Phenotyping and Genotyping a *Coffea canephora* Population, Cultivated at High Altitude, Aiming at a GWS Program for Coffee

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SUMMARY

Significant advances recently occurred in *Coffea canephora* research, such as the completion of its full genome sequencing. The information generated can be used in advanced molecular approaches for genetic improvement, like genome-wide selection programs (GWS). The purpose of this study was the phenotyping and genotyping of 403 *C. canephora* individuals cultivated at *Cerrado* climatic conditions characterized by a relatively high altitude (>1000m) and a long dry season. Phenotypic evaluations started in 2012 with the measurement of several agronomical traits such as vigor, secondary branching, leaf-rust susceptibility and precocity of fruit maturation. Production (evaluated in fruit liters) of each plant was also measured during two consecutive years (2012 and 2013), along with a detailed classification of fruits harvested in 2012 (bean size, weight, defects and sieve). In addition, predawn-leaf water potentials (Ψ_{pd}) were evaluated during the dry season of 2013. Furthermore, genotyping by sequencing (GBS) of 403 plants was performed using the nextRAD methodology, while genetic analyzes were done through Cervus and Adegenet softwares. Results obtained allowed to conclude there is a great potential for cultivation of *C. canephora* selected clones under irrigated conditions at high altitudes. The phenotypic and genotypic diversities studied with our population also seems suitable for GWS studies in coffee.

INTRODUCTION

There are approximately 120 *Coffea* species, among which two are economically important: the tetraploid *Coffea arabica* L. (2n = 4x = 44) and the diploid *C. canephora* (2n = 2x = 22). *C. canephora* is an allogamous species that displays genetic self-incompatibility. Due to its cross-fertilization properties, it has greater genetic variability than *C. arabica*, being more resistant to diseases and pests and able to adapt to different climatic conditions. It is commonly grown at low and medium altitudes, even in drought-prone areas, as it shows greater tolerance to water deficit.

Significant advances in *C. canephora* genomics have occurred in recent years resulting in new tools to help accelerate the genetic improvement of this species. As an example, one can cite the recent conclusion of the genome sequencing of *C. canephora*, a future reference for

advanced molecular genetics to be applied directly on the improvement of this species, such as genome-wide selection programs (GWS). The goal of the present study is to genotyping and phenotyping 403 individuals of a *C. canephora* population cultivated in *Cerrado* climatic conditions characterized by a relatively high altitude (>1175 m) and a long dry season under.

MATERIALS AND METHODS

Plants corresponded to a population of *C. canephora* Conilon planted in 2009 at the experimental field of Embrapa Cerrados research center (Planaltina, DF, Brazil). Several parameters such as production, predawn-leaf water potential (Ψ_{pd}), sieve, 100 grains weight, classification as to defects and fruit morphology, were evaluated for the 403 cultivated individuals. In addition, genomic DNA from leaves of these individuals was extracted to perform genotyping by means of the nextRAD approach. Ψ_{pd} were measured the 4th september 2013 after 50 days of water suspension and before the return of irrigation. Using the genotyping data, the values of genetic diversity such as, observed heterozygosity (Ho), expected heterozygosity (He), null-allele frequencies (F) and polymorphism information content (PIC), were calculated by Cervus and adegenet software.

RESULTS AND DISCUSSION

The frequency distribution of the average production data were obtained during the 2011-2012 and 2012-2013 harvests (Fig. 1). For a great part of the population (approximately 170 plants), the productivity ranged between 11.32 to 14.71 L.
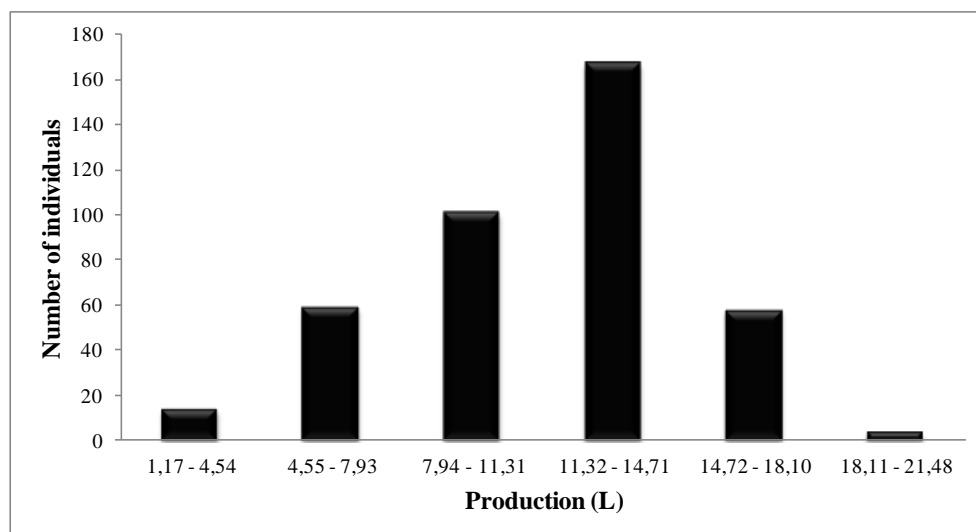

