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Review

Strategies for Robusta Coffee (*Coffea canephora*) Improvement as a New Crop in Colombia

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Abstract: Robusta coffee is mostly grown in Africa and Asia and parts of tropical America, but not yet in Colombia. The crop has potential in lowland areas of this traditional Arabica coffee producer. Compared to Arabicas grown in highland areas, the Robustas have more drought and heat tolerance. However, they differ in flavor and have higher caffeine levels. With natural resistance to some of the major pests and diseases of other coffees, such as rust and berry borers, they thrive under harsh conditions. The genetic improvement of Robusta coffee requires the understanding of its genetic resources and a good breeding strategy. This review discusses the traits of interest and selection criteria for breeding and recommends methods of varietal development for Robusta in Colombia. Most of the traits of importance in breeding are quantitative and of low or intermediate heritability. Robusta is an outcrossing species and can suffer from inbreeding depression, so mass and recurrent selection are used, followed by the clonal propagation of best plants. Colombia has limited germplasm only from the Congolese group, so the SG1/Conilon and SG2 genotypes should be introduced with quarantine. Issues to address include the timing of flowering, asynchronous fruit maturation, chemical composition and sensory quality, as well as bean size. Variability for abiotic stress tolerance exists in Robusta genotypes and needs further study. New methods of breeding include hybrid development and recurrent selection. Having adapted varieties of Robusta coffee should promote production in Colombia, as it has in regions of Brazil, and would complement Arabica coffee for this traditional and major producer.

Keywords: crop improvement strategies; disease resistance; genetic diversity; insect and nematode tolerance; quantitative traits; varietal selection

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1. Introduction

Coffee is one of the world's highest-value commodities, ranking second after petroleum. It is cultivated in more than 80 countries, with a production area of more than 10.2 million hectares in tropical and subtropical regions, particularly in Africa, Asia and Latin America. Coffee species are members of the Rubiaceae family of plants. They are mainly classified in the genus *Coffea* [1]. This genus contains more than 80 species native to the equatorial forests of East and West Africa, Madagascar and islands of the Indian Ocean (e.g., Comoro). The coffee genus currently has two economically important species: *Coffea arabica* L. and *C. canephora* Pierre ex A. Froehner [2]. These are the Arabica and Robusta coffees, respectively. Robusta is less well known in countries such as Colombia compared to Arabica, but it is of higher importance in lowland tropics and increasingly in hotter regions due to climate change. The economic impact of Robusta has grown substantially over the last half of the century, especially with the onset of production in Southeast Asia. In 2020, Robusta coffee represented around 36% of world yields mainly in Vietnam and Brazil. Other countries of significant importance in Robusta production include India, Indonesia, Ivory Coast, Mexico and Uganda [3,4].

Robusta coffee is a true diploid (2n = 2x = 22), while Arabica coffee is a segmental allotetraploid /amphidiploid (2n = 4x = 44) derived from *C. eugenioides* and *C. canephora*. Robusta is mostly outcrossing (allogamous), cross-pollinating and heterozygous. When grown from seed, Robusta plantations are made up of highly heterogeneous individual compared to Arabica, which is more than 90% self-pollinated (autogamous). Notably, cross pollination in Robusta is due to gametophytic self-incompatibility, which is thought to be monogenic in inheritance. To create more homogenous plantations in Robusta coffee, growers practice clonal reproduction (by cuttings or grafting). Meanwhile for pure plantations of Arabica, the plants are grown from self-seed.

Robusta coffee originated in the lowland level rain forests of Central Africa, from Guinea to Uganda in an altitudinal range of 0–1000 m above sea level (masl); while Arabica originates from the higher elevations of Ethiopia, Sudan and Kenya (1300–2000 masl). The plant type of Robusta is umbrella shaped compared to Arabica which is more erect. However, the former can be a taller tree with irregular structure and multi-caulate stems compared to the former which is a pyramidal shrub that is uni-caulate.

The commercial cultivation of Robusta coffees is recent and began in the eastern part of the Congo Basin in the 19th and early 20th centuries, followed by its introduction into Java [5]. Subsequently, its cultivation spread to new regions of Asia and Latin America and it is currently the second most cultivated coffee type after Arabica.

The genetic diversity of *C. canephora* was first investigated at the molecular level in the 1980s two genetic groups were recognized: (i) Congolese (from the Democratic Republic of the Congo, Central African Republic and Cameroon) and (ii) Guineans (from Guinea and Ivory Coast) [6,7]. the Congolese group was divided into five subgroups, named SG1, SG2, B, C and Uganda based on ideas regarding the domestication process [8]. In Brazil, coffees from two Congolese subgroups are found, namely Conilon (SG1 subgroup) and Robusta (SG2 subgroup). The Conilon subset is quite distinct and found in coastal areas.

Robusta coffees are less well studied or collected than Arabica coffees and collections of both types emphasize cultivated accessions [9]. Currently, the collections have a total of 11,415 *C. arabica* genotypes, but only 635 *C. canephora* genotypes. A few semi-domesticated species such as *C. liberica* (94), and wild species such as *C. eugenioides* (81) have also been collected by certain gene banks. Other relatives within the *Coffea* genus are an important part of the world germplasm, reaching 7756 accessions as of this past decade. The genetic diversity of *C. canephora* is conserved in a few ex situ collections containing genotypes from multiple diversity groups. These collections have been the starting point for genetic improvement worldwide and can be in South America too. More preservation of coffee genetic resources in situ in Central Africa is needed, especially for wild species and relatives.

