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BASIC AREAS
Article

Physiological quality of seeds of *Coffea canephora* from early and late clones during maturation

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ABSTRACT: The objective of this study was to evaluate the physiological quality of seeds from early and late maturing *Coffea canephora* clones, aiming to identify the ideal harvest time. The fruits were collected every two weeks from 188 days after anthesis (DAA) and characterized as green, cane green, cherry, and dry. The seeds were evaluated for water content, germination percentage, normal and abnormal seedlings, nongerminated seeds and dead seeds, vigor index, hypocotyl, and root length. Seed germination of the early maturing clone started at 202 DAA, while that of the late maturing clone started at 230 DAA, with both clones showing a water content of 63% and a dry mass of 37%. Although the optimum harvest point was identified at the cherry ripening stage, it was found that the late maturing clone showed a more pronounced variation in the maturation process, directly impacting the physiological quality of the seeds. Furthermore, a significant correlation was observed between the maturation stages and the physiological quality of the seeds. For the early maturing clone, the maximum physiological quality was recorded at 244 DAA, corresponding to 80% of fruits at the cherry stage, while for the late maturing clone this point was reached at 326 DAA, with 98% of the fruits ripe.

Key words: conilon, germination, vigor, phenology.

INTRODUCTION

Coffee is one of the most significant commodities globally, and Brazil is the main producer (FAO 2020). *Coffea arabica* L. (Arabica coffee) accounts for 64.3%, while *Coffea canephora* Pierre ex A. Froehner (conilon or robusta) represents 35.7% of national production (CONAB 2022). Conilon coffee stands out due to its high productivity and resistance to diseases and droughts (Partelli et al. 2019). Furthermore, *C. canephora* is an entrepreneur in the Arabica coffee blend industry due to its high content of soluble solids, caffeine, and acidity (Bastian et al. 2021, Da Costa 2023), with harvesting recommended at the cherry stage to prevent mechanical damage and preserve the commercial value of coffee (Tesfa et al. 2019, Stavrinides et al. 2020).

Coffea canephora can be categorized into different maturation cycles: early, intermediate, and late, corresponding to ripening in the months of April, May and June, respectively (Dubberstein et al. 2016). The occurrence of multiple flowerings in this species results in the coexistence of fruits at different stages of maturation on the same plant (Miranda et al. 2020, Salazar et al. 2019). The variation in maturation makes it difficult to determine the ideal harvest time, which impacts the quality of the drink (Campuzano-Duque and Blair 2022). However, studies correlating fruit development with the physiological quality of *C. canephora* seeds are scarce, and no investigation has addressed the issue in different maturation clones.

Propagation of *C. canephora* is traditionally done by cuttings, with less emphasis on seeds. However, seed propagation has increased due to the demand for rootstocks resistant to nematodes and drought conditions, and their compatibility with

C. arabica for the cultivation of more resistant seedlings (Gonçalves et al. 2021, López-García and Cruz-Castillo 2019, Spiral et al. 2023). In this context, a seminal cultivar 'Conquista ES8152' was launched, offering genotypes with desirable attributes, such as intermediate to late maturity, vigor, drought tolerance, rust resistance, and high drinking quality (Ferrão et al. 2019).

In the development of seminal varieties, the collection of seeds from different clones occurs simultaneously, presenting a challenge in synchronizing maturation and determining the ideal harvest time (Campuzano-Duque and Blair 2022). In varieties made up of mixtures of clones, it is common for seeds of optimum quality not to ripen at the same time. This often requires storing seeds until all genotypes are available for variety formation. However, *C. canephora* seeds are intermediates with low longevity under storage conditions (Stavrinides et al. 2020).

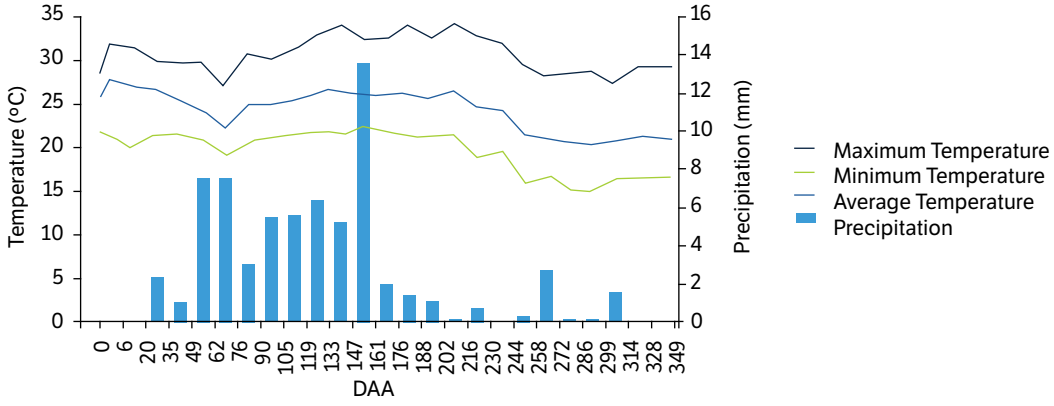
Therefore, the search for information to obtain seeds of high physiological quality emerges with the aim of improving the production of seminal seedlings. This is justified due to the impact of the presence of immature fruits on the viability of the seed lot (Dussert et al. 2018). Studies show that *C. canephora* seeds harvested at full maturity stage have higher germination rates compared to green seeds (Irma et al. 2022). However, there is a lack of information on the quality of seeds from clones with different maturation cycles.

