genetic breeding, the importance of self-incompatibility lies in the possibility of hybrid synthesis, without the need for manual pollination, and in the increase of genetic variability in plant populations, which can be used to improve these populations.

Devreux et al. (1959) suggested that in the *C. canephora* species, the self-incompatibility would be of the gametophytic type. When the pollen S allele is different from the two S alleles of the style, the pollen tube grows normally, penetrating the ovary and performing fertilization. Conagin and Mendes (1961) and Berthaud (1980) presented numerous data showing that the self-incompatibility of *C. canephora* is of the gametophytic type linked to a single gene locus 'S' with a series of interacting alleles, S1, S2 and S3.

The main consequences of self-incompatibility in *C. canephora* are the absence of self-fertilization, non-fertilization between flowers of the same plant and deficiency in crosses when using related genetic materials and the formation of highly heterozygous and therefore heterogeneous populations. Thus, in the case of development of clonal cultivars where it is necessary to define which clones should be grouped, previous studies of genetic compatibility of the component plants are of fundamental importance.

For more detail on this subject see chapter 9 of this book.

2.2 FLORAL BIOLOGY

In the literature, different works related to the floral biology of coffee are found. According to Sybenga (1960), Coste (1968) and Rena and Maestri (1984), flowering in coffee plants comprises a sequence of physiological and morphological events beginning with the phase of floral induction until the anthesis, proceeding through the intermediary phases - differentiation or initiation of the floral origins -, and ending with the flower development phase. The transition between phases is usually gradual and imperceptible, especially in the early stages, until the formation of the origins. The species *C. canephora* is tropical with gregarious flowering, that is, all the individual plants, in a certain geographical extent, flourish simultaneously.

The physiological and morphological changes of flowering in plants are promoted by internal hormonal regulators, preferably abscisic acid, as well as external factors such as soil temperature and humidity. The plants lateral branches have, in the leaves axil, reproductive or vegetative buds, according to the case, ordered in a linear series, called serial buds. They might originate floral buds or secondary side branches. Each reproductive serial bud will originate

a short axis, which will develop a flower. These axes have several nodes in which opposing bracts are inserted, in which axils are formed descending series of floral buds, which, in turn, can give rise to new short axes, similar to the mother axis, terminated equally by a flower and with several nodes, and so on (RENA; MAESTRI, 1984).

The inflorescences (Figure 1) usually develop in the leaves axil of the side or plagiotropic branches, which grow throughout the year, and rarely in the orthotropic branches (CARVALHO et al., 1991). They have several floral buds that are compressed against each other, forming a compact set, called glomerulus.

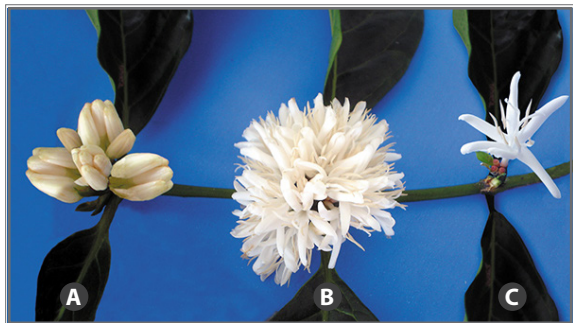


Figure 1. Floral bud (A), inflorescence (B) and conilon coffee flower (C).

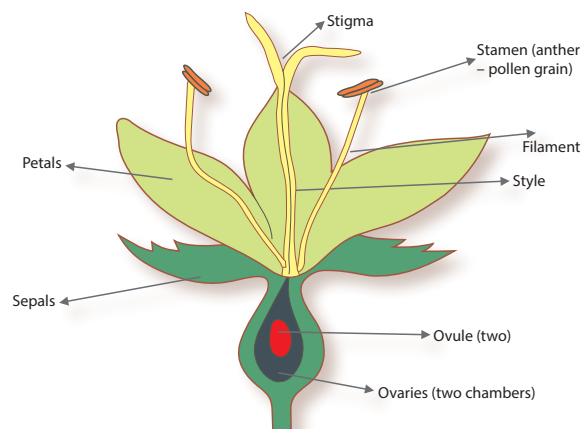


Figure 2. Schematic drawing of a *Coffea canephora* flower (cross section).

Source: Ferrão et al. (2007).

These inflorescences have short peduncles,

with two pairs of leaf bracts, with three to five terminal flowers. Each flower has short peduncle and inferior ovary, provided with two stores, each one, in general, with an ovule; long style with two stigmatic lobes; five reduced sepals; short corolla; and stamens with short fillet attached to the lower third of the anther (Figure 2).

2.3 POLLINATION

The coffee tree blossoms three to four times a year, with greater emission of flowers from August to October. The dormancy time until the flower blooms varies from four to ten days, depending on the temperature, humidity of the air and the soil. Flowering occurs after rain and/or irrigation and temperature rise. The flower blooms in the early hours of the morning and the anthers dehiscence, a few hours later, depending on temperature and sunshine. Thus, in the induction of flowering, the flower buds double in length and volume over a period of two to three days, leading to the flowers bloom which usually occurs in one day.

Pollination is favored mainly by wind, being also performed by insects, to a lesser extent. The species *C. canephora* produces more pollen than *C. arabica*, and the distance covered by the pollen grain may be greater than 100 meters, depending on the topographic conditions and the winds intensity during the period of pollen release.

Under normal conditions, the pollen remains viable in the plant for one day. The stigma remains receptacle for a period of three to four days. The pollen tube reaches the ovary in 24 hours when pollination is feasible, this is when there is no genetic incompatibility.

The fruits development varies according to the cultivar and edaphoclimatic conditions,

taking from 220 to 330 days. The seed consists of an endosperm, within which the embryo is found with two small cotyledons. The seeds are surrounded by a tanned film (remnant of the integuments and the perisperm) and protected by the fruit endocarp (parchment). Seed germination usually occurs around 45 days after being planted in the nursery (CARVALHO et al., 1991).

3 SOME ASPECTS RELATED TO CHARACTER INHERITANCE

The main objectives of *C. canephora* breeding are the cultivation of high productivity and high quality beans, adaptability to various environments, production stability, drought tolerance, resistance to pests and diseases, maturation uniformity and other agronomic characteristics of interest.

In order to define breeding strategies and plant selection, an improved knowledge about the species in question, its genetic structure and the heritability of the characteristics to be improved are required. Information about the *C. canephora* species is scarce compared to those of *C. arabica*, making it difficult to plan and implement breeding programs actions.

Therefore, some factors that have implications in the improvement of production characteristics and *C. canephora* bean quality will be discussed in the following paragraphs:

3.1 BEAN PRODUCTIVITY

The robusta coffees productivity in African and Asian countries involving clonal varieties with planting density between 1,200 and 2,000 plants/ha has been between 2.000 and 3.500 kg/ha, while in Brazil, particularly in the State of Espírito Santo (BRAGANÇA et al., 1993, 2001; FERRÃO et al., 2000, 2015b, 2015c, 2015d; FONSECA et al., 2004a; FONSECA; SAKIYAMA; BORÉM, 2015) the clonal cultivars developed by the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural - Incaper (Capixaba Institute for Research, Technical Assistance, and Rural Extension), when planted following the appropriate technical recommendations (FERRÃO et al., 2012, 2015b, 2015c, 2015d) and others developed by Embrapa Rondônia (RAMALHO et al., 2011; EMBRAPA, 2012), with a density of 2,000 to 4,000 plants/ha, have presented productivity of 4,800 to 7,200 kg/ha.

The genotypes productivity can vary greatly, with genetic material differences from two to four times between the lowest and the highest productivity, depending on the years and locations. These differences are minimized when one has greater control of the environment. As bean productivity is a polygenic quantitative characteristic, much affected by the environment action, it is recommended to evaluate the genotype as many harvests as possible, in order to obtain reliable estimates of its productive potential that, according to Ferrão et al. (2003b), Ferrão (2004) and Fonseca et al. (2004b), should be four to five harvests. One of the known ways to obtain genetic gains faster for the characteristic is to indirectly involve information about other characteristics associated with productivity and that have a high genotypic correlation with bean productivity.

3.2 COMPONENT CHARACTERISTICS OF BEAN PRODUCTION HERITABILITY

The production of the plant depends on vegetative characteristics (architecture and growth) and reproductive (flowering and fruiting). The vegetative components that interfere in the production of the robusta coffee tree are plant height, crown diameter, number and length of the orthotropic and plagiotropic branches, number and size of the plagiotropic branches internodes and plant budding capacity, thus providing an effective possibility of the productive branches replacement by pruning after three or four harvests. The reproductive components that interfere in the production of the plant are number of rosettes, number of flowers per rosette, percentage of fertilized flowers, number of fruits per node, percentage of beans wilting, percentage of mocha type, size, type and density of beans, among others.

Heritability is defined as the ratio of the phenotypic variance that is due to genetic variations. For purposes of plant breeding, heritability has dual purposes. One is to predict the reliability of the phenotypic value in expressing the genotypic value, and is therefore a selection process measure of accuracy. Another is to quantify the selection differential proportion that is expected to be gained when the selection is practiced on the defined selection unit (CRUZ; CARNEIRO, 2003). In this way, a concept different from the classical definition can be adopted in selection prediction, where heritability is expressed as the ratio between the total genotypic variance and the total phenotypic variance (heritability in a broad sense), or relation between the additive genetic variance and the phenotypic variance (heritability in the restricted sense).

Due to allogamy, there is great genetic variability in *C. canephora* species, mainly for vegetative and reproductive characteristics, which is very important in breeding programs.

In a diallel, held in Cameroon, Africa, by Boularmont et al. [19--] cited by Charrier and Berthaud (1988), involving eight genetic material of robusta coffee for five morphological and growth characteristics, three of production, susceptibility to pests and diseases and caffeine content, significant differences were verified at the level of 1% probability for General Combination Capability (GCC), for all traits studied, and not significant for Specific Combination Capability (SCC), for most characteristics. The results show the predominance of the additive effects in relation to the non-additives, evidencing that, through simple methods of breeding and selection, such as recurrent selection, there is the possibility of being successful in the breeding for production, height of the plant, crown diameter, length of branches, number of nodes, bean type and size, caffeine content and susceptibility to diseases. There were also high positive and significant correlations, with coefficients of 0.63 to 0.90 between parents and their progenies for different characteristics studied, once again showing the possibility of exploring genetic variability in order to improve the plants of interest in the species characteristics.

3.3 STABILITY AND BIANNUAL PRODUCTION

The production stability is related to the behavior predictability of a genotype. The ideal in perennial plants, such as coffee, is that the genotypes recommended for planting have low production variability over the years and good behavior in the face of divergent local conditions and cultivation, especially in unfavorable environments, and respond positively

when the technological improvement of the environment. The behavior of the genotypes in response to the differences between environments, mainly involving time and place, can be verified through genotype x environment interaction analysis (G x A). The annual production of coffee is highly correlated, among others, with climatic conditions, fertilization, management and performance that the genetic material has been presenting in previous harvests. Therefore, income selection should be based on at least four harvests, thus taking at least six to seven years to complete a work cycle. Production stability is best evaluated when genetic materials are tested for at least four harvests and in several environments and are estimated by methodologies such as Eberhart and Russel (1966), Cruz, Torres and Vencovsky (1989) and Lin and Binns (1988). The need for evaluation in the mentioned period is due to the coefficient of repeatability for productivity, which in *C. canephora*, Conilon variety, is around 0.60, with a coefficient of determination (R^2) of 85% (FONSECA, 1999; FERRÃO et al., 2003b; FERRÃO, 2004; FONSECA et al., 2004b) demonstrating to be an average value for repeatability of this characteristic in the response of the genetic material behavior from the first to the second and from the first to the fifth harvest.

In studies in the Ivory Coast, evaluating the G x A interaction with robusta coffee clones, with and without irrigation, significant G x A interaction was observed (CHARRIER; BERTHAUD, 1988). Ferrão et al. (2003), studying eight clonal genetic materials of conilon coffee in the State of Espírito Santo, in four locations, for four harvests, with and without irrigation, using the Eberhart and Russell (1966) and Cruz, Torres and Vencovsk (1989) methodologies, verified the occurrence of G x A interaction and good production stability of all genotypes, due to the regression deviation was statistically equal to zero and the coefficients of determinations (R^2) were slightly higher than 80%.

The coffee plantations present great spatial and temporal variability of productivity. The variation of production over the years, high and low, is known as biennial (CARVALHO et al., 2004), which in conilon is usually less intense than in arabica coffee. Rodrigues et al. (2013) evaluated the biennial production in three groups of conilon coffee clones of early, intermediate and late maturation of the Incaper breeding program. There was expressive genetic variability within each maturation group, with genotypes showing high biennial periodicity, while others behaved stably throughout the harvests. Biennial periodicity is a feature strongly associated with genetic material, strongly expressed by adverse climatic conditions (drought and high temperatures), pest and disease incidence and inappropriate plant management. What is desirable is to develop productive and stable cultivars with less possible biennial production.

The coffee tree tolerance to drought is a very important characteristic for the adaptation of a cultivar to the environmental conditions. Water *deficit*, at certain stages of the crop and/or in certain years, is common especially in marginal areas of *C. canephora* cultivation. A drought tolerant cultivar should have the ability to survive and also produce under water *deficit* conditions in sites zoned as suitable for cultivation. The quantity and quality of the coffee beans are more or less affected according to the time and period of exposure to stress. The phase of flowering to the filling of beans is a period of great demand of water by the crop. The lack of this resource in these phases compromises the final product quality and productivity. The four flowering

that usually occur during the year may be one of the mechanisms of partial compensation to the effects of drought. Drought, in addition to reducing the amount of fruit, significantly reduces the size of the plant, also affecting its development and, obviously, productivity in the current and the following years, compromising the average productivity and contributing to accentuate the biennial production effect. In Espírito Santo, more than 70% of cultivated areas present water *deficit* between 50 and 550 mm/year (FEITOSA, 1986). In these areas, most of the conilon coffee plantations of the State are found. Ferrão et al. (1999, 2017) developed the clonal variety of Conilon 'Emcapa 8141' - Robustão Capixaba and Marilândia ES8143, tolerant to drought, that in this conditions shows average productivity of 53.0 processed bags/ha, higher than the State estimated at 35.0 processed bags/ha (CONAB, 2014).

3.4 PRODUCT QUALITY

The improvement in coffee quality is conditioned by genetic, chemical and technological factors. Thus, quality can be affected by the species, cultivar, method of preparation, uniformity of fruits maturation, geographic origin and altitude, type of harvest, drying and processing way, attack of pests on fruits, among others (CARVALHO, 1988a; CLARKE, 1985; CLIFFORD, 1985; VINCENT et al., 1977).

There is little research on genotype interference in coffee quality. In *C. arabica*, due to the low genotype variability, there has been little variation in the quality of the different cultivars, provided that the crops are well managed and the coffee is properly harvested and prepared (CARVALHO, 1988a). However, works carried out in Kenya, Africa, showed variability in quality, when comparing breeding genetic material with the semi-wild of *C. arabica* (VAN DER VOSSSEN, 1985). In *C. canephora*, Leroy et al. (1992) and Lambot et al. (2008) found significant differences among clones for the beverage quality.

The factors associated with coffee quality in terms of size and chemical composition of the beans in the beverage are presented below.

3.4.1 Beans characteristics

Robusta coffee beans are generally smaller in size than those of arabica, weighing 12 to 15 g per 100 seeds, and can reach 18 to 22 g (COSTE, 1968). They have a shape that varies from round to canoe, are brownish and have a lower gloss than arabica coffee beans, although there is great genetic variability for these characteristics. Seed size is an inheritable trait, with great variation among genotypes. There are examples of selections success involving large beans populations. It is possible to select genotypes of 18 g per 100 seeds, of which 80% are retained in 16 sieve and 6.3 mm in diameter (CRAMER, 1957). In the State of Espírito Santo, at the Conilon Germplasm Active Bank, the genetic materials evaluated presented beans of different shapes, colors and sizes, with clones reaching the medium sieve 17 (FERRÃO, F.; FONSECA; FERRÃO, M., 2000).