In this review, we will focus on breeding of Robusta for Colombia, which would be a new region of production for this type of coffee. The highland regions of Colombia are recognized worldwide for their production of mild Arabica coffees. However, the lowlands of this same country could be major areas of adaptation for Robusta as a new crop [10]. Given their high value and multiple industrial uses, Robusta coffees could potentially supplement farms dependent on subsistence crops, replace illicit drug production and diversify income opportunities for small scale farmers. They would also satisfy the internal demand for the consumption of instant coffees in Colombia and neighboring countries, since most other coffee is exported.

For these and other reasons [10], the Ministry of Agriculture and Rural Development of Colombia, through its research arm called Agrosavia, has had the mandate since 2019 to investigate Robusta coffee production models, determine its adaptation requirements and fulfill varietal needs by starting breeding efforts. Crop improvement in a diverse country such as Colombia requires the careful consideration of soil and climatic conditions of the potential regions and the biotic and abiotic constraints that go along with the regional climates and soils. With that in mind, we review the major goals of a breeding program for Robusta coffee in this part of South America.

2. Objectives of Genetic Improvement

The genetic improvement objectives for Robusta coffee are similar to those for other plants or coffees: defined by a series of abiotic and biotic factors that reduce the yield potential. In addition to yield capacity of fruit, the ratio of pulp to green or dry beans is important in the case of both Robusta and Arabica coffees [10]. Bean quality and characteristics determine the potential economic profit of the coffee crop and will be considered in a separate section to diseases, pests and climate-influenced physiology.

The abiotic factors that influence the adaptation and yield of Robusta coffee in Colombia depend on the soils and climates where it is grown, but in general they are typical of the influencing factors in other parts of the tropical world: which include drought, high temperatures and low soil fertility. Nutrient deficiencies such as that of phosphorus (P) or toxicities of certain elements such as aluminum (Al) are not well studied for coffees. These two tend to interact in soils with low pH, that is, the acid soils of the tropics such as those of the Oxisol and Ultisol series, among others. Meanwhile, biotic factors are those caused by the interaction of coffee with biological organisms that detract performance such as insects, notably coffee berry borer; and pathogens especially fungi such as rust [10].

Other objectives are purely physiological and have to do with the earliness to flower after the rainy season or the time to mature fruit production. Production varies by harvest months and by alternating years. Since Robusta coffee is an allogamous outcrossing crop with gametophytic self-incompatibility, it requires pollen flow which means simultaneous flowering is needed. Finally, the physical, bromatological and sensory quality of the bean are of great importance for the consumer and therefore also goals of breeding Robusta coffee. Amount of caffeine and chlorogenic acids are of greater weight for selection in final phases of varietal selection [10]. Given these plant improvment objectives, several common breeding techniques exist for the selection of the best plants from populations and the best hybrid combinations and clonal varieties of Robusta coffee.

Since Robusta coffees are cross-pollinating, it is necessary to grow different seed or graft/cutting (clonal) derived genotypes in the same plot to ensure successful pollination and fruit production. This affects synchronicity of maturation as well as number of active pollinator plants over time. Thus, most open pollinated (OP) varieties of Robusta coffee are result of mass selection (MS). Multi-line varieties, which are purposefully composed of a mixture of many genotypes or clones are common. Meanwhile hybrid varieties (HV) consist of populations of plants derived from seeds of a unique crossing event.

2.1. Selection for Performance Criteria

The yield of Robusta coffee is generally calculated from the weight of the dry green beans (parchment coffee) obtained with respect to the area of the production unit [10], expressed in hectares. Generally, the volume of green coffee can be estimated from the weight of the fresh cherries and the conversion coefficient of cherry to green coffee, called "turn-out". Said value can be expressed in absolute terms or in percentage terms and is specific for each variety of Arabica or Robusta coffee depending on the amount of pulp in the cherries. In the case of Robusta, the amount of green coffee ranges from 15 to 20% of cherry yield depending on the genotype [11]. Yield is a complex criterion that depends on several factors: variety, soils, climatic conditions, plant density, fertilization, pruning technique, etc. It is generally calculated over a cycle of 4 to 5 years, from first production to first pruning [12]. In general, coffee production tends to increase over the years, however, due to the interaction with the environment and the physiological growth rate of the plant, it is possible to observe an alternation of production between one year and another. This is also known as the "biennial" effect (i.e., high production one year and low the following year). Drought events can exacerbate this biennial effect [12]. The onset of the rainy season can affect exact dates of flowering and amount of cherries produced. Two rainy seasons per year can cause bi-annual production, twice during the year. Older trees can grow too tall for harvest that provide lower yields especially when shaded.

The actual yield of Robusta materials planted in different producing regions varies greatly from region to region. In Africa and Asia, for example, clonal varieties can produce between 2 and 3.5 tons of green coffee per hectare with a density of 1200 and 2000 trees per hectare. In Brazil, on the other hand, the clones of Conilon developed by INCAPER produce between 4.8 and 7.2 tons per hectare with densities ranging from 2000 to 4000 plants per hectare [13]. Climate change is affecting yields and growing areas.