The analysis of seed maturation and development stages, aiming at identifying the optimal harvest moment, is of interest for genetic improvement, management practices, phenological changes and the overall quality of coffee (Sousa and Silva et al. 2022, Kath et al. 2023), because seed quality directly influences plant vigor and plantation productivity. Therefore, the hypothesis is that physiological and morphological maturity occurs at different intervals between early and late maturing clones. In this context, this study had the objective to evaluate the physiological quality of seeds from early and late maturing *C. canephora* clones, aiming at identifying the ideal harvest time.

MATERIALS AND METHODS

Study area

The fruits of *C. canephora* were harvested from a crop located at the Experimental Farm of Marilândia, ES, Brazil, belonging to the Capixaba Institute for Research, Technical Assistance and Rural Extension (INCAPER). The geographical location of the farm is recorded at latitude 19º 24' 19" S, longitude 40º 32' 20" W, with an altitude of 188 meters. The crop was three years and six months old, and the plants were grown in full sun, with a spacing of 3 m × 1.5 m, under a rainfed cultivation system. A management practices, fertilization and cultural treatments followed the conditional technical recommendations for the crop (Prezotti et al. 2007). The experiment was conducted between 03/15/22 and 08/23/22, with climate data recorded by the National Institute of Meteorology Meteorological Station installed at the Marilândia Experimental Farm at INCAPER (Fig. 1).



DAA: days after anthesis.

Figure 1. Climatic conditions during the maturation cycle of early (101) and late (408) clones.

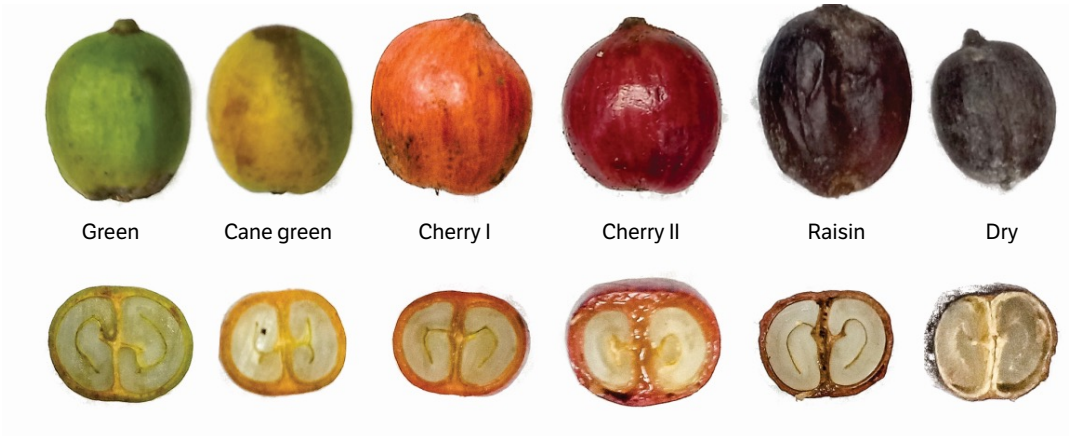
Experimental design

The experiment followed a completely randomized design, with four replications, using a split-plot scheme. The plots were composed of genotypes from different maturation cycles (early and late), while the subplots were different harvest times. The early maturing genotype was clone 101, belonging to the variety 'Diamante Incaper 8112', while the late maturing genotype was represented by clones 303 or 408, associated with the varieties 'Centenária ES8132' and 'Marilândia ES8143', respectively. Fruit collections were carried out every 14 days, starting at 188 days after anthesis (DAA).

Assessments carried out

After harvest, the fruits were transported to the INCAPER Plant Physiology and Post-Harvest Laboratory, in Linhares, ES, Brazil, for a series of evaluations. The uniformity of maturation was assessed visually, based on phenological maturation stages (green, cane green, cherry and dry), adapted from Dalvi et al. (2017) and Pezzopane et al. (2003) (Fig. 2). A sample of 200 g of fruits was randomly selected to count the phenological stages, and the values were expressed as a percentage, calculated as Eq. 1:

$$\text{Maturation stages} = \frac{\text{Total number of fruits in the stadium}}{\text{Total number of fruits in the sample}} \times 100 \quad (1)$$



Source: adapted from Dalvi et al. (2017).
Figure 2. Maturation stages: green, cane green, cherry I, cherry II, raisin and dry. Scale 1 cm.

After determining the phenological stages, the fruits were subjected to the manual pulping process, removing the skin, and fixing it in a sieve when ripe or manually when unripe, without adding water. Three samples of 10 g of seeds, still containing the parchment, were taken to determine the water content of the seeds expressed as a percentage, following the Rules for Seed Analysis (Brasil 2009). The fresh mass of the seeds was obtained using a precision scale, followed by drying in an oven at 105°C for 24 hours to determine the dry mass. Water was expressed as a percentage on a wet basis.

The parchment-covered seeds were subjected to treatment with 5% sodium hypochlorite solution for 30 minutes, followed by washing in distilled water to soften the parchment and disinfect the seeds. The parchment was then removed by hand, and the seeds were immersed in a fungicide solution Captan (1 g/100 mL) for 15 min. The germination test was carried out with four replications of 50 seeds, following the procedures described in Brasil (2009). A Germitest paper roll system moistened with distilled water (2,5 times the weight of dry paper) was used in biochemical oxygen demand germination chambers, with alternating temperature of 20/30°C and photoperiod of 12 hours.