Figure 1. Frequency distribution of average production in liters (L) of two years (2011-2012 and 2012-2013) for a population of 403 selected individuals of *C. canephora*.

Figure 2 shows the Ψ_{pd} for 403 individuals selected in the *C. canephora* population. Most of them had values ranging from -1.0 to -0.5 MPa. As weather conditions were the same for the whole population, significant variation of Ψ_{pd} values, characterized a great diversity for drought tolerance within the studied population, with the presence of drought-tolerant (-1.0 MPa > Ψ_{pd}) and drought-susceptible (-2.0 MPa > Ψ_{pd}) plants.
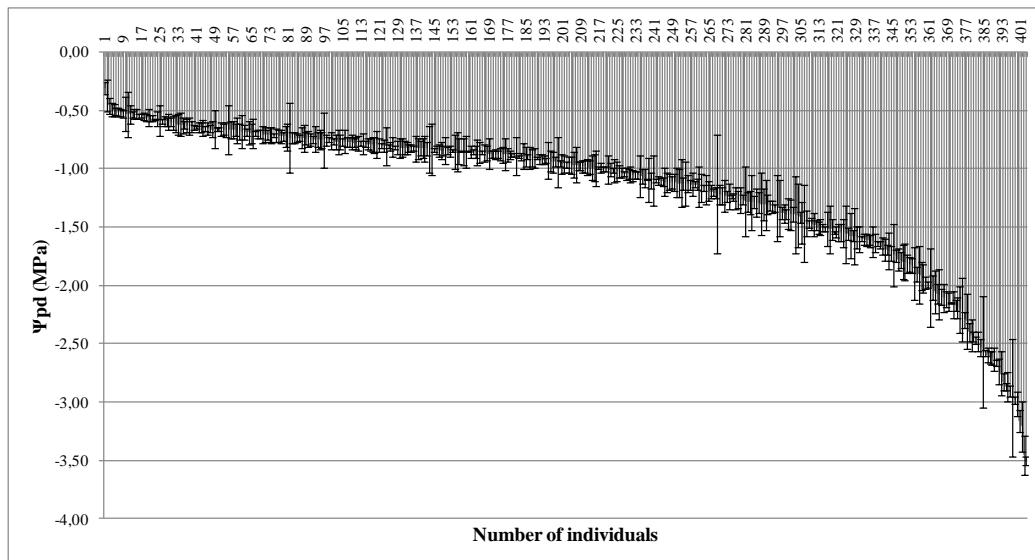
Figure 2. Predawn-leaf water potential (Ψ_{pd}) for 403 selected individuals of a *C. canephora* population.