2.2. Whether to Select for Synchronous Maturation

In either multi-line OP or hybrid HV populations, heterozygosity and heterogeneity in the population is maintained to always ensure cross pollination between trees. Given this high heterogeneity within a population, it is possible to observe a great variability for flowering period and fruit maturation within Robusta populations depending on their origin. In Brazil, "early", "medium" and "late populations have been produced to try to create more simultaneous harvesting of red berries rather than spread out harvests.

The possibility of selecting for this trait of synchronous maturation simplifies the planning of on-farm cycle. Sowing synchronously maturing germplasm allows for better defined harvesting periods and bulk post-harvesting cultural activities within "seasons" rather than across many weeks or months. Thus, on a farm with an intensive production system, the sowing of synchronous varieties allows a quick harvest of up to 80% of ripe cherries in a single harvest pass through the plantation. Timely harvesting significantly increases the final quality of the beans produced. In addition, rotation of the work teams for harvesting, pruning and other agronomics procedures can be organized consecutively; starting with the earliest plots and ending with those that are later flowering period. This is mainly applicable to large farm or plantation production of Robusta. For a small farmer, the harvest situation is different. The scarcity of labor makes the small producer tend to prefer varieties with extended maturation (not synchronous), which allows them to distribute the harvesting and postharvest activities over a longer period.

2.3. Grain Physical Quality

The physical quality of the grain is measured by its size. Although cultural and post-harvest practices can impact the physical quality of the grain, the genetic component of each Robusta variety has a significant effect to this selection criterion. There are three ways to evaluate grain size. In the field, grain size can be estimated from fruit size and the pulp to seed ratio. Therefore, when cherry size is larger, and a variety has low pulp to high seed ratio the beans are usually large. A second way to estimate grain size is through the weight of one hundred green coffee beans, which gives an accurate idea of the average weight per bean. The third way to evaluate grain size is by measuring the grain size in a sample of known weight. The number of grains retained by different sieves, each with different pore sizes, is weighed. This granulometry also provides important information on the homogeneity of grain size for a given variety.

Robusta's grain size is generally smaller than Arabica. The weight of 100 Robusta beans can vary between 12 and 15 g, while for Arabica values range between 15 and 20 g. The genetic component that determines the phenotypic expression of this trait quantitative but of high heritability (h^2 = 0.73). Grain size and pulp to seed ratio are highly correlated characters, which greatly facilitates joint selection [14,15].

2.4. Chemical Composition of the Grain and Sensory Quality

The sensory characteristics of a cup of coffee are closely linked to the chemical composition of the beans used for its preparation. Although the overall chemical composition of the coffee bean from both *C. arabica* and *C. canephora* species are similar, the relative composition of different compounds can vary considerably from one species to another. Therefore, bean quality improvement differs somewhat between the two types of coffee, Arabica and Robusta. The latter contains higher concentrations of caffeine, proteins, arabinogalactans and chlorogenic acids (except the compound 3-FQA). Meanwhile, Arabica type coffee is characterized by having high contents of carbohydrates, lipids, trigonelline and organic acids, while among some other secondary metabolites [15].

Since the chemical composition of the bean is influenced by the genetic origin of the variety, it is important to study the genetic components determining production of the different chemical compounds present in green coffee. Heritability of the compounds in Robusta coffee is variable. Caffeine and lipid content, for example, show high heritability (h^2 = 0.80 and 0.74, respectively), while trigonelline content and chlorogenic acids have relatively low heritability (h^2 = 0.38 and 0.36, respectively).

The variability in heritability values means that a selection for low caffeine content may be more efficient and faster than selection for trigonelline content. The effect of "genotype x environment" interaction may be higher for acidity and flavor characteristics than for the caffeine content. However, given the significant positive correlation between caffeine and chlorogenic acid content, a selection for lower caffeine content may indirectly favor a low chlorogenic acid, which will result in improved final quality [15].

Improvement of cup taste is another potential target for crop improvement but one that is quite subjective [15]. This is mainly because the sensory quality of a cup of coffee is a very complex concept that depends on multiple factors such as the climatic conditions of the plantation, the genetic origin of the variety, cultural practices (fertilization, use of pesticides), the post-harvest process (type fermentation and drying), roasting, and even the final extraction method (capsules, filter, etc.). Therefore, if one wants to compare the sensory quality between different varieties and select amongst different lines/genotypes or populations, it is necessary to guarantee a strict control of many factors from geographic and agronomic origin, to coffee bean storage and preparation of the green and parchment coffee of each variety at every step of the processing chain.

2.5. Resistance to Pests and Diseases

Fortunately, Robusta coffees have a large advantage over Arabica in their high level of disease and insect resistance. This is especially true for some highland diseases such as coffee rust. However, some insects and nematodes can become more serious problems of Robusta coffee as the crop grows in production area.