The first germination count occurred 15 days after sowing, followed by a second count at 30 days, according to Brasil (2009). The following parameters were evaluated: percentage of germinated seeds, normal seedlings, abnormal seedlings, dead

seeds, and nongerminated seeds (Fig. 3). The seedlings were photographed, and the images analyzed using ImageJ software to measure hypocotyl length and root length. The vigor index was calculated according to Dhindwal et al. (1991) (Eq. 2):

$$VI = \text{Hypocotyl length in cm} + \text{Root length in cm} \times \text{Final germination percentage} \quad (2)$$

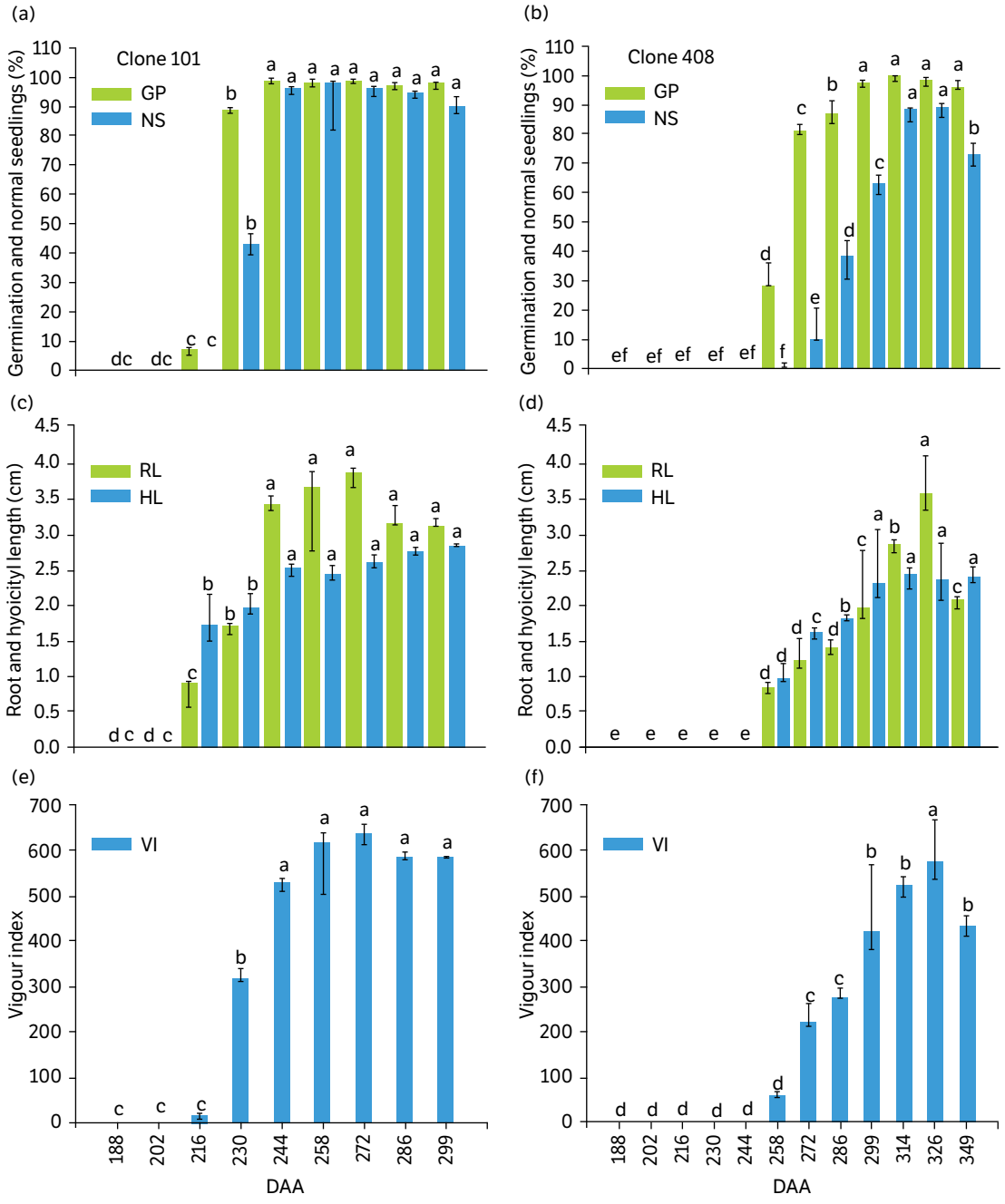
Figure 3. Normal seedlings, abnormal seedlings, dead seeds, nongerminated seeds of early (101) and late (408) maturing clones. Scale 1 cm.

Statistical analysis

The collected data were subjected to the normality test, and statistical analyses were carried out using the Sisvar program (Ferreira 2011). Means were compared using the Scott-Knott test with significance considered at the 5% probability level ($p \leq 0.05$). To evaluate the effects of maturation stages on physiological variations, Pearson correlations were performed using the PAST program (Hammer et al. 2001). The intensity of the correlation was interpreted following the scale proposed by Cohen (1988), with small (0.10 to 0.29), medium (0.30 to 0.49), and large (0.50 to 1) levels.

RESULTS

The germination process was influenced by the interaction between *C. canephora* clones and the different evaluation periods (Fig. 4). For the early clone, the physiological quality of the seeds reached its maximum point in fruits harvested from 244 DAA, demonstrating optimal performance up to 299 DAA, as reflected by germination percentage, normal seedlings, and germination vigor (Figs. 4a and 4e). On the other hand, in the first harvest periods (188 e 202 DAA), a higher percentage of dead seeds was observed, while the maximum percentage of abnormal seedlings was recorded at 299 DAA (Suppl. Table 1).



DAA: days after anthesis.

Figure 4. Germination and seedling growth. (a and b) Percentage of germination (GP) and normal seedlings (NS); (c and d) root length (RL) and hypocotyl length (HL); (e and f) vigor index (VI) of early (101) and late (408) maturing clones. Means followed by the same letter in the column do not vary statistically from each other using the Scott-Knott test ($P \leq 0.05$).

