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Enhancing Coffee Robusta Cultivation in Egypt: A Comprehensive Investigation on the Synergy Role of Shade Management and PGPR on Seed Germination and Seedling Growth

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ABSTRACT

Coffee (*Coffea spp*.) is one of the most valuable commodities globally, in Egypt, coffee farming is predominantly small-scale, the focus lies on hardier Robusta variety, due to its resilience to local climatic conditions and require less water. This study explores the influence of Plant Growth Promoters Rhizobium (PGPR) and shade levels on the cultivation and growth of coffee Robusta (C. *rubsta Pierre* or C. *canephora*) in Egypt. Conducted over 15 months from March 2022 to June 2023, the experiment utilized a split-plot design with three replicates.to assess the impact of four shade levels (0, 40, 50, 60 %) with and without PGPR. Notably, the interaction between shade and PGPR significantly influenced the boasted the highest seed germination rate %, earlier seed germination time. Seedlings under shade 60% treated with a combination of PGPR exhibited the highest plant high, root number and length was observed. For the leaves development, there was a different influence of shade and PGPR individually or combined in the various stages of plant growth. The study demonstrates that the strategic use and controlled of shade levels and PGPR can substantially and significantly enhance the growth of coffee Robusta seedlings. These findings offer an effective and promising approach thereby increasing coffee production efficiency and sustainability for coffee farming in Egyptian.

Keywords: Coffee, *C. canephora*, Robusta, shade, plant growth promoters rhizobium, caffeine, Egypt, promising tropical crops.

1. Introduction

Coffee (*Coffea spp*.), a member of the *Rubiaceae* family, belongs to a thriving tribe of about 90 evergreen giants that naturally thrive in tropical climates. These trees bask in the humid heat of the growing season and the warm dryness of harvest time. Among these giants two stand out in the fields and markets, Arabica (*C. arabica L*.) and Robusta (*C. roubsta Pierre*) or as known (*C. canephora*). These are the undisputed commercial kings, while Liberica (*C. liberica Bull ex Hiern*) and excelsa (*C. excelsa*) play more specialized roles which are often cultivated for hybridization and research purposes, particularly as rootstock for other coffee varieties. Coffee reigns supreme as the world's most favored beverage, holding a crucial role as a cash crop and export mainstay (Krishnan, 2022; Panaligan *et al*., 2021). In some developing nations, particularly parts of Africa, it can contribute to as much as half of their foreign income. Five countries - Brazil, Vietnam, Colombia, Indonesia, and India - are the leading players in global coffee cultivation and production. Others, like Ethiopia, Mexico, and Guatemala, join them in contributing to over 90% of the world's coffee supply according to FAO (2023; Kangile *et al*. 2021).

Coffee robusta is the second most consumed coffee in the world, accounting for about 43% of global coffee production and Arabica making up the remaining 1.5% except for Liberica. It is grown in the Eastern Hemisphere, especially in Africa and Indonesia, and is the largest producer of Vietnam. Robusta coffee has a strong, harsh but deep flavor, due to its high caffeine content. Robusta is a hardy

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plant that is easy to care for, has greater productivity, and is less susceptible to disease than Coffee Arabica (Sachs *et al*., 2019).

Nganda coffee beans belong to the Robusta species, one of just two coffee bean types globally (alongside Erecta). Hailing from Uganda, Nganda is highly popular there and boasts a flavor profile similar to other Robusta beans. However, its "Single-origin" status sets it apart. Grown exclusively in Uganda, Nganda develops unique characteristics due to its specific terroir, offering a distinct experience for coffee enthusiasts (Davis & Kiwuka, 2023).

Also, coffee can be propagated through various methods, including vegetative techniques like cuttings and grafting, and even through advanced in-vitro or micro-propagation. However, for commercial production, the most popular and widespread method remains the simple yet effective approach of seed propagation (Avila-Victor *et al*., 2023).

While sunlight plays a crucial role in plant growth and photosynthesis, its importance during coffee seed germination is a bit more nuanced. Unlike many other seeds that require light exposure to trigger germination, coffee seeds are classified as photo insensitive. This means they don't require light for their seeds to sprout, but it doesn't mean light has no influence at all (Guevara-Barrera *et al*., 2024).

PGPR are a diverse group of naturally occurring soil bacteria that form symbiotic relationships with plants. They colonize plant roots and promote growth in various ways, including fixing nitrogen, solubilizing nutrients, releasing hormones, increased root development, and suppressing pathogens. PGPR offer a safe and environmentally friendly alternative to chemical fertilizers and pesticides. Also, they can help small-scale farmers improve their coffee yields and income without relying on expensive external inputs. PGPR promote soil health and fertility and contributing to long-term sustainability in coffee production. They are part of a broader category of plant growth-promoting microorganisms (PGPM), which also includes Arbuscular Mycorrhizal Fungi (AMF). These microorganisms have been extensively studied for their positive impact on coffee production, from isolation to field application (Urgiles-Gómez *et al*., 2021).