2.5.1. Rust

Coffee rust, a disease caused by the fungus *Hemileia vastatrix*, is the pathogen with greatest impact on world coffee production, particularly for *C. arabica* [16]. The species *C. canephora* is the main source of known resistance genes against this disease and has been used for more than 70 years by breeders to obtain of inter-specific, rust-tolerant Arabica varieties. At least 4 resistance genes (*Sh* 6, *Sh* 7, *Sh* 8, *Sh* 9) derived from *C. canephora* have been identified thanks to genetic studies based on the segregation of resistance to different races of rust on a collection of plants called "differentials", which show known reactions to various races of the pathogen [17]. Other resistance is polygenic and quantitative. Therefore, much evidence shows that Robusta coffees are generally highly tolerant to rust and that such tolerance is due to the presence of both qualitative and quantitative resistance genes together with complete and incomplete resistance mechanisms. Observations in different Robusta coffee regions show an incidence of the disease on certain varieties of the Guinean group [17]. The increase in cultivation areas, together with the progressive prevalence of extreme and climates that are often conducive to fungal development (higher humidity and high temperature), have made some commercial varieties of Robusta begin to show signs of sensitivity to the rust. This should be a warning for those who wish to extend Robusta cultivation to new producing areas with conditions favorable to rust, that breeding for resistance will be important.

2.5.2. Coffee Borer

The coffee bean borer (CBB), is a beetle species named *Hypothenemus hampei* (Coleoptera) that attacks coffee cherries and carries out its reproductive cycle inside the bean [18]. The species *C. canephora* shows a particularly high genetic sensitivity to this pest, which is favored in certain varieties, due to the continuous presence of fruits in the field. Additionally, the high temperatures and low relative humidity of certain producing areas where Robusta coffee is grown make the insect proliferate more quickly. The numberof cherries harvested can be reduced by fruit fall of borer infested seeds. Besides affecting yield, CBB seriously affects the final quality of green coffee [11,18]. Although different levels of tolerance to CBB have been observed in some Robusta populations in the Central African Republic, there appears to be no genetic resistance against the pest in Robusta or any other known diploid species [13].

2.5.3. Nematodes

Yield losses due to nematodes in coffee vary from one producing region to another and from one country to another depending on the species. In Brazil, nematode losses reach up to 45% in certain producing regions [11]. Currently there are more than 15 *Meloidogyne* nematode species recognized as coffee pathogens, with 4 being the most frequent in commercial plantations (*M. exigua*, *M. incognita*, *M. coffeicola* and *M. paranaensis*). The first of these causes prominent root galls, which make it easily recognizable in the field [19]. This species is widely distributed in different countries of Latin America, where it causes significant damage locally. Highest incidences of nematodes occur in the species *C. arabica*. Although, *C. canephora* does not escape attack, it has some resistance. Other diploid species such as *C. liberica* have genetic resistance to nematodes, which is why they have been used as rootstocks for cultivation of Arabica in infested areas. The Nemaya variety, very popular in Central America, is a clonal hybrid that has a high resistance to nematodes and wide use by local producers [11]. Genetic studies have shown the existence of a resistance gene (*Mex 1*) with partial dominance, originating from *C. canephora*. This gene contributes to the *M. exigua* tolerance observed in varieties of Catimores, which have inherited the resistance through the Timor hybrid, a natural hybrid between arabica and robusta [19]. Polygenic resistance to nematodes cannot be ruled out.

2.6. Drought Resistance

Frequent episodes of water deficit are occurring in most Robusta production regions due to climate change. Although Robusta is quite drought tolerant [6] and largely grown in regions of moderate to high rainfall. However, these are increasingly subject to intermittent drought. Robusta productivity and vegetative development are seriously affected below 1200 mm of annual rainfall [10,12]. The selection of drought-tolerant Robusta varieties, as well as the development of rootstock genotypes highly resistant to water deficit, constitute two alternative breeding routes of growing interest among coffee breeders. Studies that were carried out in Brazil show that it is possible to use morphological and physiological criteria such as stomatal characterization to accelerate the selection of drought-tolerant Robusta coffee seedlings [13].

2.7. Tolerance to Heat, Cold and Sun Exposure

Although in general, Robusta coffee is considered tolerant to heat and direct sunshine [13] improvement could select genotypes with these tolerances for coastal production areas. Shade trees are used in Robusta coffee in Asia and sometimes Brazil. However, they have not been studied in Colombia where some regions could be hotter or cooler than Brazilian conditions. Climate change is causing an increase in areas suitable for Robusta.

Arabica coffee in Colombia was traditionally grown under shade but now is mostly produced under full sun despite higher temperatures recently. Direct solar exposure is often found in production areas in Brazil for Robusta coffees, but in Colombian tropics it will be necessary to think about shading and adaptation of varieties to strong solar radiation. On the other hand, it is doubtful that Robusta coffees will spread to the high-altitude Arabica coffee production areas due to low temperature tolerance, a problem for Brazilian production where cold and frost are limiting. Robusta coffees are generally grown at altitudes above 600 m but there are sources of lower and higher elevation adaptation in the African gene pool and in Conilon from Brazilian states of Espírito Santo and neighbors.

3. Variety Improvement Strategies

Robusta coffee genotypes all have the allogamous (obligatory) out-crossing mode of reproduction. This means that to produce fruits, each plant requires the pollen flow from another genotype of the same species that has opened its flowers at the same time as well as reproductive compatibility. This mode of reproduction favors the heterozygosity and heterogeneity of the individuals in the population, which results in high genetic and phenotypic variability, which is amplified as the plants reproduce by seed from one generation to another.

Note that because of the high variability, in any population derived from crosses between two Robusta clones it is possible to find highly productive individuals that can be selected through a clonal selection strategy. Each clone thus selected can be preserved by means of traditional vegetative propagation methods such as cuttings, or grafting on carrier plants, with good root development.