The late clone reached maximum root length and germination vigor at 326 DAA, with a subsequent decrease at 349 DAA (Figs. 4d and 4f). However, germination (first count and total percentage) (Suppl. Table 1; Fig. 4b) and the length of the hypocotyl was greater from 299 DAA (Fig. 4d). For this clone, the percentage of normal seedlings was higher at 314 and 326 DAA, with a decrease observed at 349 DAA (Fig. 4b). Up to 244 DAA, all seeds were dead, with reduction at 258 DAA, but with a maximum percentage of nongerminated seeds (Suppl. Table 1). At 272 DAA, the maximum percentage of abnormal seedlings was observed, with reduction in dead and ungerminated seeds (Suppl. Table 1).

In the case of the early clone, the lowest performance was evident up to 230 DAA, a period in which most fruits were at the green and cane green stage of maturation (Fig. 5a). In this clone, the highest percentage of dead seeds was observed when the fruits were 100% at the green maturity stage (188 and 202 DAA) (Fig. 5a; Suppl. Table 1).

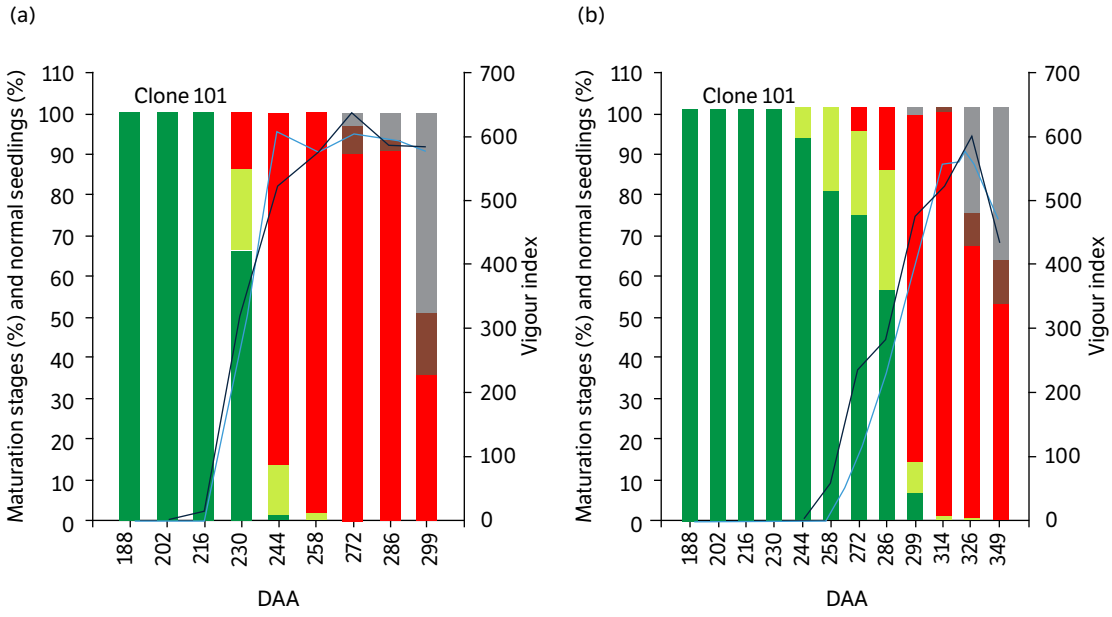


Figure 5. Maturation stages: green (■), cane green (■), cherry (■), raisin (■), dry (■), normal seedlings (——) e vigor index (——) of early (101) and late (408) maturing clones depending on the different harvests, in days after flowering (DAA).

With the progression of maturation (66% green and 20% cane green) at 230 DAA, there was reduction in dead seeds, however with a maximum percentage of abnormal seedlings. The maximum value of normal seedlings was reached from 244 DAA, corresponding to fruits harvested at the cherry stage and with less than 1% of raisins and dried fruits (Fig. 5a).

The late clone demonstrated to be more sensitive to the harvest point (Fig. 5b). With up to 93% green fruits, all seeds were dead, while with 74% green, 20% cane green and 5% cherry (at 272 DAA) a maximum percentage of abnormal seedlings was observed, with reduction in dead and nongerminated seeds (Fig. 5b; Suppl. Table 1). The maximum percentage of normal plants was recorded at 314 and 326 DAA, coinciding with the highest percentage of fruits at the cherry stage (from 98% to 66%) (Fig. 5b). For this clone, maximum seed vigor was obtained with 66% cherry and up to 8% raisin and 25% dry, decreasing at 349 DAA, when it presented 53% cherry, 10% raisin and 37% dry (Fig. 5b).

The color of fruits and seeds underwent significant changes during the maturation cycle. Up to 216 DAA for the early clone and 230 DAA for the late clone, only fruit formation at the green stage was observed (Fig. 5). At these stages, the fruits have a relatively high-water content (57.8 in media) (Fig. 6).

The dry masses of the fruits showed more pronounced increases between the first and fourth collection for the early clone (188 and 230 DAA). The periods from 202 to 230 DAA recorded the greatest gains in seed dry mass, decreasing relative water content (Fig. 6), higher percentage of fruits in the green and cane green stages (Fig. 5), reaching a maximum dry mass at 299 DAA (5.4 g) (Fig. 6), with a low percentage of cherry fruits (35%) and an increase in fruits in the dry stage (49%) (Fig. 5).

For the late clone, there was a gradual increase in dry matter between the first and fifth collection, but only in the seventh collection, at 272 DAA, germination reached a percentage greater than 80% (Figs. 5 and 4). The relative water content was gradually decreased during fruit development and found to be stable between 244 and 314 DAA (Fig. 6).