The seed size is influenced by the environment, suggesting, therefore, that it is a

quantitative characteristic, that is, controlled by several genes. Some coffee clones produced from collections in Madagascar and Ivory Coast had a weight decrease of 3 to 5 g per 100 seeds when harvested in drought years. In the State of Espírito Santo, in addition to reducing the size of the beans, there has been a significant increase in the percentage of wilting in drought years, causing a low productivity in the processing, making up to six kilos of cherry coffee to produce a kilogram of processed coffee, although there is a great variability of response to drought among the genetic materials used. Charrier and Berthaud (1988) showed that, when cross-breeding among large-bean breeders, there is a great heterogeneity in beans size and a correlation of 0.36 to 0.76 between parents and progeny among the offspring and high GCC for the mentioned characteristic, which allows to conclude on the possibility of obtaining relatively easy and fast genetic gains with the application of simple breeding methods as for bean size.

The percentage of mocha type beans is a genetic characteristic largely affected by the environment. Mocha beans are rounded due to abortion of one of the two ovule from the ovary resulting from deficient pollination, unfavorable environmental conditions or genetic problem. Generally, in arabica coffee, the percentage of mocha type beans is, on average, 10%; in *C. canephora*, between 10 and 30%, and may exceed 50% in certain cases. The characteristic varies between genetic materials and there is high correlation between parents and offspring, depending on the parents involved in the crosses.

The cultivars developed and recommended for planting in the State of Espírito Santo have presented 20 to 32% of mocha type beans (BRAGANÇA et al., 1993, 2001). In the conilon cultivated in Espírito Santo, the average is about 33%; in the cultivar Vitória Incaper 8142, is around 21% (FONSECA et al., 2004a). In clonal cultivars Diamante ES8112, ES8122 - Jequitibá and Centenária ES8132, the percentage of mocha type beans is 18.68%; 24.83% and 26.38%, respectively (FERRÃO et al., 2015b, 2015c, 2015d).

3.4.2 Caffeine content

Robusta coffee, in general, presents high caffeine content. Caffeine content vary widely when studied in wild and cultivated genetic materials, with values ranging from 1.8 to 3.4% (LEROY et al., 1993). Environmental conditions do not greatly affect the caffeine content in the beans. These contents can be determined and present intermediate heritability. The coefficient of correlation between parents and offspring is very high (0.88), and the genetic variance average value of progenies studied for the characteristic is in a higher proportion of additive nature. The great variation among the progenies allows success in selection for contents lower than 2% of caffeine (CHARRIER; BERTHAUD, 1988).

Clonal cultivars in the Ivory Coast are in average 2.8%, but expect to be reduced to 2.2% by breeding programs (CAPOT, 1977). The literature shows that the level of caffeine is variable, with extremes of 1.0 to 5.5% in progenies originating from crosses between contrasting parents involving low and high levels. The cultivars developed by Incaper's breeding program, for example, have 2.4 to 2.5% caffeine.

3.4.3 Beverage Quality

C. canephora species coffees have been characterized as neutral, full-bodied, with pronounced bitterness. They have different organoleptic and chemical characteristics than arabica. Traditionally, research institutions have invested little in equipment and trained personnel to carry out the quality evaluations of the final product by the proof of the beverage in *C. canephora* and, thus, little information was generated. In recent years, with the demands of the market and consumers for better quality products, coupled with the greater participation of the robusta coffee in *blends*, roasted and ground, *espresso* and soluble, new direction is being given to breeding programs for this characteristic analysis under different aspects.

The coffee beverage is made up of hundreds of substances, some of which are transformed during processing, leading to a change in the quality of the beverage and making it difficult to evaluate. There has been evolution regarding the basic understanding of the chemical composition of green and roasted coffees, but the relationship of organoleptic quality with the chemical elements is not yet well elucidated. It is known that different groups of substances are correlated and interfere in quality, such as: chlorogenic acids with bitterness; sugar and amino acids with taste and flavor; trigonelin with the aroma, among other substances which are being researched. The studies have shown that the quality of the robusta coffee can be improved, as well as the genotypic variability existing in *C. canephora* and the inheritance of the factors that lead to the improvement of the product quality should be better evaluated.

Moschetto et al. (1996) analyzed the genotype effect on the quality of the beverage working with different *C. canephora* genotypes and interspecific hybrids, involving the Congolense x Guineano groups and observed a significant difference between genetic materials in relation to the different organoleptic characteristics. They verified that the genotypes originated from the Guinean group were less “appreciable” than those of Congolense. And the cross involving these two groups originated superior descendants. They observed, in general, unpredictable aroma in the genetic materials and the best clones presented little body, low bitterness, natural aroma and some with low acidity. In the study of correlations among 11 characteristics studied, in 39.39% of the cases, there were positive correlations between the characteristics, with variation of 0.05 to 0.74, with the following highlights: bitterness and body (0.74); pH and bitterness (0.53); pH and volume (0.58); intensity of color and acidity (0.59); intensity of color and body (0.57). In 60.61% of the cases, the correlations between characters were negative, with magnitudes from -0.08 to -0.76, with emphasis on preference and aroma (-0.76); body and acidity (-0.47); bitterness and acidity (-0.55) pH and acidity (-0.66) and intensity of color and bitterness (-0.65).

Ferrão et al. (2015 b, 2015c, 2015d) after over 20 years of research studying more than 2000 clones of the Incaper conilon coffee breeding program, 27 superior clones for different agronomic, biochemical and sensory characteristics were selected. These clones were grouped by fruit maturation times and, thus, the first conilon coffee cultivars with superior drinking qualities were developed, which are ‘Diamante ES8112’, ‘ES8122’ - Jequitibá and ‘Centenária ES8132’, which are found registered and protected at the Ministério da Agricultura, Pecuária e

Abastecimento - Mapa (Ministry of Agriculture, Livestock and Supply).

4 OBJECTIVES AND STRATEGIES FOR GENETIC IMPROVEMENT

In order to meet most crop demands, the main objectives of *C. canephora* breeding programs in Brazil are: obtaining cultivars with high productive capacity and bean quality, as well as agronomic and botanical characteristics desirable for different cropping systems; evaluating and selecting cultivars tolerant to the adverse conditions of environments (drought, high temperature, soils with low fertility and high levels of aluminum - Al); selecting cultivars for planting under irrigation; evaluating and regionally selecting clones and cultivars with superior agroindustrial characteristics; evaluating and selecting genetic materials for mechanical harvesting, for higher altitudes, for shading; increasing the number of accesses, maintaining and characterizing the clones, varieties and synthetic hybrids in active germplasm banks; selecting genotypes with higher total soluble solids content and lower caffeine content of the beans; identify DNA markers of interest for molecular marker assisted selection; performing basic studies on reproduction biology and genetic, biometric and cytological analyzes; adjusting methodology for *in vitro* propagation of *C. canephora* genotypes and interspecific hybrids.

Even though we know the economic and social importance of coffee and also the existence of favorable *C. canephora* alleles for use in intra and interspecific breeding, the research results to date are not expressive in the literature, thus, making the planning, execution and direction of strategies for the breeding progress with the species difficult.

For greater efficiency of breeding work, it is important to know the species and its floral biology, form of reproduction and propagation; the number of chromosomes; the existing germplasm and its location and ease of use; information about genetic basis, inheritance and heritability of the characters and correlations between them. The research site (s) should also be well defined, the strategies of the most appropriate methods of breeding and the conditions under which the experiments will be carried out, always considering the research objectives, the facilities, the necessary financial and human resources, and the time available to carry out the work.

Perennial plants are classified into three distinct groups based on the reproductive system: cross-pollination, self-pollination and vegetative propagation plants. In the first two cases, there is seed formation (sexual reproduction) by the union between male and female gametes, and in the autogamy, due to self-fertilization, the progenies from the same plant are genotypically similar; in cross-pollination, due to the cross-fertilization, the progenies derived from plants are usually heterozygous and heterogeneous. In asexually propagated plants, vegetative organs such as tubers, rhizomes, stems, cuttings, branches, buds, and other parts of the plant other than the seed are used for reproduction. In *C. canephora*, vegetative propagation has been a widely used practice, with multiplication of the genetic material by cuttings, which are formed by parts of the orthotropic branches. The seedlings originated from this process are called clonal seedlings.

In the conduct of breeding programs, it is of fundamental importance to define methods that allow greater ease of conduction, with greater genetic gains possible in shorter periods, maintaining the genetic variability of the species, but with the increase of the frequency of favorable alleles in the population.

Plant selection is based on the genotypic values of the individuals to be cloned and the additive genetic values of the individuals that will be used in the recombination. For this, the additive fraction of the genetic variance is necessary to predict gains in sexual reproduction and the non-additive fraction of the genetic variance to predict gains in asexual reproduction (RESENDE, 2002; CRUZ; REGAZZI; CARNEIRO, 2004).

The great genetic variability associated with self-incompatibility and the possibility of vegetative propagation in the *C. canephora* species favors the establishment of breeding programs to get faster genetic gains in relation to many other perennial species.

The strategies that are being used in breeding programs for *C. canephora* are the introduction of germplasm, clonal selection, hybridization, recurrent selection (CHARRIER; BERTHAUD, 1988; LASHERMES; COUTURON; CHARRIER, 1994; LASHERMES; PÉTIARD, 1996; PAILLARD 1999; FERRÃO, R.; FONSECA; FERRÃO, M. 1999b; FONSECA et al., 2002; FAZUOLI et al., 2009; MISTRO, 2013).

Clonal selection and the production of intraspecific hybrids, although they are not methods that aim at the transfer of specific characteristics between species, are breeding strategies very used in *C. canephora* (CHARRIER; BERTHAUD, 1988; FERRÃO, R.; FONSECA; FERRÃO, M., 1999; FONSECA, 1999).

Plants that propagate asexually normally exhibit great vegetative vigor by maintaining heterozygosity. The cross between highly heterozygous clones will provide the appearance of great genotype variability, allowing the emergence of descendants with advantageous characteristics that, by selection and cloning, may be superior to their parents.

Vegetative propagation has the advantage of fixing a genotype at any time, without the need of advancing generations for this purpose, as with self-pollination species, and it is possible to immediately take advantage of the higher individuals that occur at any stage of the breeding program, giving rise to uniform descendants, thus facilitating all phases of crop management. However, it presents as a disadvantage the narrowing of the genetic base of the population, which is a major concern in the establishment of breeding strategies.

In order to minimize this problem, it is recommended that breeding strategies in *C. canephora* involving asexual and sexual reproduction be conducted in parallel, since the first one promotes the narrowing of the genetic base of the genotypes obtained, the second allows genetic recombination, variability and providing gene maintenance in the population, which may be important in medium and long-term breeding programs. These strategies are used in the Ivory Coast, Africa (LEROY; CHARMETANT; YAPO, 1991; LEROY et al., 1993, 1994), in the States of São Paulo (FAZUOLI; MISTRO; BRAGHINI, 2009; MISTRO, 2013) and Espírito Santo, Brazil (FERRÃO, R.; FONSECA; FERRÃO, M., 1999; FONSECA et al., 2002; FERRÃO et al., 2007, 2015a; FERRÃO, R.; FERRÃO, M.; FONSECA, 2013).

Cross schemes such as *top crosses* and *diallelus* have been used in order to estimate the

combinatorial ability of the parents, especially the general combining ability in order to obtain synthetic hybrids. In the selection of parents for crosses, when there is a large number of genotypes, genetic divergence analysis can be used in order to be more effective in this choice in terms of required allele complementarity and, consequently, to be able to better express the desired heterosis in the produced hybrids.

The development, improvement and access to techniques such as molecular markers, anther, embryo and meristem cultures, dihaploid production, somatic hybridization and gene transformation, as well as the availability of computational resources suitable for multivariate analyzes, open new perspectives for this and other species breeding programs.

Within this item, the main strategies used in the breeding of *C. canephora* will be described, with emphasis on the introduction of germplasm, clonal selection, recurrent selection, hybridization and maintenance of genetic variability.

4.1 INTRODUCTION OF GERMPLASM

The introduction of germplasm includes the entry of seeds, vegetative parts, pollen grains, *in vitro* cultures and DNA.

The introduction consists of bringing genetic materials from active germplasm banks and collections of national or even international programs to the regions of interest and evaluating them for environmental adaptation and acceptance by producers and consumers. Populations can also be introduced, evaluated and subsequently improved, as well as cultivars that, after being experimentally evaluated, can be recommended for planting.

Many of the cultivated species, mainly in marginal regions and/or where there is no breeding program, were recommended through the introduction. The introduced genetic materials that present characteristics of interest can be kept in collections, for being used in future works.

In the particular case of *C. canephora*, the introduction of germplasm played an extremely important role, allowing the establishment of populations with wide genetic variability, in which genetic material can be found with adaptability to the most diverse environmental conditions of cultivation, constituting itself in excellent quality material to breeding programs (FONSECA, 1996). Most of the *C. canephora* commercial crops of the Conilon variety planted in different parts of the country, such as in the states of Rondônia, Bahia, Pará, Mato Grosso, among others, originated from introductions of genetic material from the State of Espírito Santo.

4.2 CLONAL SELECTION

The vegetative or asexual propagation is of great importance for the fixation of the genetic constitution of the plants and allows the clones formation. A clone can be defined as a set of individuals genetically derived from a single plant and that spreads through vegetative means such as cuttings, forks and buds, among others. Plants of the same clone are therefore genetically identical to each other and to the plant that originated them.

Clonal selection is the main selection method used for vegetative propagation species,

including *C. canephora*. It is practiced sequentially, starting with the selection of individual plants with characteristics of interest, which are cloned and evaluated in an appropriate experimental design. It is an important strategy in conilon breeding programs as it is one of the quickest ways to obtain genetic gains. Once the desired allele combination has been identified for the characteristics of interest, it can be fixed and multiplied with the use of cloning.

In several countries, selections of clones that have the alleles of interest aiming at the development of superior cultivars have been relevant breeding strategies in *C. canephora* (CAPOT, 1966; DUBLIN, 1967; FERWERDA, 1969; BOUHARMONT; AWEMO, 1980; VAN DER VOSSSEN, 1985; BOUHARMONT et al., 1986; BRAGANÇA et al. 1993, 2001; FONSECA, 1996, 1999; FERRÃO, 2004; FONSECA et al., 2004a; FERRÃO et al., 2000, 2007; 2012, 2014, 2015a, 2015b, 2015c, 2015d, 2015, 2017; FERRÃO, R.; FERRÃO, M.; FONSECA, 2013).

The continuity of success in the breeding program through clonal selection depends on the magnitude of available genetic variability. Within this context, in the breeding program, strategies should be planned so that the maximum genetic gain does not occur in a single generation and include in cloning a recurrent selection scheme, whose progress in increasing the frequencies of favorable alleles can be capitalized step by step.

Some issues should be considered in the use of clonal selection as a breeding strategy: 1) initial size of the base population - due to the total release of genetic variability at one time, the initial population should be large, to increase the probability that the superior genotype is present in that population; 2) large base population - induces the need for a large uniform area for evaluation; 3) long juvenile period - this problem can be minimized through early selection using information from correlation studies; 4) high plant size - besides hampering the evaluations, there is a need to use larger spacing and, concurrently, large areas; 5) genetic variability for different selection characteristics - the population must have high frequency of superior individuals, otherwise, clones superior to those already cultivated by the producers will not be selected; 6) repeatability of clones behavior over time - repeatability is important, since the clone selected in one step must have similar behavior in the next step.

The most appropriate ways to improve the efficiency of selection by cloning in perennial species are:

1) Early selection - seeking alternatives to decrease the juvenile period in order to reduce the time for the release of the cultivar for planting. It is necessary, in this early selection, to identify the characteristics of high heritability that are manifested precociously and have a high genotypic correlation with qualitative morphoagronomic characteristics and of interest that are manifested later. For high heritability characteristics, a high selection intensity in the juvenile phase should be applied; on the other hand, for characteristics of medium or low heritability, the selection intensities must be medium and low, respectively.

2) Selection index - a selection index should be formed based on the definition of the clone evaluated in the different stages of the breeding. The efficiency of using this index is directly correlated to the characteristics of high heritability. Even providing little contribution at each stage of breeding, such index, after several selection steps, may contribute to a significant genetic gain.