Thus, clonal varieties are possible in Robusta coffees. Another strategy is to increase the existing variability through crossing between the best individuals from the same population or from different populations. In this case, the selection is carried out after examining the progeny derived from said crosses, for which the so-called recurrent selection is used. Its objective is to increase the frequency of genes carrying traits of interest within the population, through successive cycles of recombination and selection. Robusta coffee breeding goals are shown in Figure 1.

Figure 1. Illustration of important characteristics for varietal selection of Robusta coffee. (**A**), grain size; (**B**), productivity; (**C**), uniformity of flowering; (**D**), lack of rust or other diseases; (**E**), drought tolerance. Pictures: Juan Carlos Herrera- Nestle. 2021.

Genetic Basis of Robusta Coffee and Varietal Types

The genetic base of commercial cultivars of Robusta coffee across the world is mainly from the two Guinean-Congolese subgroups of *C. canephora*: SG1 called var. Conilon and SG2 called var. Robusta. The first subgroup constitutes the genetic base for plant breeding and genetic improvement of commercial varieties in Brazil, Ecuador and Mexico and the second subgroup has led to varieties in Vietnam, Uganda and India [5].

Overall, the Guinean-Congolese group germplasm is found mainly in ex situ gene banks and would be important to collect further for breeding purposes [8]. However, genetic diversity and population structure of Guinean-Congolese ecotypes of coffee have not been well investigated yet, so more testing and adaptation studies are needed.

The research process to increase productivity of Robusta and breed varieties for and in Colombia can be postulated to require the introduction of two germplasm groups: SG1 (with a focus on commercial Conilon types from Brazil) and SG2 (Robustas from other parts of original range in Africa plus countries of Latin America and Asia), as these are the groups which would guarantee wide diversity for selection.

A diverse set of introduced Robusta and Conilon germplasm is needed to begin genetic improvement for the various disparate regions of the country. It should be in seed form so that segregating populations are introduced to Colombia, rather than as single plants or clones of varieties. However, care should be taken for the quarantine of seed imports as certain pests and diseases are seed borne in coffees.

Starting in the 1970s, selection methods have allowed the large-scale propagation of Robusta coffee clones of interest (clonal varieties), as well as the release of varieties derived from hybrid crosses between two or more selected clones (hybrid varieties). The development of vegetative propagation methods in Robusta and the discovery several years later of the existence of two large genetic groups of *C. canephora*, namely the var. Conilon and var. Robusta groups described above, were the starting point to propose new strategies for breeding this type of coffee.

Vegetative propagation, on the one hand, opened the possibility of creating highly productive clones from existing populations available in different producing countries. On the other hand, it was a precursor to fixation of hybrid vigor in useful F_1 progeny from promising parental combinations.

This last strategy represents an ideal way of exploiting heterosis in Robusta coffee, but requires selecting somewhat homozygous parents for crosses as a starting point, which as discussed above is made difficult by low levels of inbreeding. Overall, creating hybrid clone varieties is a route that requires more time and more access to good sources of genetic variability.

Despite all this, the number of clonal varieties and hybrid Robusta cultivars available to growers is relatively large, after more than a century of improvement in various world regions and specific countries. All these improved or wild populations, which constitute a rich genetic source for the different selection programs that work on the development of future varieties, can be divided into two large groups related to SG1 and SG2.

Use of wild accessions should also be considered and is found in two groups: the first group of populations is represented by populations of wild origin originating from Java, Gabon (Kouillou subgroup) and Uganda (*C. ugandae*; *C. bukobensis*), mainly. These populations are characterized by little previous selection and therefore contain a good part of the original genetic variability of the existing wild groups [12].

The second group of germplasm is represented by all those populations derived from controlled or natural crosses between distant wild or related semi-wild genetic groups. From a practical point of view, the derived populations are of great interest to the breeder insofar as they conserve a good part of the original variation of the wild founder populations from which they are derived. All this germplasm is of interest for breeding by various methods (recurrent or mass selection) and with different resulting variety types such as clones, bi-clonal and poly-clonal hybrids.

4. Selection Methods

In the following paragraphs, the types of varieties existing in Robusta are described, as well as the main selection strategies and breeding methods that have been used by different coffee research groups that would also be useful to establish Robusta as a new crop in Colombia.

4.1. Development of Clonal Varieties

Given the allogamous (cross-pollinating) nature of *C. canephora*, selection of uniform inbred genotypes with high yield and a high level of homozygosity is a difficult process and is neither achievable nor desirable for the most part. Robusta coffees are obligatory out-crossers and more than one variety must be cultivated in an area to ensure pollination. After multiple cycles of inter-breeding and selection within a given population, it is possible to identify genotypes as clones with stable agronomic characteristics which can be fixed by vegetative multiplication. For this, different methods, such as propagation by cuttings, propagation by grafting, or propagation by in vitro culture of somatic tissues (i.e., somatic embryogenesis) are used. A detailed review of each procedure used in coffee and their relevance and advantages has been previously described [20]. Vegetative propagation generally requires a high investment in terms of facilities, labor and multiplication area, which translates into a higher cost per plant produced. In the case of opting for a multiplication method such as somatic embryogenesis, which also requires skilled labor, the costs can be up to 10 times higher than the cost of a plant produced from sexual seed. However, the result is a uniform clonal variety.