Averages of production (2011-2012 and 2012-2013 crop/year) and Ψ_{pd} were compared for 17 plants (Figure 3). This allowed the identification of highly productive plants and also drought-tolerant ($\Psi_{pd} \pm$ -0.5 MPa), like L12P8 and L8P42. It is worth noting that the plant L5P47 showing the lowest Ψ_{pd} value had an average production above 12L.
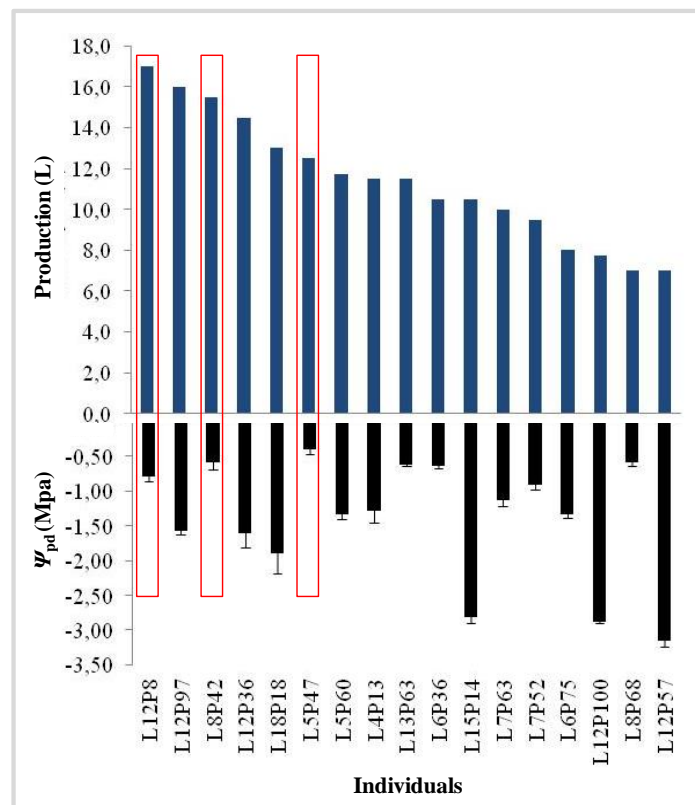
Figure 3. Comparative graph of average production (top) and average Ψ_{pd} (bottom) of 17 plants in a population of *C. canephora*. Red outlines indicate plants that stood out for both indices.

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Through GBS approach, a total of 5,412 SNPs was identified. These SNPs were distributed along the 11 chromosomes of *C. canephora*, some of them in genes and others in intergenic regions. The mean Ho for all loci was 0.343, ranging from 0.006 to 0.993; for 1,413 loci, Ho was greater than He, presenting negative values for (F). Such behavior is expected at loci with excess of observed heterozygous genotypes. The lowest PIC, 0.063, was registered for SNP 4722, while the greatest, 0.5, related to SNP 525 (Figure 4). The average PIC was of 0.28 and 53% of SNPs had values greater than or equal to 0.3 PIC, indicating a good level of information content.
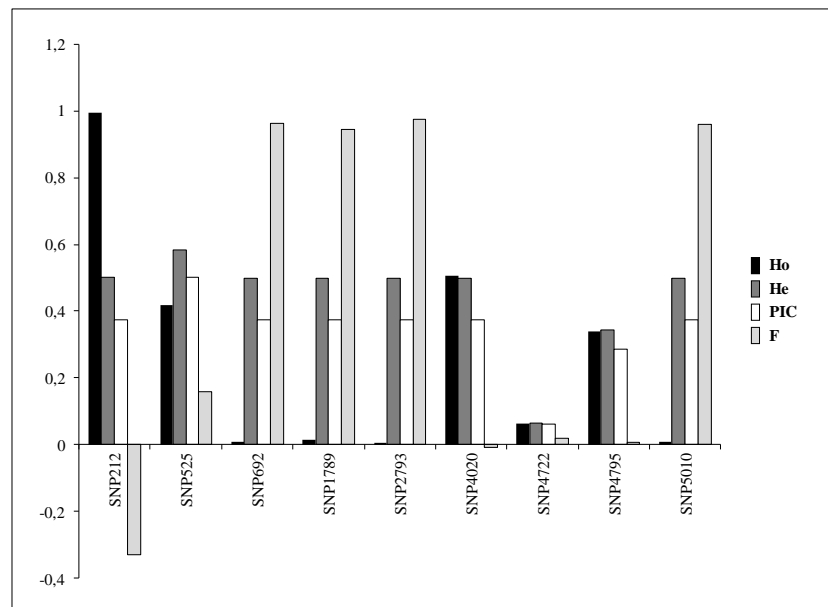
Figure 4. Frequency distribution of Ho, He, PIC and F for SNPs markers in *C. canephora*. Results show that genotype diversity of the studied population seems suitable for genome-wide association studies in coffee.

Results obtained so far, indicates that the population under study is adequate for further association studies as the analyzed individuals display sufficient phenotypic and genotypic diversity.

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