3) Families selection - the basic assumptions are that genetic variation occurs both between and within families and presents a standardized normal distribution. In this situation, in the first place, the selection is made between, identifying the superior families and, later, the selection inside, when the plants of interest within the prominent families are selected.

The success of clonal selection also depends on the way it is performed, that is, whether based on phenotypic characteristics or whether based on the behavior of its progenies, the latter procedure being the method that has provided the greatest success in selection. The evaluation of the progenies in different environmental conditions is of great importance, since it has been verified that the variability in *C. canephora* can be attributed, in part, to the environmental variation (FERWERDA, 1969).

The main characteristics practiced in the selection are the production potential, estimated from four harvests; the adaptability and stability of production for diverse environments; tolerance to drought, pests and diseases; the uniformity of fruit maturation; larger bean size with low percentage of mocha type beans; the high relation between cherry coffee and processed coffee; the low percentage of wilted beans; the biennial production; and low caffeine content (FONSECA, 1999; FERRÃO, 2004; FERRÃO et al., 2012).

In clonal selection, special attention is given to considering the adaptability and stability of the genotypes, expressed by the superiority of the genetic material and the maintenance of its behavior over time, respectively. The lower biennial variation is as important as the productivity potential in the selection of higher production stability clones.

In the case of the genetic self-incompatibility phenomenon among clones that have similar genetic constitutions in order to guarantee a satisfactory pollination, Charrier and Berthaud (1988) suggest to test the genetic compatibility before forming a clonal variety and also, that the cultivar should be made up of a minimum of eight clones to ensure the activity sustainability and avoid risks of genetic vulnerability. The nine clonal conilon coffee cultivars developed by Incaper for Espírito Santo are formed by the grouping of 9 to 14 clones (BRAGANÇA et al., 1993, 2001; FERRÃO et al., 1999, 2000, 2014, 2015a, 2015b, 2015c, 2015d, 2017; FERRÃO, R.; FERRÃO, M.; FONSECA, 2013).

In the development of these clonal cultivars, the following procedures were used: crop selection or base population with genetic variability; identification of the higher individuals; cloning of the selected higher individuals; experimental evaluation of the selected clones together with local witnesses, in experimentally designed trials, installed at representative places of the crop, for a minimum period of four harvests; selection and grouping of the superior materials for the different characteristics; genetic compatibility test among the clones of each group; multiplication of the superior clones in the clonal garden; and launching of the new cultivar with the availability of cuttings and/or seedlings for producers, nurseries, cooperatives, producers associations, city halls, teaching institutions, among others. The productivity values achieved by clonal cultivars are generally higher than those obtained by hybrid varieties. However, Charrier and Bertahaud (1988) stated that it is possible to obtain hybrid varieties producing from 75% to 100% of clonal cultivar productivity, although it is not possible in this case to maintain desirable uniformity among individuals due to heterozygosity of the parents.

By means of breeding programs in Brazil and worldwide, different clonal cultivars with average productivity higher than the hybrids and varieties spread by seeds were developed (DUBLIN, 1967; FERWERDA, 1969; CAPOT, 1977; CHARRIER; BERTHAUD, 1988; FERRÃO, R.; FONSECA; FERRÃO, M., 1999; FONSECA, 1999; FONSECA et al., 2002, 2004a; EMBRAPA, 2012; FERRÃO et al., 2015b, 2015c, 2015d, 2017) Special registration can be given to the conilon coffee breeding program in the State of Espírito Santo conducted by Incaper, whose clonal cultivars have reached productivity in different locations of the State, higher than 60 processed bags/ha under non-irrigated conditions and 120 processed bags/ha when irrigated, besides originating a better quality product (FERRÃO et al., 2014, 2015a).

Ferrão, R., Ferrão, M., Fonseca (2013) show that for obtaining the superior clonal cultivars of Incaper, at least 12 years of field research in environments representative of the crop were necessary for at least four crops, with evaluation of about 20 characteristics associated with the production and final quality of the product. The following is a brief description of the ten steps for obtaining a clonal cultivar, the illustration of which is shown in Figure 3.

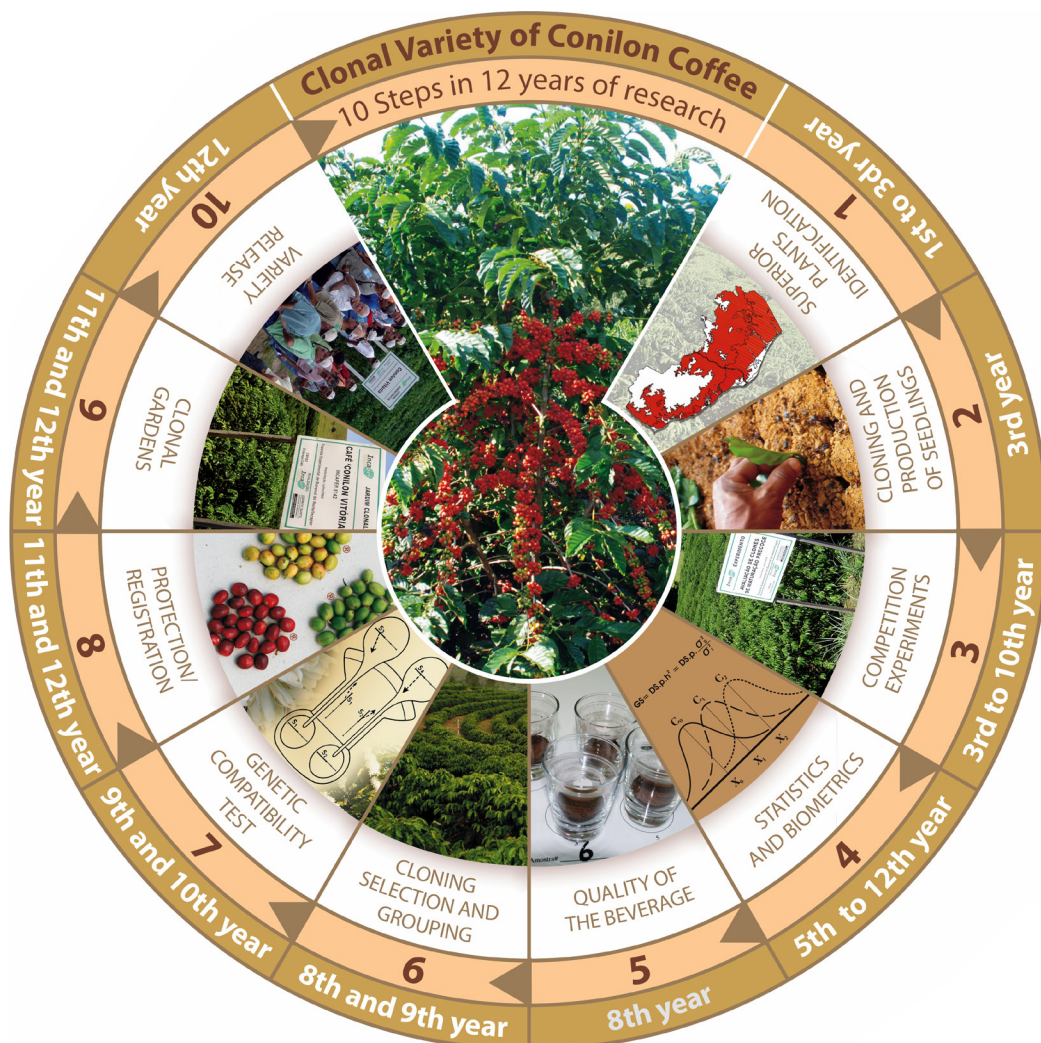


Figure 3. Flowchart for the development of conilon coffee clonal cultivars.

Source: Ferrão, R., Ferrão, M. and Fonseca (2013).

1. Identification of higher plants - the genetic variability (differences between plants in relation to the several characteristics) found in commercial crops of producers, spread by seeds, in introductions, in populations from fields of recombination and controlled crosses is used. Based on this variability, the superior plants, with high vegetative vigor, high productivity, drought tolerance, resistance to diseases, adequate architecture, large beans, uniformity of fruit maturation, among other characteristics are identified, marked and monitored.

2. Cloning and production of seedlings - the upper plants are identified and then cloned and taken to nurseries for the production seedlings aiming at evaluation in field experiments.

3. Competition experiments - the superior clones are evaluated in field experiments, in three representative environments of the culture (Experimental Farms of Marilândia, Sooretama, Bananal do Norte/Incaper), located in the Municipalities of Marilândia, Sooretama and Cachoeiro de Itapemirim, in the State of Espírito Santo, respectively, for at least four harvests, for about 20 characteristics associated with the production and quality of the beverage following the scientific method.

4. Statistics and biometrics - different statistical and biometric analyzes of the experiments involving genotypes, locations and years for different characteristics are carried out. The results provide reliability and scientific rigor to the work, thus constituting an important tool for the definition of future breeding strategies, sexual (seed), asexual (clonal) and in the clones definition and grouping for the formation of cultivars.

5. Quality of the beverage - chemical and sensory analyzes of the promising clones bean are carried out, with the objective of evaluating the contents of its chemical components and the sensory attributes associated to the quality of the beverage.

6. Clones selection and clustering - selection and clustering of the superior clones are based on observations and statistical and biometric analyzes for different environments and years considering the characteristics associated with coffee production and quality.

7. Genetic compatibility test - Controlled crosses between superior clones involving all possible combinations are performed, with the objective of evaluating fertilization and fruit formation. This test aims to avoid the formation of cultivars with self-incompatible clones.

8. Protection/Register - cultivar characterization is done based on the recommended descriptors for the species aiming at the protection of the cultivars. On the occasion of the launches, the cultivars are registered and protected at the Ministry of Agriculture, Livestock and Supply (Mapa). Registration is an indispensable requirement for the commercialization of seedlings of a cultivar. Protection guarantees the intellectual rights and knowledge associated with the technologies developed.

9. Clonal gardens - a network of clonal gardens, which are fields of vegetative multiplication, are implemented in partnership with city halls, cooperatives, nurseries, producers associations, educational institutions and research looking for making the cultivars available to producers.

10. Cultivar launching - the presentation of the cultivar to the society (lectures and technical scientific publications) is performed. This is the last phase before the application of different technology transfer methodologies (technical visit, field day, demonstration units), which aims at their inclusion in the productive systems, contributing to a positive evolution in

the activity.

The classical breeding scheme, which can be applied to the *C. canephora* species, is shown in Figure 4, when the ultimate goal of the work is to develop clonal varieties or varieties or synthetic hybrids.

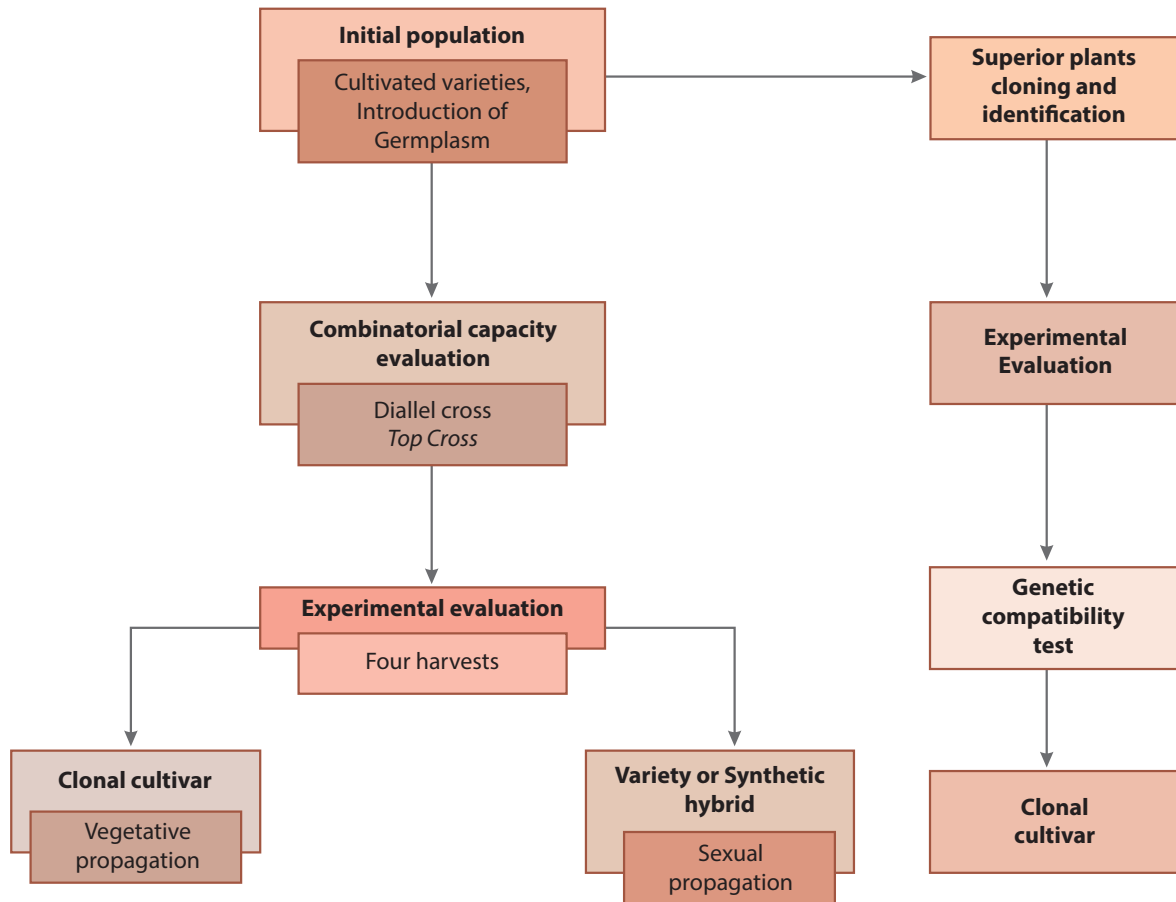


Figure 4. Classic scheme of *Coffea canephora* breeding for the obtaining of cultivars.

Source: Adapted from Ferrão et al. (2007).

4.3 RECURRENT SELECTION

In perennial plants, because of the greater complexity in conducting breeding programs, methods capable of producing practical results are needed within a relatively short period of time. Thus, improved cultivars should be created during the different stages of population breeding.

The continuous selection in a population can cause different problems, among them: narrowing of the genetic base due to the reduction of the genetic variability and the less probability of selection of genotypes superior to those already extracted in the previous generation (SOUZA JÚNIOR; ZINSLEY, 1985) being possible to lead to its exhaustion (GERALDI, 1997; PEREIRA; VENCOVSKY, 1988). The great challenge of the breeder is to monitor changes in the population in order to obtain the continuous gains for the selection, without characterizing

and reducing the genetic variability and the genetic base of the population.

Most economically important characters have quantitative inheritance, that is, they are, in general, controlled by several genes. The breeder difficulty is to seek breeding strategies aiming at increasing the frequency of the genes of interest favorable alleles in the population and consequently improving the character expression. The challenge is that the greater the number of genes involved in the expression of a given characteristic, the less likely it is to be able to use them all, mainly because, together with this difficulty, there is also the environmental effect interfering in the final expression of the characteristic. Thus, the frequency of favorable alleles can be increased continuously and gradually by successive cycles of selection, through the recurrent selection (HALLAUER, 1992; RAMALHO, 1994; GONÇALVES; SOUZA SOBRINHO, 1999).

Recurrent selection can be defined as the systematic selection of desirable plants from a population, followed by their recombination and evaluation to form the new improved population which in turn is used as the base for a new selection cycle and thus, successively. The difference between the means for the different characteristics evaluated from the improved population and the initial population indicates the efficiency recurrent selection. The selected individuals are expected to be superior than the original population and the genetic variance is, at least, maintained at an appropriate level.

The objective of recurrent selection, as already mentioned, is to continuously and progressively increase the frequency of favorable alleles with successive selection cycles, maintaining and even recovering genetic variability and thereby promoting the populations breeding, which may be used as a cultivar per se or as a source of superior individuals, in the process of clonal selection, hybridization or as progenitors of improved varieties. In this way, new superior genotypes can be extracted again, repeatedly fed back into the system and thus providing the long-term sustainability of the program. For Borém (1997), the success of the method results in the breeding of a population with superior performance to the base or origin population.