The most used breeding strategy to select outstanding individuals in Robusta derived populations is mass selection (MS). This method seeks to identify the best trees within the different derived populations available, thanks to an intense intra-population selection that favors the identification of the best existing genetic combinations that will be used to build the new clonal variety. MS is the most promising approach for improving Robust for Colombia, given the outcrossing nature of Robust and the high genetic load of recessive lethals that can be removed by simple phenotypic observation. This technique has been widely used in Cameroon, Madagascar and Mexico. The best example of the potential of MS is the work carried out since the mid-1970s in Brazil using different source populations and parents of Conilon coffee. The selection of clonal varieties made from Conilon has allowed parts of Brazil to obtain high genetic gains with drought tolerance, which have resulted in highly productive commercial varieties [12,13]. Progeny analysis of crosses have been used to determine the best parents in diallelic design [21]. However, for each selected individual to maintain its characteristics when forming the new variety, it is essential to propagate it through vegetative multiplication.

4.2. Development of Hybrid Varieties

One of the great advantages of developing hybrid Robusta varieties is that they are seed based and thus offer an ease of propagation that clonal methods do not offer. Due to their self-incompatibility, two parental clones can easily be grown in a relatively isolated plot to produce the hybrid seed. Under these conditions, all the seeds collected from the cherries from each tree in the plot will come from the cross between the two parental clones. Given the reduced maternal effect observed for most of the characteristics of agronomic interest, the direction of the cross between the parental trees does not have a significant effect on the homogeneity and stability of the new hybrid variety.

The most widely used method currently to produce so-called hybrids can be based on elite plants, or selection. The number of elite genotypes to combine into a clonal hybrid variety is theoretically limited to the number of trees in the seed plot. However, currently propagated clonal hybrid varieties usually consist of two (bi-clonal) parents or many more clones (poly-clonal) as parents. The results range from uniform hybrid varieties to much more diverse hybrid open-pollinated (OP) populations.

Studies carried out suggest that the use of at least 8 parental clones would guarantee good fertility and low genetic vulnerability to deleterious mutation and high genetic load [22]. It should be noted that the greater the number of clones used in crosses, population development and multi-lines, the greater the phenotypic heterogeneity of the resulting variety. The best way to reduce this variability is to select clones that are uniform and even in flowering, productivity and, if possible, of high sensory quality. Obtaining homogeneous clones is a complex task; however, this can be made easier with double haploids. This methodology produces trees with high homozygosity, from elite clones through tissue culture and plant regeneration. Despite advances in in vitro culture techniques, currently the only way to regenerate homozygous clones is by selecting double haploid that are found spontaneously inside poly-embryogenic seeds, which are of relatively low occurrence most likely due to deleterious recessive genes and high inbreeding depression [23].

Another alternative strategy used for the selection of both clones and hybrids of high genetic value is the reciprocal recurrent selection (RRS) first practiced with great success between the 1980s and 1990s in Ivory Coast. Its objective was to use the genetic diversity discovered in the two genetic groups known at that time, the Congolese and the Guinean, to breed for new hybrids. Thanks to this strategy, it was possible to obtain high genetic gains through rapid accumulation of genes of interest for complex characteristics such as production and resistance to diseases. Indeed, the populations and resulting hybrids developed using this strategy were shown to be twice as productive as many of the Robusta hybrid selections produced from the 1970s.

As the use of RRS is beyond the current state of Robusta selection in Colombia we propose a general protocol for simple recurrent selection (RS) alone in three steps repeated in cycles (Figure 2). In a first stage, the breeder will seek to identify the best individuals from the population (in our case from SG1), by means of crossing between plants to then obtain some selected genotypes. Secondly, the best individuals derived from these within genepool crosses are then crossed with more distant genotypes to increase intra-group genetic diversity. When we have done this with additional SG2 material or exotic germplasm from the center of origin, the next step would be selecting hybrids to generate a new group of introgressed individuals. That way, the cycle begins again from step one. After three or more cycles, the inter-group diversity increases rapidly, and the inter-group hybrids accumulate a higher level of heterosis that significantly increases the probability of selecting individual plant clones with greater vigor and better productivity.

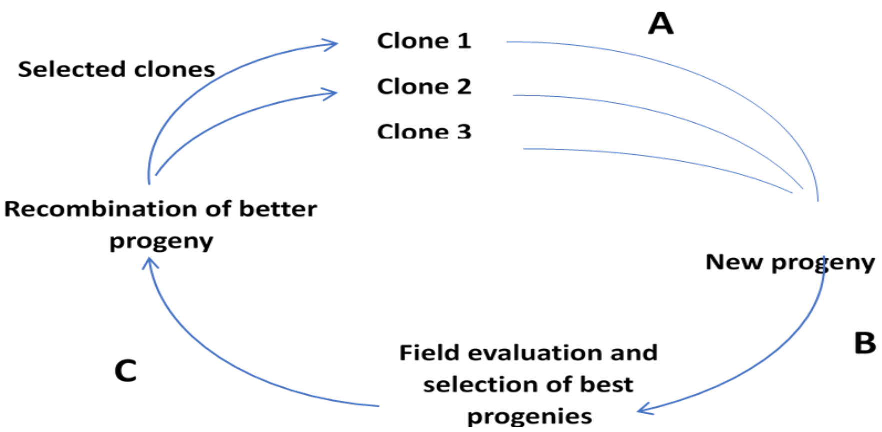

Figure 2. General diagram of the recurrent selection (RS) process in Robusta coffee. The cycle begins with the crossing of a group of clones (A); the derived progeny is evaluated in different environments to select the best individuals; (B) the individuals are crossed with each other to produce a new population from which the best clones are identified; (C) the process begins again with a new group of improved clones.