The physiological quality of the seeds correlated significantly with the stage of fruit maturation (Table 1). Fruit maturity at the cherry stage showed a strong positive manifestation with the first germination count, germination, normal seedlings, root length, hypocotyl length, seed vigor and dry mass, and a negative observation with dead seeds (Table 1).

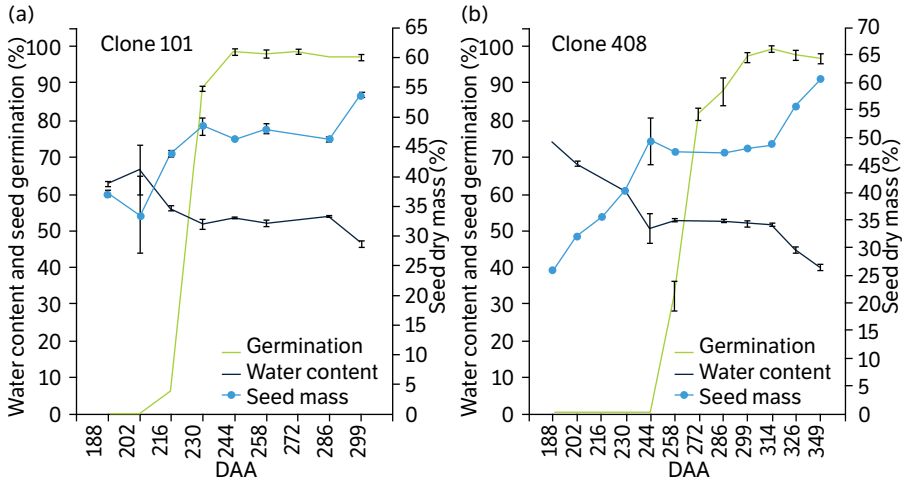


Figure 6. Relationship between percentage of germination, percentage of dry mass accumulation and water content of developing seeds of early (101) and late (408) maturing clones. Bars mean standard deviation from the mean.

Table 1. Pearson's linear correlation matrix between the variables green fruits, cane green, cherry, raisin, dry, first count germination (FCG), germination percentage (GP), normal seedlings (NS), abnormal seedlings (AS) , dead seeds (DS), nongerminated seeds (NGS), root length (RL), hypocotyl length (HL), dry mass (mass) and water content (WC) of seeds, vigor index (VI) of early (101) and late (408) maturing clones. Small correlation between 0.10 and 0.29; medium between 0.30 and 0.49; and large between 0.50 and 1.

Variables	FCG	GP	NS	AS	DS	NGS	RL	HL	VI	MASS	WC
					Clone 101						
Green	-0.91	-0.93	-1.00	0.10	0.91	-0.22	-0.98	-0.85	-0.98	-0.74	0.75
Cane green	0.18	0.34	0.10	0.82	-0.36	0.61	0.09	0.16	0.10	0.21	-0.23
Cherry	0.80	0.81	0.90	-0.20	-0.79	0.10	0.91	0.72	0.88	0.50	-0.51
Raisin	0.45	0.42	0.48	-0.13	-0.41	0.09	0.46	0.48	0.52	0.68	-0.66
Dry	0.34	0.31	0.35	-0.08	-0.31	0.12	0.31	0.38	0.37	0.58	-0.57
FCG		0.93	0.92	0.16	-0.93	0.39	0.96	0.99	0.93	0.89	-0.90
GP			0.95	0.28	-1.00	0.53	0.94	0.88	0.97	0.82	-0.83
NS				-0.02	-0.94	0.28	0.99	0.86	0.99	0.76	-0.77
AS					-0.31	0.86	-0.01	0.17	0.05	0.32	-0.31
DS						-0.56	-0.94	-0.89	-0.96	-0.83	0.84
NGS							0.27	0.39	0.36	0.46	-0.45
RL								0.91	0.99	0.80	-0.81
HL									0.88	0.92	-0.92
VI										0.80	-0.81
MASS											-1.00
WC											
					Clone 408						
Green	-0.96	-0.91	-0.98	-0.27	0.84	0.12	-0.95	-0.95	-0.98	-0.76	0.75
Cane green	0.12	0.29	-0.13	0.82	-0.43	0.67	0.01	0.22	0.01	0.20	-0.23
Cherry	0.87	0.79	0.94	0.10	-0.70	-0.26	0.87	0.84	0.91	0.57	-0.56
Raisin	0.59	0.50	0.66	-0.04	-0.45	-0.17	0.64	0.55	0.61	0.67	-0.65
Dry	0.57	0.48	0.62	-0.01	-0.43	-0.17	0.59	0.53	0.57	0.67	-0.64
FCG		0.97	0.95	0.47	-0.91	-0.12	0.92	0.98	0.97	0.76	-0.75
GP			0.88	0.63	-0.97	0.06	0.91	0.99	0.94	0.75	-0.74
NS				0.19	-0.80	-0.22	0.96	0.91	0.98	0.71	-0.70
AS					-0.72	0.49	0.32	0.55	0.35	0.40	-0.40
DS						-0.29	-0.87	-0.96	-0.88	-0.75	0.75
NGS							-0.04	0.08	-0.13	0.15	-0.15
RL								0.94	0.98	0.74	-0.73
HL									0.96	0.77	-0.76
VI										0.73	-0.72
MASS											-1.00
WC											

The water content of the seeds showed a positive demonstration with fruits in the green stage and dead seeds, although it exhibited high negative correlations with other parameters and stages (Table 1). Dry mass was inversely proportional to water content in both clones (Table 1).