The recurrent selection proposed by Hull (1945) has been extensively used in the breeding of cross-pollinated plants in different cultures, initially with corn (COMSTOCK; ROBINSON; HARVEY, 1949). The success in the use of recurrent selection depends on the existence of base populations with genetic variability for the characteristic (s) of interest, allowing the identification and selection of superior genotypes after the evaluation of parents and/or plants and, at the same time, to maintain the population with sufficient variability to continue obtaining gains in the subsequent cycles (RAMALHO, 1994; DIAS; RESENDE, 2001; PEREIRA et al., 2002).

The different methods of recurrent selection are classified in recurrent intrapopulation and interpopulation selection. The first method consists in the breeding of the population per se, while the second one aims at the breeding of an interspersed population with another of interest.

Recurrent intrapopulation selection can be used in mass selection methods, selection between half-sibling families and complete siblings. The choice of the method depends on the characteristic to be improved, its heritability, the place and time available for program execution,

staff training, population variability and the type of reproduction and the genetic material propagation and the knowledge of the information in the literature as well. It capitalizes on the additive effects of alleles and thus, is a powerful tool for population breeding. It is easy to be conducted, possible to work with larger populations and, depending on the characteristic (s) being improved. It can provide compatible genetic gains, with less time and cost, compared to other more sophisticated breeding methods. It should be applied as an initial selection method of breeding when the main objective of the work is the populations breeding to obtain varieties propagated sexually or when one does not yet have information about the levels of dominance associated with the main characters of the selection.

According to Paterniani and Miranda Filho (1987) and Hallauer (1992), recurrent selection consists mainly of the following phases: identification of the superior individuals of the population to be improved; evaluation of superior genotypes in experiment with repetitions, if possible in more than one environment; and recombination or intercrossing of the superior individuals in an isolated free pollination field to form the next selection cycle (Figure 5). The genetic gains for the characteristics prioritized in the surveys are obtained with the advances of the selection cycles (Figure 6).

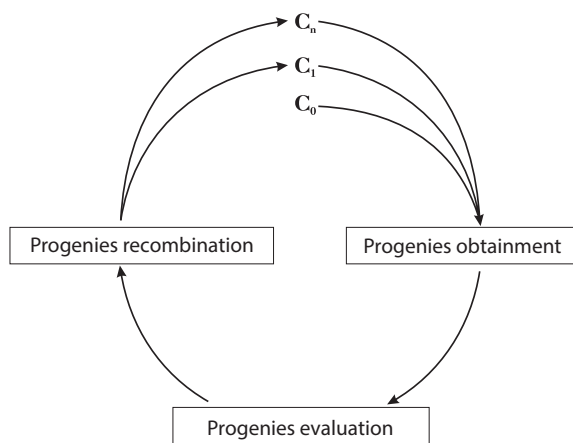


Figure 5. Demonstrative scheme of recurrent selection.

Source: Borém (1997).

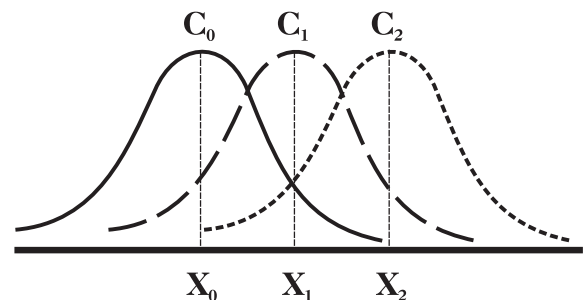


Figure 6. Genetic gains obtained by recurrent selection.

Source: Adapted from Ferrão et al. (2007).

Recurrent selection has been successfully applied in different tropical perennial species, such as in cocoa (LOCKWOOD; PANG, 1993; BARADAT; LABBE; BOUVERT, 1994; PAULIN, 1994; PIRES et al., 1999), in eucalyptus (RESENDE; HIGA, 1990; NAMKOONG; KANG; BROVARD, 1998; RESENDE, 1999; RESENDE; FERREIRA; MUNIZ, 1999) and in *C. canephora* (LEROY et al., 1993, 1994, 1997; FERRÃO, R.; FONSECA; FERRÃO, M., 1999; FAZUOLI; MISTRO; BRAGHINI, 2009; FERRÃO et al., 2012, 2015).

In the State of Espírito Santo, Incaper has been working with the recurrent selection strategy since 1997 in populations of early maturation, intermediate maturation and late maturation. The populations were formed by the recombination in isolated fields of clone groups with different maturation periods. In each population, superior clones were recombined and each progeny

of the different half-sibling families was evaluated for different characteristics over consecutive years. Populations are in the second selection cycle. After biometric evaluations to verify the genetic changes of each population, the superior individuals were identified, incorporated into asexual (clonal) breeding strategies, and grouped to advance in new selection cycles and also, to be used for the formation of new seed propagated cultivars.

Vicentini (2013) and Vicentini et al. (2015), carrying out biometric analyzes in the late fruit maturation of the Incaper conilon coffee breeding program, verified the existence of genetic variability for different characteristics in the set of evaluated progenies, allowing the continuity of the recurrent selection process, with the possibility to succeed. The analyzes of genetic divergence results showed dissimilarity among genetic materials, indicating the possibility of success in obtaining hybrids.

4.3.1 Recurrent selection with the use of vegetative propagation

The success in the use of vegetative propagation occurs when, in the base population, superior and desired genotypes are present; otherwise, the effort expended for the clones evaluation will not result in gains in the development of superior cultivars.

Several schemes could be used in recurrent phenotypic selection with the use of vegetative propagation. Figure 7 shows a very efficient scheme in perennial culture that can also be used for *C. canephora*.

The beginning of the program occurs with the definition of the base population, which can be obtained by the use of genetic material from producer crops, where great genotype variability is found for the different characteristics studied; by controlled crosses using the pollen mixture of all parents; by controlled crosses involving the parents of interest; or by recombination of a group of genotypes with characteristics of interest. The superior plants, after being vegetatively propagated, are evaluated in experimental tests. The clones that stand out undergo the genetic compatibility and clustering test to later be recombined in an isolated field, and then constituting a recurrent selection cycle. The cultivars or other superior clones of different breeding programs, or even generated in the program itself, might enter, at any moment, in the recombination scheme, contributing with favorable alleles for the target population of the selection.

4.3.2 Reciprocal recurring selection

Reciprocal recurrent selection is a breeding method that has been widely used in cross-fertilization since 1960, mainly for selection of quantitative inheritance characteristics under polygenic control, such as bean productivity. It is used when there are two divergent populations and with complementary genes, in which selection and recombination process there is an increase in the frequency of favorable alleles in the two breeding populations.

Reciprocal recurrent selection should be used when the objective of the work is to seek the population breeding per se and in crosses aiming at exploring heterosis. It consists of the

identification of productive populations with characteristics of divergent and complementary interests. This breeding method makes it possible to explore both the GCC, associated with the additive genetic variance, and the SCC, related to the non-additive genetic variance.

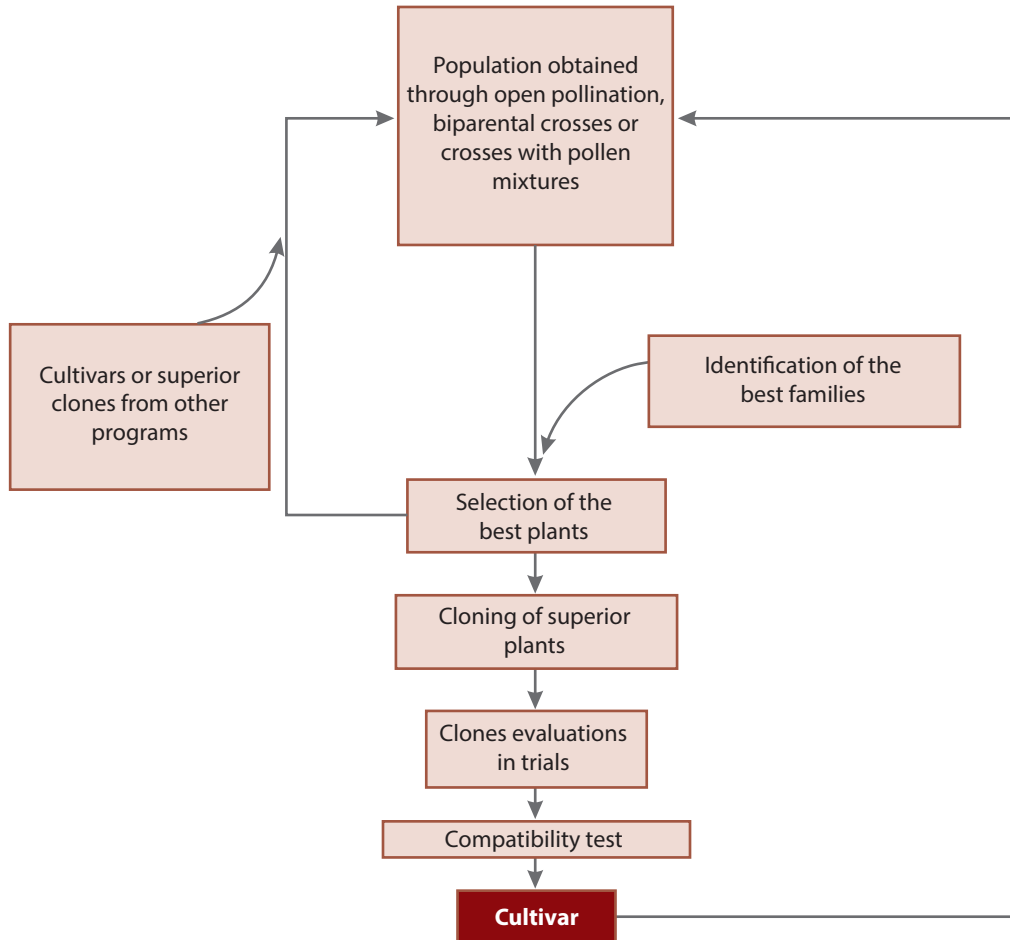


Figure 7. Recurrent selection scheme with the use of vegetative propagation.

Source: Adapted from Ferrão et al. (2007).

Thus, this type of selection must be used under the following conditions: when it is desired to simultaneously explore the GCC and SCC, when genetic divergence is known among the base populations, when one has information about the allelic dominance in the loci that control the character of interest, when the program aims at obtaining hybrids and when there is training, structure and skilled labor to carry out the work. Attention should also be paid to the final objectives of the work - whether it is aimed at obtaining commercial hybrids and/or clones and whether the effect of specific ability of combination is statistically significant. In species of some crops, such as robusta coffee, coconut, eucalyptus, rubber tree, cocoa, recurrent reciprocal selection is the suitable breeding strategy.

Reciprocal recurring selection works in *C. canephora* began in 1984, in the Ivory Coast, using two divergent populations of different geographical origins, known as Congolese (Group 1 - Central Africa) and Guinean (Group 2 - West Africa) (BERTHAUD, 1986). In this study, the

genotypes of each population, combined with several testers of the reciprocal population (LEROY et al., 1993), were evaluated, since there is a predominance of the general combining ability (CHARRIER; BERTHAUD, 1988). The best hybrids and clonal cultivars currently planted in the Ivory Coast are derived from breeding involving the crosses of these groups (LEROY et al., 1993, 1994, 1997). According to the authors, the main characteristics of each population are the following: Guinean - susceptibility to leaf rust, high caffeine content, low seed weight, drought tolerance, good sprouting, short internodes, slightly elongated leaves and early maturation. Congolese - resistance to leaf rust, medium caffeine content, larger size seeds, susceptibility to drought, low sprouting, long internodes, large leaves and late maturation of fruits.

The main results of these studies were the presence of genotypic and phenotypic variability within and between the Guinean and Congolese populations for plant architecture, resistance to drought, vigor, organoleptic characteristics, resistance to pests and diseases; superiority of the hybrids descended from the intergroup cross in relation to the intragroup cross, justifying the efficiency of reciprocal recurrent selection; efficiency of recurrent selection in populations breeding per se and in obtaining superior hybrids; and variation from 0.22 to 0.78 of heritabilities for characteristics related to plant architecture, from 0.13 to 0.40 for plant vigor and from 0.15 to 0.28 for those associated with beans weight. The phenotypic and genotypic correlations between plant productivity and vigor were high; there was no high correlation of bean weight with vigor and production; there was a good correlation between the crown diameter of the four year old plants with the productivity of two to five year old plants. Regarding the estimates of genetic gains for different characteristics, Leroy et al. (1997) verified significant progress, whose productivity of hybrids involving elite parents from the both populations were from 16% to 140% higher than the two commercial clonal varieties, and the most productive cross was more vigorous. By selecting 5% of the best plants, the work showed high genetic gain expectations with a value higher than 60% for production; moderated with rates of 14% to 18% for young plant vigor and low for crown diameter, being possible to predict 60% of the genetic gains in selected plant productivity compared to the most productive clone used as control.

Reciprocal recurrent selection can be illustrated by the Figure 8 flowchart.

4.4 HYBRIDIZATION

Hybridization has been one of the best breeding strategies in order to aggregate in a single individual characteristics of interests found in different genetic materials. There are many examples of success involving hybridization in species of economic and social importance in the world scenario, such as wheat, potatoes, vegetables such as tomatoes and cabbage, sugarcane and fruit plants, which multiply vegetatively. The best example is the hybrid corn, developed and cultivated in different regions of the world.

In breeding programs through hybridization it is of fundamental importance to select the breeders with base on agronomic characteristics and biometric studies, such as diallel cross and genetic divergence, adaptability and stability.

Hybridization in *C. canephora* may be interspecific or intraspecific. The second is greatly

facilitated in *C. canephora* by the existence of the gametophytic system of self-incompatibility, which can dispense the emasculatation.

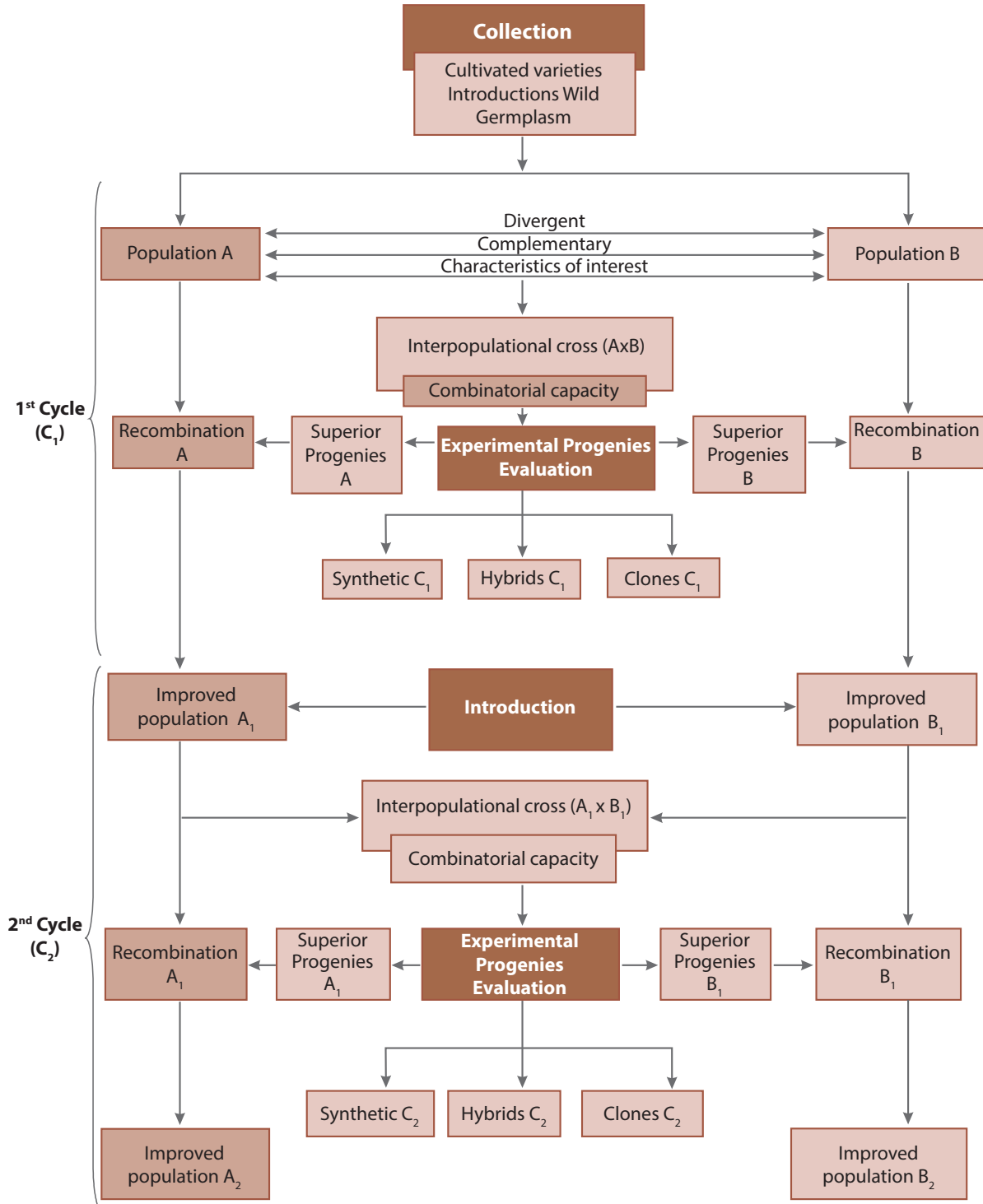


Figure 8. Reciprocal recurrent selection flowchart.