An alternative, longer and more complex way of improving Robusta coffee would be to cross it with Arabica coffees. This would be based on interspecific hybridization of the two species which are at different ploidy levels. Although this would allow the introgression of characters between the species it is very difficult to achieve due to low success of diploid x tetraploid crosses. To avoid incompatibility due to the difference in DNA content between the two species (2x vs. 4x), the Robusta plants must be duplicated in chromosome number through application of colchicine and selection of allotetraploids which are later crossed with Arabica genotypes. This laborious process has been done mainly to improve the sensory and quality characteristics of the Robusta coffee. The only interspecific hybrids derived from the cross *C. canephora* × *C. arabica*, other than the famous 'hybrid of Timor' are called "Arabustas". These were multiplied and evaluated in different producing regions in Africa. Despite the high vigor shown by the Arabustas, their success is very limited largely due to their high phenotypic heterogeneity, but also because of fertility problems, a consequence of marked genetic instability [24].

5. Development of Robusta Coffee in Lowland Colombia

Robusta production in Colombia is very limited so far to date. This is mainly because *C. canephora* gerplasm availability is very limited in Colombia, mostly due to the need to protect the Arabica coffee industry and because of a lack of knowledge about the potential of Robusta coffee for lowland regions. The only entity to import Robusta coffee seeds or clones has been via the official national agricultural research program AGROSAVIA/ICA and in between CORPOICA or coffee growers' federation's research arm, CENICAFE. Original imports were from CATIE in Costa Rica which has some exchange with the Robusta breeding program in Mexico and other Latin American countries.

Recent interest by the breeding program of Nestlé-France, a multi-national based in Europe, has provided support for the germplasm introduction of four Ecuadorian, four Mexican and four Nicaraguan clone sources to Colombia of Robustas in the Nestle breeding program. That French led breeding has been based on limited diversity in the SG1 subgroup [6,25] that traces back to Congolese group germplasm (Table 1). Apart from SG1, discussions have started by AGROSAVIA for access to Conilon type SG1 subgroup from EMBRAPA and INCAPER national and regional programs or with university researchers (e.g., UFES) in Brazil. Other germplasm groups based on microsatellite markers such as B, C and Ug from Eastern and Southern range of *C. canephora* diversity in Africa [26] or Guinean from West Africa will be difficult to import directly to Colombia for breeding.

Table 1. Germplasm of interest to the Colombian breeding program and sources.

Phenotypic Groups	Isoenzyme Marker Groups	Microsatellite Marker Groups/Heterosis	Geographical Origin
Guinean	Guinean	Guinean	Ivory Coast, Guinea, Sierra Leone
Congolese	SG1	SG1/Conilon	Coast Central Africa (Gabón, DRC) bred into an Improved Coastal Brazilian type
	SG2	SG2	Interior of Central Africa to Southern Congo River Basin
		B	Southern Africa
		C	Cameroon
		Ug	Uganda

References: for column 1: Berthaud, 1986 [6]; column 2: Montagnon et al., 1992 [25]; and column 3: Cubry et al., 2008 [26] and Alkimim et al. [27].

Current quarantines double as observation trials and are conducted in two stations: C.I Turipaná and C.I El Mira. These research stations belong to AGROSAVIA and are near ports of entry and isolated from production zones. Monitoring for disease or pests has been conducted by ICA as regulatory agency for the quarantine process. The 12 Latin American clones (4 each from Ecuador, Mexico and Nicaragua) along with 3 additional French clones (FRT 65, FRT 97 and FRT 101) were introduces as populations and planted out in replicated experiments with varying plot sizes (3 × 2 m; 3 × 1 m; 3 × 1.5 m and 3 × 2.5 m). In total 3212 cutting based plants are currently (as of 2021) found in these nurseries. Thus far, the plants have tested negative for *Xylella fastidiosa*, with no other diseases or pests of concern found. Screening for pathogens from other Latin American regions such as *Gibberella xylarioides*, *Pseudomonas syringae* pv. *garcae* and *Sclerotium coffeicola* were done weekly. Insects pests that were monitored for included *Leucoptera coffeella*, *Oligonychus ilicis*, *Thrips hawaiiensis*, *Xylosandrus compactus* and *Xyphinema americanum*. None have been found so far after two years of screening.

The development of a Robusta breeding program in Colombia is justified for local adaptation in the Caribbean coast, Llanos, Pacific coast and Piedemonte regions with or without crosses to Arabica coffees and independent of breeding in other areas of South America [27]. It is notable that regions in Colombia where the Robusta coffee would be tested and bred are different in agro-ecology and in farming systems from the Brazilian regions which have been used for testing.

Despite diversity of Robusta in Colombia being limited due to import restrictions and quarantining process, the growth of production can be contemplated based on germplasm from Nestle-Ecuador (FRT105, FRT107, FRT109 and FRT141) that have passed all requirements for seed to be distributed from initial plants. The breeding for Amazonian conditions in the Brazilian state of Rondônia (Figure 3) provides hope that similar success would be obtained in lowland regions of Colombia. The Brazilian program in the Amazon was successful making new varieties using hybridization between coastal Conilon (SG1) and forest region Robustas (SG2) with selection for local adaptation [27–29].