Regarding the raisin and dry fruit stages, different results were observed between the clones. For the early clone, the raisin stage showed high correlation only with the vigor index and the dry mass of the seeds. As for the late clone, the dry stage showed high and positive correlation with the first germination count, germination, normal seedlings, root length, hypocotyl length, vigor, and seed dry mass. This trend was also observed in the passata stadium, except for germination (last count), which showed medium correlation. For the early clone, the dry stage was positively correlated only with the dry mass of the seeds.

DISCUSSION

The study investigated the maturation process and seed quality of two coffee cultivars, 101 (early) and 408 (late), over different evaluation periods. Assessments took place during a period of precipitation deficit, from late summer to winter. Anthesis began in July 2022, lasting until September 8, 2022, with full flowering (maximum anthesis). According to Souza et al. (2017), a main flowering normally occurs in the months of July or August, approximately six days after the first rains following a period of water deficit. Both coffee flowering and fruit growth rates are affected by climatic and genetic factors, which influence the uniformity of maturation and harvest period (Kath et al. 2021, Espíndula et al. 2018).

The results of the study revealed that seed maturation occurred in the months of May and June for the early cultivar and from July and August for the late cultivar, respectively with nine and 11 months of fruit development. Genetic characteristics played a significant role in the maturation process. The early maturing clones have greater photosynthetic capacity and reach the maturation point earlier compared to the late maturing clone (Morais et al. 2012). This is due to the fact that early maturing clones tend to have a faster accumulation of dry matter and nutrients, while late maturing clones have a rapid expansion phase at the end of the filling period, generally coinciding with the season dry (Marré et al. 2015, Morais et al. 2012).

However, it is important to note that seeds harvested at immature stages may have slow and irregular germination. It may be due to the presence of fruits that are not fully formed, and with insufficient accumulation of reserves (Oliveira et al. 2020). Variability also depends on seed size, physical quality, seed health, storage time, and materials (Anteneh et al. 2014). Obtaining high quality seeds is critical for the storage process, as seeds harvested at the right time have maximum physiological quality (Sripathy and Groot 2023).

Determining the optimal harvest stage to maximize seed quality is complex due to the asynchronous nature of fruit development and the influence of genetic and climatic factors. The presence of different genotypes in the same batch of *C. canephora* contributes to the diversity of seed maturation and size, impacting the final quality (Espíndula et al. 2018). Furthermore, coffee growers harvest fruits with different proportions of maturation stages, from cherry to green cane (Irma et al. 2022). However, a cherry fruit harvest is recommended to ensure seed quality, once the maturity stages positively correlated with characteristics such as germination, vigor, root length and dry mass.

The analyses also revealed that the harvest of ripe and dry fruits affected seed quality differently in the two cultivars studied. In the early clone, the presence of fruits in senescence stages reduced seed quality, while in the late clone there were no negative effects. This highlights the importance of considering the harvesting stages in obtaining high quality seeds, mainly in relation to the uniformity of the seeds in the lot.

Furthermore, physical maturity, characterized by the stabilization of the dry mass of the seeds, coincided with maximum germination values for the early seed. However, the late maturing clone took about 28 days to reach maximum germination, even after reaching physiological maturity. According to previous studies, physiological maturity generally coincides with the end of the seed filling phase (Sripathy and Groot 2023). However, the ability of the embryo to germinate and develop is a genetically determined characteristic in the seed (Dussert et al. 2018, Stavrinides et al. 2020). The period between physiological and morphological maturity is crucial in obtaining quality seeds, as it indicates the ideal time for harvesting.

The results obtained provide crucial insights into how fruit maturation and harvest stages affect seed germination and quality of different coffee cultivars. Understanding these factors is essential to improve the production of coffee seedlings and promote sustainable agricultural practices. The study highlights the need to consider both the genetic characteristics of the cultivars and the ideal harvest stages to guarantee the quality of the seeds used in coffee cultivation.

CONCLUSION

The analysis of fruit color as an indicator of the ripening stage proved to be effective in determining the harvest time.

In both clones, the cherry stage stands out as the ideal time for harvesting, reflecting the maximum physiological quality of sensations. It is worth mentioning that the early clone presented a longer time window in which the fruits reached the cherry stage, indicating a more uniform and lasting harvest point compared to the late clone.

The physiological maturation of the seeds of the early clones was reached between 244 and 299 DAA, period that proved to be suitable for harvesting due to the seeds presenting high rates of germination, vigor, dry mass, normal seedlings, root length, hypocotyl length, and average water content of 51.47%.

The seeds of the late clone reached morphological maturity at 244 DAA, with 74% of fruits still green, and physiological maturity at 272 DAA, showing germination of 80% and 70% of abnormal seedlings. However, the optimum harvest point for the late clone was at 326 DAA, marked by lower values of abnormal seedlings, higher vigor and a significant percentage of fruits at the cherry stage, reaching 98%.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Methodology: Crasque, J., Dousseau-Arantes, S., Comério, M., Volpi, P. S., Arantes, L. O., Brandão, T. M. S., Cerri Neto, B. and Milanez, C. R. D.; **Supervision:** Comério, M., Volpi, P. S. and Dousseau-Arantes, S.; **Writing – Review and Editing:** Crasque, J., Dousseau-Arantes, S.; **Project administration:** Dousseau-Arantes, S., Arantes, L. O. and Machado Filho, J. A.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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REFERENCES

Anteneh, M., Atilaw, A. and Kufa, T. (2014). Investigation of coffee seed physical purity, seed health and effect of storage time on viability. Malaysian Journal of Medical and Biological Research, 1, 85-96. https://doi.org/10.18034/mjmbr.v1i2.380

Bastian, F., Hutabarat, O. S., Dirpan, A., Nainu, F., Harapan, H., Emran, T. B. and Simal-Gandara, J. (2021). From plantation to cup: Changes in bioactive compounds during coffee processing. Foods, 10, 2827. https://doi.org/10.3390/foods10112827

[Brasil] Ministério da Agricultura, Pecuária e Abastecimento (2009). Regras para análise de sementes. Brasília: Ministério da Agricultura, Pecuária e Abastecimento / Secretaria de Defesa Agropecuária.