Source: Adapted from Leroy et al. (1993) by Ferrão et al. (2007).

4.4.1 Intraspecific hybridization

In order to perform artificial (manual) intraspecific hybridization, the following steps are taken: definition in advance of the parents; protection with appropriate bags of productive branches of plants that will be used as male and female parents, about three to five days before the anthesis; elimination of flower buds that are already open; and monitoring of the parents as to the correct timing for the artificial pollination.

In order to carry out the crosses, the plagiotropic branches containing the open flowers, which will work as male parent, pollinators of other plants, properly identified, should be detached and taken to the female parent, also identified, of another plant. With the manual rupture of the both sacks, the male inflorescences containing the open flowers with large amounts of pollen are placed in contact with the female inflorescences. Since the flowers of the female parent are also open, by an easy process of pollen grain detachment and there being no genetic incompatibility between the parents, artificial pollination is performed, thus initiating the fertilization process for the fruit formation. Each cross must be identified by a label with the numbering of the parents involved, the procedure date, among other important information.

Artificial pollination, in general, should start between eight and ten o'clock in the morning. The technician should monitor the unprotected flowers of the same plant or adjacent plants. This will give a good indication of the best time to perform the pollination.

The commercial production of hybrid seeds is carried out in isolated fields involving bi or multiclinal fields. Parents should be arranged in a way that facilitates natural pollination and that the flowering of the parents occurs synchronously. In a field of one hectare cultivated, it is possible to produce more than 1.000 kg of seeds, which are sufficient to install a coffee plantation in about 2,000 ha.

4.4.2 Interspecific hybridization

Hybrids among different species have aroused great interest from plant breeders. Many important attributes lacking in cultivated species are found in exotic and wild species. Thus, interspecific hybridization is a potential basic procedure for the transfer of alleles responsible for improving the characteristics of cultivated species.

The genetic structure of the genus *Coffea* favors breeding by introgression. Natural and artificial hybridization between species of this genus are possible, often giving rise to fertile hybrids (CHARRIER; BERTHAUD, 1988). Interspecific hybridization is an appropriate procedure for improving productivity, rusticity, beverage quality and other characteristics of interest.

Several superior cultivars originated from interspecific hybridization between *C. arabica* and one of these: *C. canephora*, *C. dewevrei* and *C. congensis*, developed, recommended and widely used by producers. Due to the different number of chromosomes of *C. arabica* in relation to the other species, interspecific hybridization, always involving it with the others of the genus, is difficult and prevents the formation and direct use of hybrids F_1 , being, in this situation, important the doubling of the chromosomes number. Researchers from several countries have

paid special attention to hybrids between *C. arabica* and *C. canephora* for two main reasons: improving the quality of *C. canephora* and, by reciprocal action, introducing alleles that confer vigor and resistance to diseases in *C. arabica* (CARVALHO; MEDINA FILHO; FAZUOLI, 1984).

Particular importance has also been given to the descendants of natural or artificial hybrids among the species *C. arabica* and *C. canephora*, as the 'Timor Hybrid', which has been widely used as a parent for resistance to rust of different cultivars developed in several countries and also in Brazil, including: Iapar 59, Tupi IAC 1669-33, Oeiras – MG 6851, Paraíso MG H419-1, IPR 98, Obatã IAC 1669-20, Pau Brasil, Sacramento IAC 125 RN and others (MONACO; CARVALHO, 1975; CARVALHO et al., 1991; FAZUOLI et al., 1996, 2002; PEREIRA et al., 2002). Several authors have described the 'Timor Hybrid', which may be cited (BETTENCOURT, 1981; CHARRIER; BERTHAUD, 1985; BETTENCOURT; RODRIGUES JUNIOR, 1988; CARVALHO, 1988a; PEREIRA et al., 2002).

Hybrids known as arabustas, originating from the cross between *C. arabica* and *C. canephora* tetraploids, have received special attention in many countries, especially in the Ivory Coast and Brazil (CAPOT, 1977). The production of tetraploids in *C. canephora* is done by treating seeds or ends of branches with colchicine (CARVALHO et al., 1991). These hybrids, besides vigor, present resistance to rust (CHARRIER; BERTHAUD, 1985).

The Icatu cultivars, originating from the *C. canephora* cv. Robusta x *C. arabica* cv. Bourbon and successive backcrosses with *C. arabica* cv. Mundo Novo, exemplify the success of interspecific breeding. The Icatu populations have great variability for several agronomic characteristics, presenting genotypes with resistance to many physiological types of the rust causing agent, being also rustic and productive (FAZUOLI, 1986; FAZUOLI et al., 2002).

The populations called 'Catimor' originate from the breeding involving the 'Timor Hybrid' (*C. arabica* x *C. canephora*) x 'Caturra'. They present many progenies resistant to rust (PEREIRA et al., 2002).

As already mentioned, many other species can be used in breeding work for presenting characteristics of interest, such as: drought tolerance (*C. racemosa*), low caffeine content (*C. eugenioides*) or even absence of caffeine in species of the *Mascarocoffea* section and resistance to nematodes (*C. salvatrix*) (CHARRIER; BERTHAUD, 1988).

The species *C. canephora*, *C. congensis*, *C. racemosa* and *C. dewevrei* have been used in crosses with *C. arabica* as important sources of alleles for resistance to pests, diseases, nematodes and tolerance to adverse environmental conditions. The resistance to the leaf miner (*Leucoptera coffeella*) is found in *C. stenophylla*, *C. racemosa*, *C. salvatrix*, *C. liberica*, *C. kapakata*, *C. eugenioides* and *C. dewevrei* (CARVALHO et al., 1991).

For the success of programs to obtain interspecific hybrids, it is of fundamental importance knowledge about the inheritance of these characteristics, as well as the dominance relationship (CARVALHO et al., 1991).

Caffeine content, although it is a qualitative characteristic, presents intermediate heritability (CHARRIER; BERTHAUD, 1988). The Laurina variety, possibly originated by mutation of the cultivar Bourbon of arabica has about half of the caffeine content of this species genetic material (KRUG; MENDES; CARVALHO, 1939). This characteristic, according to Costa (1965), cited by Carvalho et al. (1991), is governed by only one pair of genes (I_r I_r), constituting another

possibility for the reduction of this component in *C. canephora*, by interspecific breeding.

The interclones cross may constitute the simplest strategy to reduce the caffeine content in *C. canephora*, since the species presents great variability for the characteristic, with contents between 2% and close to 4%. For this, it is necessary to evaluate and select clones with lower contents, which can later be grouped together to form a new clonal variety as well as used in crosses to incorporate the alleles of this characteristic into other genetic material of better commercial performance .

The transfer of alleles responsible for characteristics of interest governed by few genes, found in related species, to *C. canephora* can be done using initially simple crosses, and later one or two backcrosses with the recurrent parent to recover the other important original characteristics of this recurrent.

For the characteristics governed by a greater number of genes, as is the case of bean production, it is necessary that the selection be performed after the performance of the progenies from the crosses evaluation. Despite the expressive gains obtained by phenotypic selection, this strategy has been more effective for high heritability characters and its efficacy in *C. canephora* breeding may be associated with the great variability in its original populations.

4.5 MAINTENANCE OF GENETIC VARIABILITY

The success of any breeding program depends on the existence of genetic variability in the base population. The choice of divergent parents used in the crossbreed for the formation of these populations ensures such variability. It is also desirable for such chosen parents to perform satisfactorily per se in relation to the characters of interest. Thus, previous analyzes about the general and specific ability of combination of the probable parents are of great value in determining the best possible choice of parents. Another concern is the maintenance of genetic variability during repeated selection cycles. It can be maintained through recombination with the use of appropriate samplings, so that the population effective size is not reduced (CRUZ; CARNEIRO, 2003).

In the breeding program of the species *C. canephora* germplasm Conilon of Incaper, different agronomic characteristics are evaluated. In the works of genetic divergence carried out by Fonseca (1999) and Ferrão (2004), high conilon variability was verified for different characteristics. From the phenotypic evaluations, the following average results were obtained: cycle (from flowering to harvesting) - from 227 to 300 days; plant height - from 1.90 to 3.30 m; crown diameter - from 2.35 to 3.08 m; maturation uniformity - from high to low; wilted beans- from 3.80 to 24.00%; flat beans - from 60.90 to 89.00%; mocha type beans - from 12.44 to 39.10%; weight of 1,000 flat beans - from 71.40 to 144.50 g; productivity index of 3.55 to 6.70; sieve of 17 or more - from 0.70 to 41.00%; sieve 15 - from 6.20 to 54.00%; sieve 13 - from 13.85 to 62.10%; sieve 11 - from 1.53 to 43.84%; caffeine content - from 1.20 to 4.00%; resistance to rust (*Hemileia vastratrix*) - from resistant to susceptible among other characteristics.

In order to maintain the genetic resources and to evaluate the genetic variability of the germplasm conilon of the State of Espírito Santo, Incaper has used phenotypic descriptors and

DNA markers to characterize the 500 genotypes present in the Active Germplasm Bank of the Institute, located in Marilândia/ES.

5 BIOMETRIC ANALYSIS: APPLICATIONS AND RESULTS

One of the great challenges for plant breeders is to overcome the productivity of today's best cultivars. In the different conduction stages of the breeding program, several factors interfere in the optimization of the expected gains, such as: the long cycle, which generates a prolonged exposure to several abiotic and biotic stresses, facilitating the appearance of new diseases, pests and nematodes; the long juvenile time, that prevents to accelerate the cycles of selection; the perennial culture of high size, which makes necessary the use of larger spacing and, concurrently, large experimental areas, making difficult the selection of uniform areas; the need to manage the crop by pruning, which makes it difficult to standardize in the field the same number of stems per plant for all treatments and parcels. Because of these factors and the pressures of the coffee production chain, which require breeding programs to solve the different problems, greater efforts should be directed to biometrics and biotechnology studies, associated to the methods and strategies of classic breeding to have more effective responses.

Biometric information is important in breeding as follows: in the identification of genetic variability of germplasm; in the definition of basic genetic material for intra- and inter-population breeding; in the definition of the parents for crosses; in the characterization of germplasm; in the definition of experimentation places; in the adaptation and stability of the genotypes to be released to the plantations per se or for being grouped in the cultivar formation; in determining the number of harvests needed to be accurate in defining the real value of the genotype; in the prediction of genetic gains; in the early selection of individuals or progenies involving characteristics that manifest in the early years and have facilities to be determined, with high heritability and desirable genotypic correlation with characteristics of low heritability.

5.1 MIXED MODELS

The success of a breeding program depends on the breeder's ability to select superior performance individuals. In order to achieve this goal, it is common to use statistical genetic models in the understanding of a particular biological phenomenon, to which one is interested. In plant breeding, for the estimate of treatment averages (eg genotypes), it has been common to use linear models in which all effects, with the exception of the residue, are considered of fixed nature. For specific cases of ordering higher individuals and considering balanced and orthogonal datasets, the use of conventional methods, such as Analysis of Variance (ANOVA), work relatively well. The difficulty arises when this procedure is performed in a set of unbalanced and unorthodox data (common fact in field experiments). For these situations, the use of the average for comparison and ordering of treatments is not considered ideal. In this scenario, the use of so-called mixed models, whose formal definition will be presented later, is considered a more suitable approach to allow the analysis of unbalanced data and to explore the existence

of biological covariance.

The use of this statistical approach for breeding purposes is due to the pioneering work of the American researcher C. R Henderson, who in the 1940s developed and applied BLUP (*Best Linear Unbiased Prediction*) for the selection of superior animals. The understanding of BLUP and, consequently, of the term mixed model goes through the definitions of fixed and random effects.

Formally, a factor (treatment, block, year ...) in a linear model is considered to be a fixed effect if it is constant and attributed to a finite set of treatments. In this case the conclusions obtained in the study are restricted only to the levels of the considered factors. In other words, the biometric results can not be expanded to other scenarios. On the other hand, when considering a random effect factor it is assumed that it was sampled from a representative population, whose responses are accomplishments of a random variable with distribution function of a predefined probability. Commonly, it is assumed that these effects are normally distributed around a common average and with specific variances (McCULLOCH; SEARLE, 2001). The practical result when considering the effect of treatments (genotypes) as random, for example, is that the ordering of the best genotypes is performed more accurately, considering factors other than just the averages.

Defined the effects nature, models will be considered mixed if they are made up of fixed and random effects factors. For this situation, the fixed effects prediction will be called *Best Linear Unbiased Estimator* (BLUE) and the random effects prediction will be called the best linear unbiased predictor (BLUP). The term 'Best' is in the sense that estimators minimize sample variance; 'linear' in the sense of being functions of the observed phenotypes; and 'unbiased', because the expectation of the estimate is equal to the parameter (LYNCHE; WALSH, 1998).

Resende (2002) defines as mixed model the one that contains fixed and random effects in the same model, whose general average and experimental error are always classified as fixed and random, respectively.

Resende (2002, 2007), even recognizing that minimum squares method is the most usual, does not recommend its use to analyze data from perennial plants breeding, for the following reasons: the presence of fixed and random effects in the same model; imbalance caused by death of plants; possibility of obtaining negative estimates of variances; repeated measurements in the same individual over several years or times may cause correlated measurements over time, which damages the independence and homogeneity of the errors, which are basic presupposition to perform the analysis of variance. Another point to consider is that the data obtained in a same individual, over several years and analyzed in factorial structure in the arrangement of subdivided parcels, violate two presuppositions required by analysis of variance: the lack of randomization between treatments and harvests and the non-independence of errors due to the fact that the measurements are taken on the same experimental units over time (RESENDE; FERREIRA; MUNIZ, 1999).