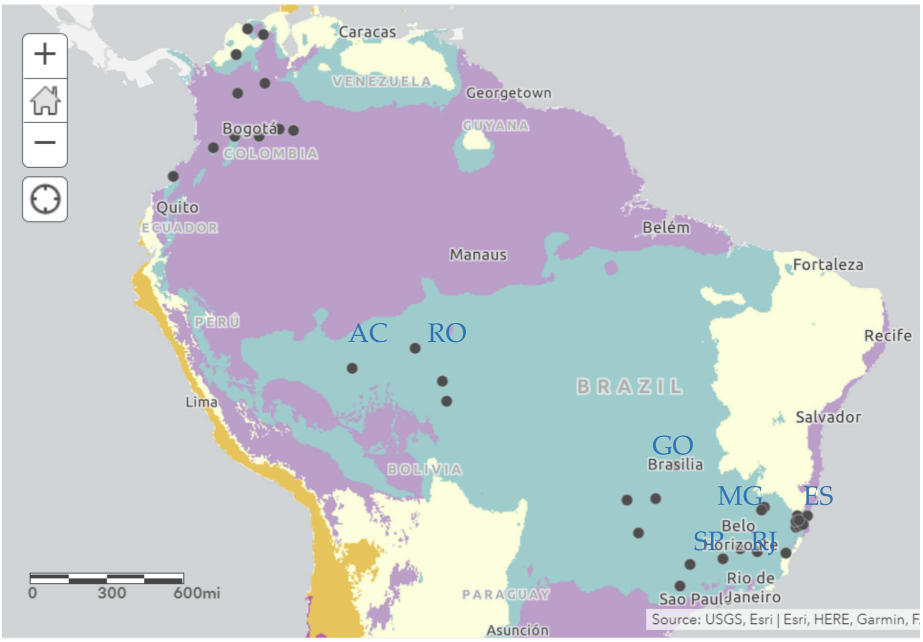

Figure 3. Map of South America comparing agro-ecologies of testing sites used in Brazil for breeding and those proposed for Colombia (all black dots). Brazilian sites are from a 2000–2022 search of publications and Colombian sites were those listed as adequate or ideal in the review by Campuzano-Duque et al. (2022) [10]. Two letter codes in blue text indicate the state in Brazil that are also listed in the text below.

Brazilian areas of Robusta breeding have been in the states of Espírito Santo (ES) and adjoining Rio de Janiero (RJ), Sao Paulo (SP), Minas Gerais (MG) and to a lesser extent Goias (GO). In more recent times Robusta breeding and production has spread to the Western Amazonian states of Acre (AC) and Rondônia (RO). These are new lowland tropical forest areas for production with different germplasm [28,29] than in more coastal and highland regions of Brazil. All the sites for breeding are indicated on the map along with regions that are tropical pluvial with seasonality (light green color) and without (purple). Bimodality in rainfall versus the long dry season found in unimodal rainfall regions affects the adaptation of Robusta coffee, their maturation period and obviously their yields. Climate change is likely to exacerbate the differences between near tropics production in Brazil and Mexico compared to equatorial production in Colombia

6. Recommendations and Conclusions

In summary, a breeding program for Robusta is needed for Colombia, given its unique situation as a mountainous country near the equator. Germplasm is available from original Robusta stocks and improved Conilon varieties, but local adaptation will be key in this highly heterogeneous crop. Various methods are available for breeding Robusta coffee including MS and RRS as major ways for planning crosses and making selections in the mixed populations of heterozygous plants. To create a pure variety, cuttings can be taken of the best plant and then multiplied by continued vegetative propagation as an elite clone. Notably, the distribution of seed rather than clones is often considered better for breeding Robusta coffee in a new location as a new crop, because seed contains the genetic diversity needed to start new populations. The high genetic diversity from introduced seed is better than the limited diversity introduced when only a few clones can be brought into a country by cuttings. These recommendations apply to the breeding of this type of coffee in Colombia, where there is are at least four regions of possible production for Robusta, each varying in rainfall pattern and soil conditions from lowland Caribbean to the acid and hilly soils of Orinoquia and Piedemonte, to high-rainfall Amazonian forests and lowland Pacific coastlines [10]. In each agroecology and for the various farming systems practiced by Colombian farmers, the evaluation of enough genotypes to make best-bet selections is needed and can be assisted by excellent physiological tools developed for Robusta coffee in Espírito Santo, Brazil. As when Conilon coffees moved from the coastal states of Brazil to the inland state of Rondônia, germplasm collection and hybridization, proper varietal selection and ecological management must be put into place for success. Initial studies including plant spacing and shade tree are also needed. As in the case of Brazil, Robusta coffees are likely to find a ready internal market with many varied uses [30]. In conclusion, climate change will probably increase the need for Robusta coffee as a heat-tolerant crop to supplement the declining Arabica coffee production likely to occur in the Andes, and that is well documented worldwide. In doing so, it is important to select for Robusta coffee varieties that meets the needs of consumers, processors and markets that are well developed in and for Colombian coffee. Newer products such as instant coffees, single-dose capsules, gourmet mixes, candies and deserts, or energy drinks will help to disseminate the use of Robusta coffees even in a traditional Arabica producer such as Colombia.

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