Campuzano-Duque, L. F. and Blair, M. W. (2022). Strategies for Robusta Coffee (*Coffea canephora*) Improvement as a New Crop in Colombia. Agriculture, 12, 1576. https://doi.org/10.3390/agriculture12101576

Cohen, J. (1988). Statistical power analysis for the behavioral sciences. 2. ed. Hillsdale: Lawrence Erlbaum Associates Publishers.

[CONAB] Companhia Nacional de Abastecimento (2022). Acompanhamento da safra brasileira. Brasília: Companhia Nacional de Abastecimento.

Da Costa, D. S., Albuquerque, T. G., Costa, H. S. and Bragotto, A. P. A. (2023). Thermal Contaminants in Coffee Induced by Roasting: A Review. International Journal of Environmental Research and Public Health, 20, 5586. https://doi.org/10.3390/ijerph20085586

Dalvi, L. P., Sakiyama, N. S., Andrade, G. S., Cecon, P. R., da Silva, F. A. P. and Oliveira, L. D. S. G. (2017). Coffee production through wet process: Ripeness and quality. African Journal of Agricultural Research, 12, 2783-2787. https://doi.org/10.5897/AJAR2017.12485

Dhindwal, A. S., Lather, B. P. S. and Singh, J. (1991). Efficacy of seed treatment on germination, seedling emergence and vigour of cotton. Gossypium Hirsutum, 19, 59-61.

Dubberstein, D., Partelli, F. L., Dias, J. R. M. and Espindola, M. C. (2016). Concentration and accumulation of macronutrients in leaf of coffee berries in the Amazon, Brazil. Australian Journal of Crop Science, 10, 701-710. https://doi.org/10.21475/ajcs.2016.10.05.p7424

Dussert, S., Serret, J., Bastos-Siqueira, A., Morcillo, F., Déchamp, E., Rofidal, V., Lashermes, P., Etienne, H. and Joët, T. (2018). Integrative analysis of the late maturation programme and desiccation tolerance mechanisms in intermediate coffee seeds. Journal of Experimental Botany, 69, 1583-1597. https://doi.org/10.1093/jxb/erx492

Espíndula, M. C., Botelho, F. J. E., Clemente, A. D. C. S., Teixeira, A. L., Alves, G. Q. F. and Oliveira, R. M. E. (2018). Quality evaluation of *Coffea canephora* 'Apoatã' seeds for rootstock production. Coffee Science, 13, 1-8. https://doi.org/10.25186/cs.v13i1.1270

[FAO] Food and Agriculture of the United Nations (2020). Statistical Databases. FAO.

Ferrão, R. G., De Muner, L. H., Da Fonseca, A. F. A. and Ferrão, M. A. G. (2019). Conilon coffee. 3. ed. Vitória: Incaper.

Ferreira, D. F. (2011). Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia, 35, 1039-1042. https://doi.org/10.1590/S1413-70542011000600001

Gonçalves, W., Andrade, V. T., Dos Reis Fatobene, B. J., De Brito Caixeta, L., Padilha, L., Oliveira, C. M. G., Rosa, J. M. O., Rodrigues, L. M. R. and Guerreiro Filho, O. (2021). Selection strategy of a *Coffea canephora* rootstock with simultaneous nematode resistance to Meloidogyne exigua, M. incognita and M. paranaensis. European Journal Plant Pathology, 160, 81-95. https://doi.org/10.1007/s10658-021-02225-8

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). Past: Paleontological statistics software package for education and data analysis. Paleontologia Eletronica, 4, 1-9.

Irma, I., Syamsia, S., Idhan, A., and Firmansyah, A. P. (2022). Pertumbuhan Bibit Kopi Berdasarkan Tingkat Kematangan Buah dan Aplikasi Cendawan Endofit Growth of Coffe Seed Based on Ther Rate of Fruit Murability and Endophite Fungi Aplication. Jurnal Galung Tropika, 11, 86-96. https://doi.org/10.31850/jgt.v11i1.941

Kath, J., Byrareddy, V. M., Mushtaq, S., Craparo, A. and Porcel, M. (2021). Temperature and rainfall impacts on robusta coffee bean characteristics. Climate Risk Management, 32, 100281. https://doi.org/10.1016/j.crm.2021.100281

Kath, J., Byrareddy, V. M., Reardon-Smith, K. and Mushtaq, S. (2023). Early flowering changes robusta coffee yield responses to climate stress and management. Science of the Total Environment, 856, 158836. https://doi.org/10.1016/j.scitotenv.2022.158836

López-García, F. J. and Cruz-Castillo, J. G. (2019). Yield of *Coffea arabica* grafted onto *Coffea canephora* in soils infested with nematodes in Mexico. Coffee Science, 14, 308-314. https://doi.org/10.25186/cs.v14i3

Marré, W. B., Partelli, F. L., Espindula, M. C., Dias, J. R. M., Gontijo, I. and Vieira, H. D. (2015). Micronutrient accumulation in conilon coffee berries with different maturation cycles. Revista Brasileira de Ciência do Solo, 39, 1456-1462. https://doi.org/10.1590/01000683rbcs20140649