Thus, the simultaneous solution for fixed effects estimation and random effects prediction is obtained through the so-called mixed model equations (MME) developed by Henderson in 1984. The matrix formulation of the statistical model, as well as the MME representation are

given below.

$$\mathbf{y} = \mathbf{XB} + \mathbf{Zu} + \mathbf{e}$$

where \mathbf{y} is the vector of phenotypic observations (data on productivity, disease, quality of drink ...); \mathbf{B} and \mathbf{u} are the fixed and random effects vectors, respectively; \mathbf{X} and \mathbf{Z} are the design matrices associated with the effects contained in \mathbf{B} and \mathbf{u} , respectively; and \mathbf{e} is the residual error vector of the model. It is assumed that the random effects of the model are distributed: $\mathbf{u} \sim N(0, \mathbf{G})$ and $\mathbf{e} \sim N(0, \mathbf{R})$ where \mathbf{G} and \mathbf{R} are the variance-covariance matrices of vectors \mathbf{u} and \mathbf{e} , respectively. Furthermore, it is assumed that $E(\mathbf{y}) = \mathbf{XB}$ and $\text{Var}(\mathbf{y}) = \mathbf{ZGZ}' + \mathbf{R}$. The equation of mixed models for simultaneous solution of fixed and random effects is given by:

$$\begin{pmatrix} X'R^{-1}X & X'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}Z + G^{-1} \end{pmatrix} \begin{pmatrix} \hat{\beta} \\ \hat{u} \end{pmatrix} = \begin{pmatrix} X'R^{-1}y \\ Z'R^{-1}y \end{pmatrix}$$

Note that for the solution of the represented equation it is necessary to know the variance-covariance matrices \mathbf{G} , \mathbf{R} and \mathbf{V} . Rare are the situations in which these matrices are known a priori and therefore are replaced by their estimates, which can be calculated based on the methods of the moments (FISCHER, 1918; HENDERSON, 1953), and the maximum likelihood method (ML) (HARTLEY, RAO, 1967) and restricted maximum likelihood (REML) (PATTERSON; THOMPSON, 1971). Commonly, methods based on REML are preferred in the case of mixed models. The use of estimated variance-covariance matrices makes the terms BLUE and BLUP no longer adequate, and it is appropriate to replace them with eBLUE (*Empirical Best Linear Unbiased Estimator*) and eBLUP (*Empirical Best Linear Unbiased Estimator*), that is, empirical BLUE and BLUP. The term 'empirical' is added to explain this approximation that is being carried out in the computation of the effects (DUARTE; VENCOVSKY, 2001).

When genotypes can be considered random, genetic effects become part of vector \mathbf{u} and can be estimated via BLUP. The genetic effect is defined as the decomposition of the genotypic value into components with genetic interpretation. A commonly performed decomposition is the division of genetic effects into additive and non-additive parts (dominance and epistasis). One of the primary objectives in breeding is to predict these genotypic values in order to identify those superior performance plants. One of the important properties of BLUP is the ability to maximize the correlation between true genotypic values and those predicted ones. In practical terms, this means reliability that, at the time of selection, the breeder is working with the best plants (PHIEPO et al., 2008).

A frequent question is when a factor should be considered fixed or random. In the literature, there are several texts that approach such dichotomy. However, it is common to consider that the choice of an effect nature is guided by two important information. The first one refers to the way in which the factor was sampled, that is, whether or not it constitutes a random and representative sample of a population (McCULLOCH; SEARLE, 2001). The second one is related to the purpose of the study (SMITH; CULLIS; THOMPSON, 2005). For the case of breeding studies, the definition of greater interest is about the nature of the genetic effects. Although non-genetic effects (blocks, year, environments, etc.) should not be neglected, they will not enter into the discussion of this chapter.

In breeding, for selection purposes, the objective is to identify the best materials among those evaluated ones. In such situations, it is interesting for the breeder that the genotype ordering is performed based on the true genetic effect of each plant. By definition, this case suggests the use of eBLUPs for ordering indicating, as soon as the effects are of a random nature. However, if the objective is only to compare genotypes pairs, then the use of eBLUPs is inappropriate and therefore the genetic effects should be treated as fixed (SMITH; CULLIS; THOMPSON, 2005). In practice, it is natural for breeding programs to initially consist of populations with a large number of plants, diverse and often unbalanced. In these situations, the data structure itself suggests that the genotype effect is of random nature. After several cycles of selection and in the final stages, in the called competition tests, the objective starts being the comparison between performance and materials. For this, the genotype effect is commonly considered as fixed. Although this is a common practice, there are authors who always argue in favor of using genetic effects as random. The argument is that results to be obtained are more realistic, given the properties of BLUP (SMITH; CULLIS; THOMPSON, 2005).

Another important argument in favor of the mixed models use, especially in the breeding of perennial species, is the measurement of what is called as repeated measures, which are defined as those performed in the same treatment, or in the same experimental unit, more than once. Longitudinal data are special cases of repeated measurements, in which observations are ordered by time. In coffee, an example of longitudinal data is the observations of productivity measured in the harvests, in different years for the same individual. An important point of the biometric analysis in this case is that, in general, the observations are correlated over time, which hurts the basic assumptions of independence and homogeneity of the classic methods of ANOVA errors. In this scenario, the use of mixed modeling is considered more appropriate because it allows the random part to be modeled with the inclusion of variance-covariance matrices that better portray the phenomenon. For this, the choice of these matrices is considered a key point and, therefore, different structures are tested in order to select the one that best adapts to the response standards of the observations (SMITH et al., 2007; MALOSETTI; RIBAUT; VAN, 2013).

Although it is a proven efficient method, the use of BLUP has not gained the same popularity in plant and animal breeding. Piepho et al. (2008) have argued that a likely reason for this is the high number of phenotypic data obtained per individual in the experiments with plants. According to the authors, for these situations, BLUE and BLUP do not offer very different results, giving the false impression that the estimator choice is not important. In addition, the same researchers punctuate the inaccurate estimates that can be obtained for the components of variance in the case of experiments with plants. One reason for this is the complexity of the structure that variance-covariance matrices can assume.

For Resende (2007), the use of mixed models has the following advantages: it models both fixed and random effects simultaneously; does not violate the assumption of independence, since it models the intraindividual correlation; in unbalanced data analyzes, estimates of the treatment effects are more accurate; it is not affected by translation (changes in the classification of effects in the model); does not generate negative estimates of variances.

In coffee, the number of studies involving mixed models is considered modest. In conilon, Cilas, Mongagnon, Bar-Hen (2011) used such an approach in the analysis of longitudinal data of *C. canephora* clones in order to investigate the temporal effect under beans productivity. For this, several structures of residual variance-covariance matrices were investigated. The best fit was obtained when considering the Compound Symmetry (CSH) structure, which indicated that the correlations between the production in the different years were constant. For this situation, the authors concluded that there was no evidence of biennial production effect.

For means of genotype selection and ordering, Mistro (2013) used mixed models to analyze a set of data from the progeny evaluation experiment of a robusta coffee population introduced from Costa Rica by the Instituto Agronômico de Campinas - IAC (Agronomic Institute of Campinas). The objective of the study was to quantify the genetic variability of genetic material and to verify the genetic potential of this population to submit it to recurrent selection. The results of the analyzes provided a safe definition of breeding strategies by sexual and asexual propagation for the genetic materials studied. The conclusions of this study will be used in the selection of superior genotypes that, in the future, may constitute a robusta coffee cultivar. A similar study was carried out by Carias et al. (2014) considering a set of Incaper conilon coffee clones with different fruit maturation times, which provided important results to assist in the future strategies of their breeding program. More detailed studies, involving not only the temporal effects modeling, but also the incorporation of spatial effects and complex interactions, such as the Genotype x Environment interaction, are being conducted in experimental populations of Incaper. It is expected that this information can be used in the more accurate selection of superior genotypes thus maximizing the genetic gains.

Despite advances in the number of coffee conilon studies in the last decade, the results are still not considered significant, especially considering the practical potential of such an approach. The existence of imbalances and the longitudinal structure, on which the experiments in *C. canephora* are based, are two strong arguments for this methodology to be incorporated in the breeding programs of plants. Detailed reviews about the importance and potential of this approach in plants are presented by Duarte and Vencovsky (2001), Smith, Cullis and Thompson (2005), Piepho et al. (2008), Malosetti, Ribaut, Van (2013).

5.2 ESTIMATES OF GENETIC PARAMETERS

Estimates of genetic parameters, such as phenotypic variance, genotypic variance, coefficient of genotypic variation and heritability, allow to know the structure and genetic variability of the population. These studies, therefore, may provide inputs to predict genetic gains and possible success in the breeding program. Such estimates are also important in redefining the breeding and selection methods to be used; in identifying the nature of the action of the genes involved in the control, especially the quantitative characters; and in the definition of efficient breeding strategies to obtain continuous genetic gains, but at the same time, without neglecting the maintenance of suitable genetic base in the population for future selection (CRUZ; CARNEIRO, 2003).

Estimates of genetic variances are generally obtained from analysis of variance mean squares, made according to the genetic and experimental design used. Once the expectations of the mean squares are known, the estimators of the variance components are obtained with some ease.

Estimates of genetic parameters can be obtained from certain genetic designs, such as COMSTOCK designs I and II; ROBINSON; HARVEY, (1949), diallel, family test, among others. These designs are of great importance because they provide estimates of the genotypic components of variance, which are used to calculate genetic parameters that are indispensable in the evaluation of work populations, orientation of more appropriate selection schemes and prediction of the breeding programs.

The additive variance has deserved great prominence because it expresses the similarity between related individuals and, therefore, is one of the components that determine the covariance between these individuals. Thus, it becomes an indispensable variance component to evaluate the success of a breeding program that is based on the covariance between the evaluated experimental material and the genetic material transferred to compose new breeding cycles or for marketing.

Most of the works available aiming at obtaining estimates of genetic parameters in the genus *Coffea* have occurred with *C. arabica*. The information of these works is not always appropriate to be applied in *C. canephora*, since the mentioned species are different and also that the main information of *C. canephora* are of African countries and obtained from genetic material groups distinct from the Brazilian conilon. Therefore, it is of great importance that this estimation is made in robusta coffee, since, as exposed by Falconer (1981) and Vencovsky (1987), the estimates of genetic parameters can be influenced by different breeding methods, genetic materials used, different environmental conditions and time and age of evaluation, among other factors.

In *C. canephora*, Leroy et al. (1994) found the following estimates of heritability: plant height; 0.37; stem diameter; 0.24; number of plagiotropic branches; 0.43; bean weight in the first harvest; 0.28; bean weight in the second harvest; 0.27; bean weight in the third harvest; 0.15; bean weight in the fourth harvest; 0.14; and weight of accumulated beans; 0.38. Montagnon et al. (1988) obtained these values of heritabilities: 0.73 for seed weight; 0.80 for caffeine content; 0.74 for fat content and 0.11 for sucrose content in the beans. According to the last authors, it is difficult to interpret the estimates of genetic parameter in *C. canephora*, since much of the genetic material used comes from different origins. For example, in this same study, the heritability for productivity in young plants was high (>0.70), but with adult plants it was low (<0.20). The characteristic weight of 100 beans was low to medium (0.15 to 0.30). The phenotypic and genetic correlations between plant vigor and productivity are high (> 0.60), but the correlation between 100 seed weight and productivity, important characteristics in the breeding, did not present a high and positive magnitude. Le Pierrès (1988) has stated that the caffeine content is an additive transmitted characteristic.

Fonseca (1999), Ferrão, R., Ferrão, M. and Fonseca (2003), Ferrão (2004), Ferrão et al. (2008) and Ramalho et al. (2011), in studies of estimates of genetic parameters involving different

characteristics and genotypes of conilon and robusta coffee, verified that in the analysis of variance there were significant differences ($P < 0.01$) among the genotypes, for most of the characteristics, indicating the existence of genetic variability among genetic materials; the genotypic coefficient of determination (H^2) was higher than 70% for most of the characteristics, reaching a value higher than 95%.

In studies of genetic parameters estimation of 60 Incaper conilon coffee clones, for 14 characteristics, Oliveira (2010) verified a low coefficient of genotypic determination for 78.50% of the characters indicating a great environmental influence and low genetic variability among the genetic materials studied.

Rodrigues (2010) and Rodrigues et al. (2012) verified the estimation of genetic parameters for three groups of clones of the Incaper conilon coffee breeding program, with different fruit maturation times for different years and characteristics. The estimates of coefficients of genotypic determinations (H^2) presented values between 71.49% and 84.86% for the maturation cycle; 74.10% and 93.94% for fruit size; 60.63% and 92.86% for plant size; 59.10% and 89.04% for plant vigor; 54.75% and 89.08% for visual evaluation index and 69.78% and 95.56% for beans productivity. The high average productivity, associated to these results, shows expressive genetic variability of the genetic material that might be successfully explored in the breeding.

Mistro (2013), studying progenies of a robusta coffee population of IAC, observed high genetic variability, possible to be explored both for clones and for recurrent selection.

Vicentini (2013) studied a late maturation population of Incaper conilon coffee fruits, which has been submitted to recurrent selection. Through the estimation of genetic parameters for five characteristics, significant genetic variability was observed for productivity, bean size and maturation uniformity. The results evidenced the possibility of continuous genetic gains using this breeding method for the mentioned characteristics.

Carias et al. (2014) carried out genetic parameter estimation for a set of Incaper clones with different fruit maturation times by the REML/BLUP procedure. They verified a low magnitude of the genetic values among the clones of the different maturation groups, being the highest value of the average heritability of the clones for the intermediate maturation group, followed by the early and late maturation, respectively. The value of selection accuracy of clones was 0.68; 0.69 and 0.18 for the early, intermediate and late groups, respectively.

These results expressed the existence of greater genetic variability in relation to the environmental variables, which, together with a coefficient of genetic variation (CV_g) that exceeds the coefficient of environmental variation (CV_e) for most of the characters, reinforce the suitable conditions for the breeding program execution and to be successful in selection work.

5.3 CORRELATIONS BETWEEN CHARACTERS

In the analysis of correlations, we try to determine the degree of relationship between two variables. The correlations between two characteristics may be phenotypic, genotypic or environmental.

Correlation studies have great importance in breeding programs, especially when the selection of the desirable character presents difficulty because it is a character of low heritability and/or problems of measurement or identification. The simple correlation allows to evaluate the magnitude and the meaning of the relations between two characters, being very useful in the breeding because it allows to evaluate the practical viability of the indirect selection, which, in some cases, can lead to faster progress than the direct selection of the desired characteristic. Correlations are shown as auxiliary tools in studies that aim at reducing the number of characteristics used in analyzes, such as in genetic divergence studies, in which the available characteristics are those that are redundant for being correlated with others that are easier to measure or that demand less cost and/or time for evaluation (CRUZ; REGAZZI; CARNEIRO, 2004).

In *C. canephora*, Conilon variety, phenotypic, genotypic and environmental correlations were estimated for eight characteristics, two by two, in terms of average of 80 genotypes evaluated by the Incaper Conilon coffee breeding program (FONSECA, 1999; FONSECA et al., 2003b). The results showed that the magnitudes of the genotypic correlations tended to surpass those of the phenotypic correlations, evidencing that the genetic factors had greater influence than those of the environment in more than 60% of the cases, thus allowing the simultaneous selection of several characteristics, since that the interest of the breeder is almost always in a group of them. The number of stems per plant correlated positively with the average crown diameter (0.657) and plant height (0.265) and null with average bean productivity (-0.084); the average plant height correlated positively with bean production (0.218); the growing positive values of the genotypic and phenotypic correlations of the first to the fourth harvest, especially with the last two harvests, with the average bean production, allowed to conclude that the greater accuracy in selection occurs after the second harvest; there were positive correlations with magnitudes of 0.842 and 0.820 between the third and fourth harvest, respectively, with the average bean production, thus showing that additional harvests may increase the reliability level of the real value prediction.

Ferrão (2004), studying the correlations between plant characteristics and evaluating different genotypes in two sites in the State of Espírito Santo, verified that in 95.45% of the cases the magnitudes of the genotypic correlations tended to overcome the phenotypic correlations, showing that the genetic factors had greater influence than the environmental ones. In 72.73% of the cases, the phenotypic, genotypic and environmental correlation coefficients presented accord of signs, indicating, therefore, the low environmental influence in the association between the characters.

Cilas et al. (2006), after studying six clones of *C. canephora*, concluded that the architectural traces of these have high heritability and some of them were strong and genetically correlated with the production. In particular, the number of nodes in the plagiotropic branches proved to be a good indicator of production for more than two consecutive harvests with genetic correlation (0.884).

Esther and Adomako (2010), aiming at the selection for high productivity in experiment in Ghana, studied the genotype and environmental correlations between agronomic variables

and the production of 18 genotypes of *C. canephora*. The correlation between production and growth vegetative variables of plants from the seventh harvest was positive with: plant height (0.65), crown diameter (0.60), stem diameter (0.55) and the number of primary branches (0.53). They observed that the average production of the first three harvests correlated better with the average production of the last three harvests (0.79), indicating its possible use for predicting the productive capacity in robusta. The results show that selection of genotypes for high productivity should be based on a selection index involving plant height, crown and stem diameters and number of primary branches of the plant during the first three years of production.

5.4 ESTIMATES OF REPEATABILITY

Repeatability studies are of great importance in breeding programs for animals and perennial plant species. The repeatability estimate aims at determining the number of measurements needed in an individual, a cultivar or a clone to predict their real value with some level of probability. Such an estimate is possible to obtain when the measurement, both in time and space, of a given characteristic is made repeatedly in the same individual.

For Turner and Young (1969), when successive measurements of a characteristic are taken in a group of individuals, the initial superiority or inferiority of the individual in relation to the others is expected to be maintained in subsequent measurements. For these authors, checking the consistency of the relative position of individuals during successive measurements has traditionally been called repeatability. It is a function of the general average, the genotypic effect on the individual's characteristic, the temporary and/or localized effect, or the environment on that individual and expresses the proportion of the total variance, which is explained by the variations provided by the genotype and the changes attributed to the common environment. When the variance provided by the permanent effects of the environment is minimized, the repeatability approximates heritability (CRUZ; REGAZZI; CARNEIRO, 2004). According to Falconer (1981), repeatability depends on the population genotypic properties, the character under study and the environmental conditions in which the individuals were kept.

A relevant application of the coefficient of repeatability estimation is that it allows to determine the number of phenotypic observations that must be made in each individual so that the selection can be carried out with adequate accuracy and reduction of time, cost, efforts and skilled labor. The greater the coefficient of repeatability, the greater the regularity of the character expression from one measure to another, and consequently one can predict the real value of the individual, with a relatively smaller number of repeated measures.

The coefficient of repeatability can be estimated by different methodologies, such as analysis of variance, principal component analysis - effectively obtained by means of the covariance and correlations matrix - and by the structural analysis method using correlation and covariance matrices. These methodologies were described in detail by Cruz, Regazzi and Carneiro (2004).

According to Abeywardena (1972), when genotypes show cyclic behavior along the

evaluations in relation to the studied characteristics, the most suitable estimate of this coefficient is that one obtained by the method based on the main components, both through the phenotypic matrix of variances and covariance and by the matrix of correlations. The varieties of *C. canephora* present cyclic production behaviors, and, in fact, the estimation of repeatability by the main components method has been more efficient (FONSECA, 1999; FERRÃO et al., 2003b; FONSECA et al., 2003c, 2004b; FERRÃO, 2004).

Resende (2001) presented estimates of the coefficient of repeatability for several perennial species, such as eucalyptus, rubber tree, cacao, coconut, cupuaçu, guaraná involving different characters. The average coefficient of repeatability was 0.66, with a variation from 0.45 to 0.92. This coefficient magnitude depends on the heritability of the studied characteristics and the method used for its estimation. The author classifies the coefficient of repeatability into three classes: high repeatability, $r > 0.60$; moderate repeatability, $0.30 < r < 0.60$; and low repeatability, $r < 0.30$.

Fonseca et al. (2003c, 2004b) estimated the coefficient of repeatability for bean production in *C. canephora*, based on the behavior of 80 genotypes of the Conilon variety in Marilândia, in the State of Espírito Santo, using analysis of variance methods with a temporary effect of environment removed from the error and main components obtained by these two procedures, correlation matrix and covariance matrix. The methods used provided different estimates of repeatability, and the highest value was obtained by the third method. The precision in the prediction of the real value of the individuals, demonstrated by the coefficient of determination (R^2), based on four harvests, ranged from 65.32% to 81.59%, depending on the method. By increasing the number of harvests from four to six, the precision increased to R^2 values between 73.84% and 86.92%. From the sixth harvest, however, this increase became unimpressive, no longer justified, considering the time required and the costs incurred (Figure 9).

Ferrão et al. (2003b) estimated the coefficient of repeatability for seven harvests in 50 conilon coffee genotypes in the State of Espírito Santo using the analysis of variance methods, principal components using correlation and covariance matrices, structural analysis using correlation and covariance matrices, in addition to estimating the number of harvests needed to express the genotype real value. It was verified that the main component method, using a covariance matrix, showed the highest coefficient of repeatability ($r = 0.662$) and the highest accuracy with $R^2 = 93.19\%$, with five harvests being enough to have a reliability of 90% to predict the real value of the individual.

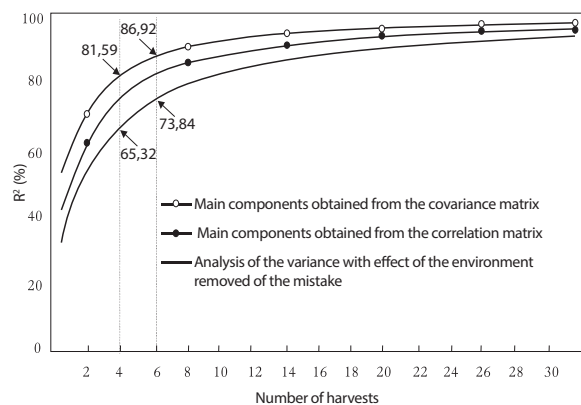


Figure 9. Estimation of the coefficient of determination (R^2) to verify the accuracy of three estimate of repeatability methods according to the number of harvests, Marilândia/ES.

Source: Fonseca (1999).

In a repeatability study for the productivity characteristic, for 40 genotypes, using different methods, for seven harvests at two sites in the State of Espírito Santo, Ferrão (2004) verified that the main components method for the covariance matrix presented the highest coefficients of repeatability, that is, 0.501 in Sooretama and 0.432 in Marilândia, with coefficients of determination of $R^2 = 87.56\%$ and $R^2 = 84.19$, respectively (Tables 1 and 2). These results indicate that this method of the main components was more adequate to estimate the coefficient of repeatability, because it provides greater accuracy in the expression of the genotype real value, since it took into consideration the genotypes behavior regarding the biennial trait of the studied characteristic. Still in the study, with this same method, it was noticed that in order to have a high accuracy in the real value of the genotypes, in the order of 80%, four harvests were required in Sooretama and five in Marilândia (Tables 1 and 2 and Figures 10 and 11).

Table 1. Coefficient of repeatability (τ) and determination for seven harvests and estimates of the number of harvests needed to obtain different R^2 in the five methods, in Sooretama/ES, 2004

| Methods of estimation | Number of measurements (n) * for different R^2 | | | | | | |
|---------------------------------------|--|-------|---------|---------|-----------|-----------|-------------|
| | τ | R^2 | 0,80 | 0,85 | 0,90 | 0,95 | 0,99 |
| 1) ANOVA | 0,386 | 81,48 | 6,36(6) | 9,01(9) | 14,32(14) | 30,20(30) | 157,50(157) |
| 2)) Main components - covariance | 0,501 | 87,56 | 3,98(4) | 5,64(6) | 8,95(9) | 18,90(19) | 98,49(98) |
| 3) Main components - correlations | 0,425 | 83,79 | 5,42(5) | 7,67(8) | 12,19(12) | 25,73(26) | 134,05(134) |
| 4) Structural analysis - correlations | 0,407 | 82,81 | 5,81(6) | 8,23(8) | 13,08(13) | 27,61(28) | 143,84(144) |
| 5) Structural analysis - covariance | 0,386 | 81,48 | - | - | - | - | - |

*Approximate number.

Source: Ferrão (2004).

Table 2. Coefficient of repeatability (τ) and determination (R^2) for seven harvests and estimates of the number of harvests needed to obtain different R^2 in the five methods, in Marilândia/ES, 2004

| Methods of estimation | Number of measurements (n) * for different R^2 | | | | | | |
|---------------------------------------|--|-------|---------|-----------|-----------|-----------|-------------|
| | τ | R^2 | 0,80 | 0,85 | 0,90 | 0,95 | 0,99 |
| 1) ANOVA | 0,352 | 79,19 | 7,36(7) | 10,43(10) | 16,56(17) | 34,96(35) | 182,16(183) |
| 2) Main components - covariance | 0,432 | 84,19 | 5,26(5) | 7,45(7) | 11,83(12) | 24,97(25) | 130,09(130) |
| 3) Main components - correlations | 0,395 | 82,07 | 6,12(6) | 8,67(9) | 13,76(14) | 29,05(29) | 151,38(151) |
| 4) Structural analysis - correlations | 0,379 | 81,06 | 6,54(7) | 9,27(9) | 14,72(15) | 31,07(31) | 161,89(162) |
| 5) Structural analysis - covariance | 0,352 | 79,19 | - | - | - | - | - |

*Approximate number.

Source: Ferrão (2004).

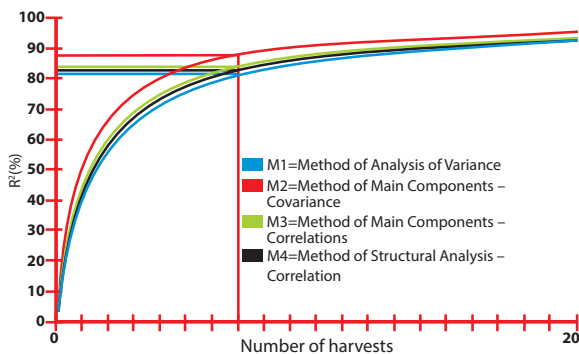


Figure 10. Graph illustrating the coefficient of estimation (R^2) in percentage, according to the number of harvests, by four methods of estimating the coefficient of repeatability of the coffee conilon productivity characteristic, in Sooretama/ES.

Source: Ferrão (2004).

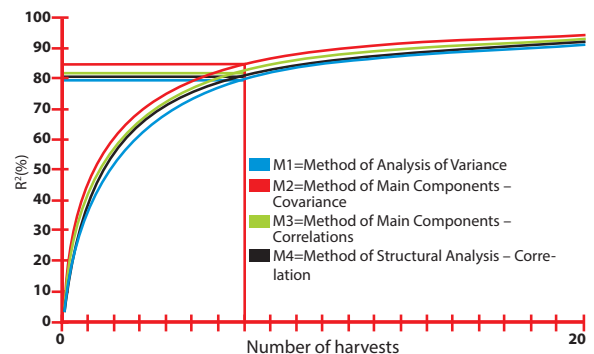


Figure 11. Graph illustrating the coefficient of estimation (R^2) in percentage, according to the number of harvests, by four methods of estimating the coefficient of repeatability of the coffee conilon productivity characteristic, in Marilândia/ES.

Source: Ferrão (2004).

5.5 GENETIC DIVERGENCE

The success of a plant breeding program lies in the existence of genetic variability in the work population. The breeders have recommended, for the base populations formation, the intercross between superior and divergent cultivars.

Studies of genetic divergence have been of great importance in breeding programs that include hybridization by providing parameters for the identification of parents who, when crossed, make it possible to express a greater heterotic effect and a greater probability of recovering superior genotypes in the segregating generations. It is also of great use in evolutionary studies of species for generating information about available genetic resources, as well as helping to locate and exchange genetic material (FALCONER, 1981; DIAS; KAGEYAMA, 1998; CRUZ; REGAZZI; CARNEIRO, 2004).

Genetic divergence has been assessed by means of biometric techniques based on the quantification of heterosis or by predictive processes. Among the methods based on biometric models that are used to evaluate the parents divergence, it is mentioned the diallel analyzes, which inform both the specific combining ability and the heterosis manifested in the hybrids. Predictive methods of divergence between parents, since they do not require hybrid combinations, have deserved considerable emphasis. Predictions are based on morphological, physiological, and molecular agronomic differences to determine divergence between the parents, which is usually quantified by a measure of similarity or dissimilarity. Inference based on ecogeographic diversity is also an example of a heterosis predictive method (CRUZ; REGAZZI; CARNEIRO, 2004).

Multivariate statistical analysis has been widely used to quantify genetic divergence.

Having an available database involving different variables of interest for the species under study, coming from experiments, it is possible to integrate the multiple information and to choose the most divergent parents and who are more likely to promote superior results in a breeding program.

In the genetic divergence study, several multivariate methods can be applied. Among them, the main component analysis, canonical variables and agglomerative methods are mentioned. The agglomerative methods differ from the others because they depend fundamentally on measures of dissimilarity previously estimated by the Euclidean distance or by Mahalanobis distance, among others. In the main component method by the analysis of canonical variables, the objective is to evaluate the dissimilarity among the individuals through a graphic dispersion, in which two Cartesian coordinates are considered. The choice of the most appropriate method has been determined by the precision desired by the researcher, by the ease of the analysis and by the way the data were obtained (CRUZ; VENCOVSKY; CARVALHO, 1994; CRUZ; CARNEIRO, 2003; CRUZ; REGAZZI; CARNEIRO, 2004).

For Cruz, Vencovsky and Carvalho (1994), of the different measures of similarity or dissimilarity that quantify the distances between two populations, the Euclidean distance and the generalized distance of Mahalanobis are the most used. The first technique is more used for the characterization of genetic material maintained in collections of germplasm, being the database without repetitions. The use of the generalized distance of Mahalanobis is recommended for data coming from tests with experimental design with repetition.

The estimation of the genetic divergence in the *C. canephora* culture presents importance in the identification of parents with maximum genetic divergence that might be destined to crosses and in the identification of productive parents with maximum similarity that, when being propagated vegetatively, can be grouped, thus resulting, in uniform, high productivity, synthetic populations or cultivars.

Molecular markers have been widely used in studies of genetic divergence of existing materials in active germplasm banks, as well as in accesses that are found widely in natural environments (FERRÃO et al., 2007; FERRÃO, M., 2009a; SOUZA, 2011). With these studies, it is possible to characterize the genetic materials in terms of their genetic dissimilarity or similarity, to identify repeated accesses and also to respond to the requests of breeders to send germplasm according to their needs (LASHERMES et al., 1993).

As examples of research on the identification of genetic diversity in the genus *Coffea* in *C. canephora* with the use of molecular markers, we can cite the following authors: 1) Lashermes et al. (1999), who studied the genetic diversity of African germplasm by grouping the species in different regions of Africa; 2) Ruas et al. (1999) analyzed the genetic diversity of the germplasm collection of the Instituto Agronômico do Paraná - Iapar (Agronomic Institute of Parana) involving the varieties of *C. arabica* and *C. canephora*, and verified similarity between species ranging from 0.58 to 0.84 and showing that *C. arabica* has an association of 0.76 with the Robusta variety and 0.68 with the Kouillou, both *C. canephora*, thus observing the possibility of succeeding in interspecific hybridization programs involving these species; 3) Teixeira et al. (1999), studying the accesses of the Active Bank of Coffee Germplasm of UFV, verified variations in similarity of

0% to 67% among the analyzed genetic materials, and the species *C. arabica* and *C. canephora* showed considerable genetic divergence; 4) Berthaud (1986) estimated the genetic diversity of a collection of more than 1,000 accesses of *C. canephora* in the Ivory Coast involving genetic material from different countries and identified two divergent groups, the Guinean, from West Africa and the Congolese, from Central Africa. He observed that when crosses involving clones descended from these groups there was a significant heterosis, which led Leroy, Charmetant and Yapo (1991) to initiate a breeding program through reciprocal recurrent selection involving these groups.

Fonseca et al. (2003a) analyzed the genetic divergence of the breeding program clones of *C. canephora*, Conilon variety, of Incaper, using multi varied analysis. In the cluster analysis, the Tocher method was used. For dissimilarity, the generalized Mahalanobis distance and the Euclidian distance were used. For graphic dispersion, the analysis of canonical variables was used. From these analyzes, the following results were obtained as a priority: by the Tocher method, three groups of genotypes were formed, the first one being subdivided into ten subgroups; definition of ten divergent clones with characteristics of interest, which were selected for diallel crosses; selection of the ES 25 and ES 92 clones as the most suitable to obtain heterotic hybrids, considering their productivity, genetic divergences and agronomic characteristics of interests.

In a study of genetic divergence, using 40 conilon coffee genotypes in two environments in the State of Espírito Santo, Ferrão (2004) verified, by the generalized distance of Mahalanobis, dissimilarities between the genotypes from 1.28 to 211.70. The genotypes clustering by the Tocher technique indicated that, in Sooretama, the genotypes were distributed in ten groups, and in Marilândia, five. In the analysis of graphic dispersion by the technique of canonical variables, four more divergent genotypes were identified in the first environment and seven in the second. Figure 12 shows the clustering of conilon coffee genotypes by the closest neighbor method, obtained from the generalized Mahalanobis distance, estimated based on 14 characteristics.