Miranda, F. R., Drumond, L. C. D. and Ronchi, C. P. (2020). Synchronizing coffee blossoming and fruit ripening in irrigated crops of the Brazilian Cerrado Mineiro Region. Australian Journal of Crop Science, 14, 605-613. https://doi.org/10.21475/ajcs.20.14.04.p2118

Morais, L. E., Cavatte, P. C., Detmann, K. C., Sanglard, L. M., Ronchi, C. P. and DaMatta, F. M. (2012). Source strength increases with the increasing precociousness of fruit maturation in field-grown clones of conilon coffee (*Coffea canephora*) trees. Trees, 26, 1397-1402. https://doi.org/10.1007/s00468-012-0685-8

Oliveira, L. A., de Souza, G. A., Silva, B. T., Rocha, A. A. G., de Toledo Picoli, E. A., de Souza Pereira, D., Pereira, D. S., Donzeles, S. M. L., Ribeiro, M. F. and Ferreira, W. P. M. (2020). Histochemical approach of the mobilization of reserve compounds in germinating coffee seeds. Coffee Science, 15, e151704. https://doi.org/10.25186/.v15i.1704

Partelli, F. L., Golynski, A., Ferreira, A., Martins, M. Q., Mauri, A. L., Ramalho, J. C. and Vieira, H. D. (2019). Andina-first clonal cultivar of high-altitude conilon coffee. Crop Breeding and Applied Biotechnology, 19, 476-480. https://doi.org/10.1590/1984-70332019v19n4c68

Pezzopane, J. R. M., Pedro Júnior, M. J., Thomaziello, R. A. and Camargo, M. B. P. D. (2003). Escala para avaliação de estádios fenológicos do cafeeiro arábica. Bragantia, 62, 499-505. https://doi.org/10.1590/S0006-87052003000300015

Prezotti, L. C., Gomes. J. A., Dadalto, G. G. and Oliveira, J. A. (2007). Manual de recomendação de calagem e adubação para o Estado do Espírito Santo. 5. ed. Vitória: SEEA/Incaper/CEDAGRO.

Salazar, B. M., Lagrimas, A. J. M, Santos, P.J.A., del Rosario, E. E (2019). Profiling and Analysis of Reproductive Phenology of Four Coffee (*Coffea* spp.) Species in the Philippines using the BBCH Scale. Philippine Journal of CropScience, 44, 10-19.

Sousa e Silva, J., Moreli, A. P., Soares, S. F., Donzeles, S. M. L., do Carmo, K. B. and Vitor, D. G. (2022). Qualities of coffee beans, from ripe fruit, kept in clean water for up to five days. Brazilian Journal of Animal and Environmental Research, 5, 3961-3971. https://doi.org/10.34188/bjaerv5n4-040

Souza, C. A. D., Rocha, R. B., Alves, E. A., Espindula, M. C., Ramalho, A. R., Teixeira, A. L. and Ferrão, M. A. G. (2017). Componentes genéticos do desenvolvimento e maturação de frutos de *Coffea canephora* Pierre ex A. Froehner. Coffee Science, 12, 355-364.

Spiral, J., Ouazzani, S., Vial, N. H., Michaux, S., Barro, L., Darracq, O. and Arigoni, F. (2023). Reciprocal grafting reveals differential metabolic responses between robusta clones with contrasting tolerances to drought. Agricultural Research, 12, 72-82. https://doi.org/10.1007/s40003-022-00622-x

Sripathy, K. V. and Groot, S. P. (2023). Seed Development and Maturation. In M. Dadlani and D. K. Yadava (Eds.), Seed science and technology: biology, production, quality (p. 17-38). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-5888-5_2

Stavrinides, A. K., Dussert, S., Combes, M. C., Fock-Bastide, I., Severac, D., Minier, J., Bastos-Siqueira, A., Demolombe, V., Hem, Sonia., Lashermes, P. and Joët, T. (2020). Seed comparative genomics in three coffee species identify desiccation tolerance mechanisms in intermediate seeds. Journal of Experimental Botany, 71, 1418-1433. https://doi.org/10.1093/jxb/erz508

Tesfa, M. (2019). Review on post-harvest processing operations affecting coffee (*Coffea Arabica* L.) quality in Ethiopia. Journal of Environment and Earth Science, 9, 30-39. https://doi.org/10.7176/JEES/9-12-04

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Average values of first germination count (FCG), abnormal seedlings (AS), dead seeds (DS), nongerminated seeds (NGS) of early (101) and late (408) maturing clones.

DAA	Clone 101				Clone 408			
	FCG	AS	DS	NGS	FCG	AS	DS	NGS
	(%)							
188	0 e	0 b	100 a	0 a	0 f	0 f	100 a	0 c
202	7 d	0 b	100 a	0 a	0 f	0 f	100 a	0 c
216	54 c	6 b	91 b	0 a	0 f	0 f	100 a	0 c
230	71 b	45 a	6 c	6 a	4 e	0 f	100 a	0 c
244	93 a	3 b	2 d	0 a	5 d	0 f	100 a	0 c
258	97 a	8 b	0 d	2 a	10 d	31 c	29 b	40 a
272	99 a	4 b	1 d	1 a	49 c	67 a	6 c	13 b
286	97 a	3 b	0 d	3 a	73 b	50 b	4 c	10 b
299	98 a	7 b	1 d	2 a	90 a	34 c	3 c	1 c
314					91 a	12 e	1 c	1 c
326					90 a	10 e	0 c	1 c
349					96 a	24 d	2 c	2 c
CV (%)	5.19	73.70	8.67	133.89	9.68	27.43	8.30	50.20

Means followed by the same letter in the column do not vary statistically from each other using the Scott-Knott test ($P \leq 0.05$).