Ivoglio et al. (2008), aiming at identifying genotypes for crosses and for obtaining segregating or heterotrophic hybrid populations, evaluated the genetic divergence among 21 half siblings progenies of IAC *C. canephora* germplasm using multivariate analyzes based on 14 morphoagronomic characteristics. Progenies IAC 2262, IAC 2290, IAC 2286, IAC 2292 and IAC 2291 were indicated for use in intercrosses programs.

Ferrão, M. et al. (2009b) studied genetic diversity among 49 conilon clones of Incaper using *Random Amplified Polymorphic DNA*

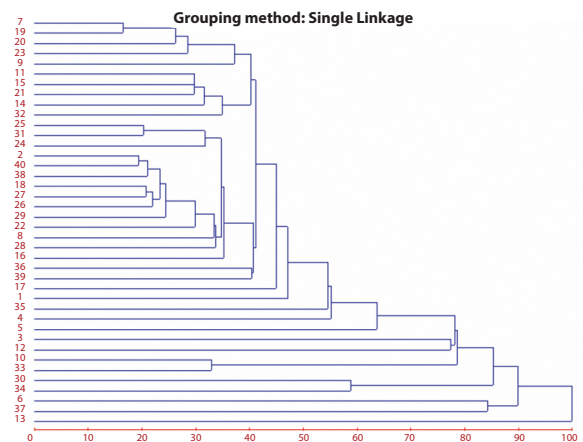


Figure 12. Dendrogram of clustering of conilon coffee genotypes by the closest neighbor method, obtained from the generalized Mahalanobis distance, estimated based on 14 characteristics, in Sooretama/ES.

Source: Ferrão (2004).

(RAPD) molecular markers. The greatest genetic distance found was between genotypes 7 and 38 (0.398), more dissimilar pair. The relatively wide genetic diversity observed shows the importance of carrying out hybridization among the studied germplasms.

Souza (2011) used 20 microsatellite *primers* to genotype 127 accessions of *C. canephora* from the germplasm collections of São Paulo, Minas Gerais and genetic material grown in Espírito Santo and Rondônia. There was a high polymorphism among the genotypes. The Conilon from Espírito Santo and Rondônia group and the Robusta from São Paulo and Minas Gerais group were formed. Both showed significant but limited variability. The results suggest additional efforts in the breeding program looking for increasing the genetic diversity of the Brazilian *C. canephora* collections.

Dalcomo (2013) evaluated the genetic divergence of 22 conilon coffee clones of Incaper, selected in the south of Espírito Santo, for 17 morphoagronomic characteristics associated with plant growth after programmed cycle pruning. It was used the method of the main components to evaluate the relative contributions of the characteristics. The genetic dissimilarity matrix was obtained through the generalized distance of Mahalanobis and the genotypes clustering by the *Unweighted Pair Group Method with Arithmetic Mean (UPGMA)*. A broad genetic variability was verified, separating, by similarity, five groups of genotypes. Higher genotypes were identified with potential to be used in future breeding program, aiming both for their inclusion in clonal variety and in hybridization.

5.6 SIMULTANEOUS CHARACTER SELECTION

In order to obtain superior genetic material that really distinguishes from the others, it is necessary that the selected genotype simultaneously brings together a series of favorable attributes that give them higher productivity and that present other superior characteristics compared to the genetic material hitherto cultivated.

Cruz and Carneiro (2003) show that selection based on one or a few characteristics has proved to be inappropriate because it leads to a superior final product only for this characteristic and at the same time inferior to other genotypically important ones that were not considered during the selective practice of that isolated characteristic.

Thus, one way to increase the chance of success in a breeding program is through the simultaneous selection of an important set of characteristics, mostly economic ones.

For such purpose, the use of selection index can be an efficient alternative since it allows to combine the multiple information contained in the experimental unit so that it is possible the selection based on a complex of variables that brings together several attributes of economic interest. Thus, the simultaneous selection index is an additional procedure, established by the great linear combination of several characteristics (CRUZ; REGAZZI; CARNEIRO, 2004).

The prediction of genetic gains offers great contribution to quantitative genetics. Through the information using this procedure, it is possible to predict the success of the adopted selection scheme and, with scientific basis, to decide which alternative techniques may be most effective.

Genetic gains can occur directly and indirectly. Paula (1997) has described that direct selection is a strategy used by the breeder who is interested in gaining a single character on which will practice the selection. This is the easiest and most practical way to obtain gains for a single characteristic, being the answer to the direct selection.

Indirect selection is a strategy used by the breeder who is initially interested in obtaining gains in a characteristic Y when selection is applied on another characteristic X. Cruz and Carneiro (2003) reported that the assessment of the magnitude of the correlated response has been of great interest when it is desired to obtain gains in characteristics of great importance, but because of complexity, difficulties of identification and/or measurement, the selection is practiced in auxiliary characters. However, they pointed out that the selection in certain characteristics can cause undesirable changes in others, when there are unfavorable correlations between the target characteristics of the selection.

Ferreira et al. (2004) performed the genetic gain prediction using 14 phenotypic characteristics evaluated in 40 conilon coffee clones at Sooretama and Marilândia sites. They verified gain in different characteristics, with emphasis on prediction of genetic progress in beans productivity, which was in Marilândia of 22.69%, and in Sooretama of 25.41%.

5.7 SELECTION INDEX

The selection index is a valuable technique in which there is an optimal combination of several characteristics, which allows the simultaneous selection of multiple characteristics to be efficiently performed.

Cruz, Regazzi and Carneiro (2004) have argued that the use of indexes as selection criteria provides superior results when compared to direct selection. Through the use of this technique, it is possible to obtain favorable genetic gains and better distributed in different characteristics, under study.

Cruz and Carneiro (2003) highlighted different selection indexes that are used in breeding. These indexes represent different selection alternatives and, even considering a single type of index, there are different methodologies with which it is possible to work the data using economic weights, desired gains or degree of restriction imposed to the calculation of the index coefficients. Thus, through these processes of working with the data, the breeder can identify, in a fast and efficient way, genetic materials more suitable for breeding purposes. With the existing computational resources, the estimation of indexes became a simple operation and it is therefore recommended to use them as an important procedure in the search for efficiency in the selection (CRUZ, 2001).

The classical selection indexes of Smith (1936) and Hazel (1943) and the Pesek and Baker Index (1969) are widely used in several cultures breeding programs.

Ferreira (2003) and Ferreira et al. (2005) used the economic weight of the genetic standard deviation (CVg) and predicted earnings by the Smith (1936), Hazel and Lush (1943) and Pesek and Baker (1969) indexes in 14 characteristics of 40 conilon coffee clones, evaluated in two places, Sooretama and Marilândia. Balanced predicted gains were obtained for the studied

characteristics and 60% coincidence of the clones selected in the two places.

5.8 GENOTYPE INTERACTION X ENVIRONMENT, ADAPTABILITY AND PRODUCTION STABILITY

The cultivars performance in different environments is differentiated, that is, a cultivar with a higher value for a characteristic in a given environment does not always maintain that same superiority when cultivated in another environment. The differentiated responses of the cultivars to the environment variation are called genotype x environment interaction. If this interaction is significant this indicates that the genetic and environmental effects are not independent, since the phenotypic responses of the genotypes would be differing with the environmental variations.

The existence of differentiated responses of genotypes to environmental variations has been frequently observed in the various species of cultivated plants. This interaction is a complicating factor in the execution of breeding programs, as it influences the obtainment of genetic gains, increases the length of the research, and makes it difficult to recommend cultivars for dissimilar environments.

Robertson (1959) classified the genotype x environment interaction into simple and complex interaction. The first one is caused by the difference of variability between the genotypes in the environments, so that there is no change in the genetic material classification, in the cultivars recommendation. The second occurs due to the lack of correlation between the genotypes performance, so that they present different responses to the environmental variations, causing a change in their classification, considering the diverse environments. The simple interaction does not bring complications in recommending cultivars or choosing genotypes. The complex one causes problems to the breeder, because as mentioned before, the best genotypes in one environment are not, in general, in others.

In the breeding planning, as regards the choice of the experiment (s) environment (s) or selection field (s), the breeder is subject to predictable and unpredictable variations, which have caused difficulties in selection. The predictable variations are due to the permanent factors of the environment, such as soil type, length of days, technological level used, altitude, topography, latitude and longitude, spacing, planting depth and presence or absence of irrigation. The unpredictable variations are those difficult to control by the researcher, such as climatic factors involving temperature and flooding due to excessive rainfall (CHAVES, 2001). Unpredictable factors, also called temporal, are the ones that affect the producers the most and, in general, are less prioritized in the breeding planning and execution.

Researchers' efforts to study the different species have been focused on formulating strategies to circumvent the problems caused by the genotype x environment interaction. In cases of significant interaction, it is generally recommended to carry out detailed studies of environmental stratification (MORAIS, 1980) and cultivar behavior by means of adaptability and stability analysis and/or development of cultivars of wide adaptability and stability (EBERHART; RUSSELL, 1966). In the case of environmental stratification, it is sought to identify,

among the chosen environments, patterns of response similarity of the cultivars, in such a way that it is possible to evaluate the level of representativeness of the tests in the range of culture adaptation and to make decisions regarding the inclusion of environments when there are technical problems or scarce resources.

For the adaptability and stability studies using the different methodologies, it is possible to evaluate the genotype in relation to the environment improvement and its behavior predictability, thus providing information on its adaptability and stability, respectively (CRUZ, 2001; CRUZ; CARNEIRO, 2003; CRUZ; REGAZZI; CARNEIRO, 2004).

The adaptability and phenotypic stability analyzes are aimed at supporting plant breeding programs and refer to the evaluation of the differential response of genotypes to the environmental conditions variation. Nowadays, there are around a dozen methods to evaluate the genotypic performance of cultivars. The differentiation between the methods and the choice of their use depends on the available database, the number of environments evaluated, the type of information required and the desired detail of the information (CRUZ; CARNEIRO, 2003).

Knowledge of interactions genotypes x locations, genotypes x years and genotypes x locations x years guides the researcher in the survey planning, in defining breeding strategies, in the definition of site(s) for breeding, besides being determinant in the evaluation of the phenotypic stability of the cultivars, aiming at its recommendation for a certain region.

There are few specific studies of the genotype x environment interaction for *C. canephora* (MONTAGNON et al., 2000). Some preliminary works have shown the interaction between site x varieties in the Ivory Coast (CHARRIER; BERTHAUD, 1988; SNOECK, 1983). Holguin et al. (1993) and Montagnon et al. (1994) studied the interaction between genotype x environment for coffee rust, and Charmetant and Leroy (1986) for bean size. According to Charrier and Berthaud (1988), the interaction genotype x environment for productivity has always been significant, which shows the researchers the need to mix clones with the purpose of recommendation for certain regions of Cameroon and the Ivory Coast. Studies carried out in this last African country confirmed the existence of genotype x environment interaction and, therefore, reveal the need to evaluate genetic material in different agroclimatic conditions.

Moschetto et al. (1996) evaluated different genetic materials of *C. canephora* and interspecific hybrids in different environments, harvest periods and beans processing types, in order to identify the interference of these variables in the quality of the beverage. It was verified a better quality of coffee beverage in the washed treatment better than in the dry process, but variation of this behavior among genotypes was identified. The clones had more uniform behavior when wet processed. There was no significant difference in the behavior of the genotypes for environments and harvesting periods.

Montagnon et al. (2000) studied the interaction of genotype x environment and adaptability and stability by the parametric methods of Wricke (1965), Eberhart and Russel (1966) and non-parametric methods (NASSAR; HÜHN, 1987) for *C. canephora* bean production. The work was conducted in the Ivory Coast, where 16 clones were evaluated in nine sites. There were significant production differences between clones and sites and clones x sites interaction. The

methods used showed complementarity.

Ferrão et al. (2003a) and Ferrão (2004) studied the interaction genotypes x environments, for productivity using eight experimental conilon coffee varieties, evaluated by two harvests in four locations in the State of Espírito Santo. There were significant differences between varieties ($P < 0.05$) in different environments. In the joint analysis involving the eight environments, there were significant interactions ($P < 0.01$) for the variation sources varieties (T), years (A), local (L), T x A, and A x L.

Subsequently, Ferrão et al. (2003a), studying the behavior of the same experimental varieties of conilon coffee for productivity, evaluated over four harvests and in four places, in the State of Espírito Santo, by the analyses of joint variance, significant differences were verified by the test F ($P < 0.01$) for locals (L), year (A) and interactions T x A, T x L, A x L e T x A x L. In the adaptability and stability analysis, using the methodologies of Eberhart and Russel (1966) and Cruz, Torres and Vencovsky (1989), the genotypes showed general adaptability ($\beta_1 = 1$). For most genotypes, there was good predictability, indicated by high coefficients of determination ($R^2 > 80\%$) and regression deviation statically equal to zero ($\sigma^2_d = 0$). The ideal genotype that presented all the attributes, such as high average, positive response to environment improvement ($\beta_{1i} < 1$, $\beta_{1i} + \beta_{2i} > 1$) and high predictability ($\sigma^2_d = 0$) was not identified. There was good accord of the results with the use of both methodologies.

Ferrão (2004) and Ferrão et al. (2007) studied the interaction genotype x environment and the adaptability and stability of production in 40 genotypes of conilon coffee, evaluated by seven harvests, in two sites of Espírito Santo, that is, Marilândia and Sooretama. Significant differences were observed, by the analyses of joint variance, for genotypes (G), years (A), G x L, A x L and G x A x L, which indicated the existence of differences between the genetic material and their differentiated behavior in relation to the years (harvests), thus leading to the need, for the purpose of recommendation, to evaluate the genetic material in more than one place and in more than one harvest and also to the need of adaptability and stability production works. In the four methodologies studied, Eberhart and Russel (1966), Cruz, Torres and Vencovsky (1989), Lin and Binns (1988) and Carneiro (1998), the ideal genotype was not identified. Six genotypes with general adaptation were identified, ten with adaptability to favorable environments and three with adaptability to unfavorable environments. Most of the genetic material presented low predictability for the first two methodologies, although the coefficients of determination (R^2_i) were slightly higher than 80%.

6 FINAL CONSIDERATIONS

Greater emphasis should be given to the *C. canephora* breeding, since it is a species of great economic and social importance in the Brazilian and world scenario and presents important genetic patrimony to be explored in the inter and intrapopulation breeding.

Important results have been obtained in the development of cultivars from the *C. canephora* species to improve the relevant characteristics, mainly productivity. However, due to the

presence of genetic reserve, new progress can be continuously achieved for productivity and other characteristics demanded, such as bean and beverage quality, maturation and bean size, drought tolerance, diseases and pests. Therefore, new breeding strategies will be necessary, especially involving the classical methods of breeding associated with biotechnology techniques and procedures, with the support of biometric analyzes, to give greater boost and chance of success in shorter periods in achieving these goals.

Sexual reproduction breeding conducted in association with asexual breeding techniques, has provided good genetic gains and helped protect the species from genetic vulnerability.

The use of improved cultivars along with other cultural and phytosanitary practices has caused a significant increase in plant productivity. As a record, we mention the work carried out by Incaper in the State of Espírito Santo, with the participation of other regional development agents related to coffee cultivation, which in the last 22 years have increased the productive potential of cultivars planted by 60 to 120 bags/ha, when properly used the recommended technologies.

There is also a need to intensify basic studies related to biometrics and biotechnology, such as estimates of genetic parameters, genetic divergence, repeatability, heritability, and number of genes in the expression of characteristics, correlations between characteristics, genotype x environment interaction, and assisted selection by molecular markers, among others, aiming to capitalize information for an appropriate planning of research programs, in order to justify and continue guaranteeing the allocation of resources for the *C. canephora* breeding and, consequently, to allow sustainability in the activity.

Due to the specificities of the experiments and breeding strategies used in *C. canephora*, it is recommended to prioritize studies and statistical and biometric analyzes, as well as the use of the mixed models method, thus providing a better accuracy in the results and in the development of breeding programs of the species.

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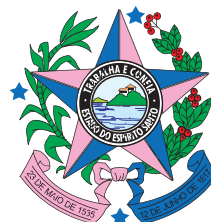


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ISBN 978-85-89274-32-6



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