Egypt's coffee volume of imports in 2023 amounted to approximately 136.292 million US dollars, an increase of 1.6% from 2022. Unroasted, decaffeinated coffee constituted 93.4% of Egypt's total coffee imports. Moreover, in 2023 Egypt imported approximately 60,000 tons of coffee, a significant figure in the country's food and beverage industry. It’s estimated that per capita coffee consumption in Egypt is about 2.5 kg per year (Trading economics website, Egypt Imports of Coffee, tea, mate and spices - 2024 Data 2025 Forecast 1994-2023 Historical (tradingeconomics.com).

It is clear that the future of coffee cultivation in Egypt is seen as bright, as the country enjoys favorable environmental conditions for coffee cultivation, such as fertile soil and a moderate climate. In addition, there is an increase in demand for coffee in Egypt, as coffee is becoming more popular among the population. Coffee cultivation in Egypt has the potential to become an economically successful industry, providing new job opportunities, contributing to economic growth, and providing the value of what is imported annually. This has led to a number of initiatives working on this, such as the Egyptian Ministry of Agriculture’s program to develop coffee cultivation, and the private sector to establish agricultural complexes for coffee.

Coffee (*Coffea spp*.) is the world's most beloved beverage, faces challenges in balancing production and sustainability. Generally, this study compares two key propagation methods; traditional seed propagation and micro-propagation or in vitro technique. Furthermore, this work assessed the impact on economic and sustainability within the context of Egyptian coffee agriculture. Through rigorous analysis and field trials, this research aims to identify the most sustainable propagation method for Egyptian coffee, paving the way for a thriving industry that balances economic growth with environmental and economic responsibility.

2.Materials and Methods

2.1. Study Area Site Data and Plant Materials

This experiment was carried out at Horticulture Research Institute (HRI) research farm. Within coordinates 59.00° South and 37.00° East at an altitude of approximately 1548 meters above sea level, and private farm in El-Ismailia Governorate, at 30.61° North latitude, 32.28° East longitude, and 14 meters elevation above sea level, as it is shown in Fig 1, during the seasons of 2022 and 2023. Seeds resource was taken from fruit in the stage of “Cherry” collected of adult trees of the Robusta variety “Nganda” CV. This plant germplasm belonged to an orchard under supervision of Makrere University

in the region of Greater Masaka, south-west of Kampala, Uganda. With coordinates 0°20'28.0"S, 31°44'10.0"E, Latitude 0.341111 and Longitude: 31.736111.

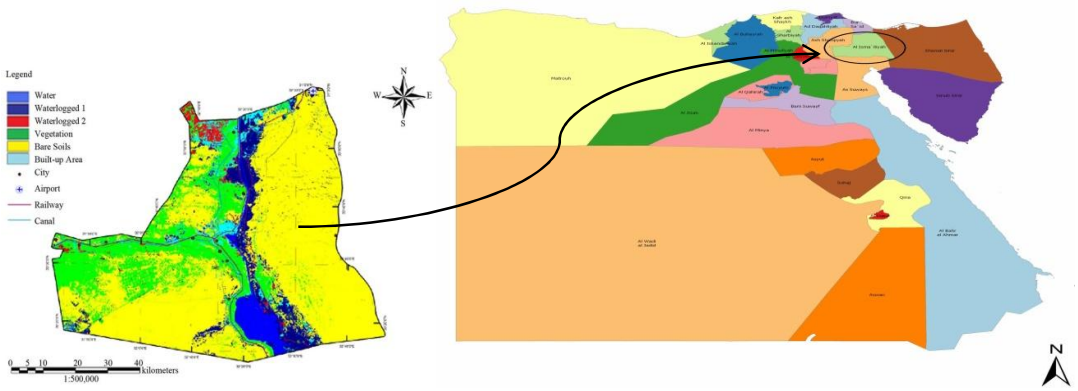

Fig.1: Photo credit to Omran, (2012). Using detailed satellite imagery to track changes in land use and surface temperature. and Governorates of Egypt – Provincial Divisions of Egypt. Mapper. https://www.mappr.co/counties/egypt-provincial-divisions-governorates/

2.2. Soil Composition

A mix of soil was prepared (40% clay, 30% sand, 20% compost, and 10% perlite) with treat it with a suitable, save, and certified fungal solution.

2.3. Pre- Treatments

2.3.1. Coffee Cherry Soaking

Through soaking coffee fruits in clean water for 24-48 hours which helps with hydration and improve removing fruit flesh or the pulp (Exocarp and Mesocarp).

2.3.2. Seed and Parchment Removal

Coffee beans are typically encased in a hard outer shell called the parchment. For most planting methods, it's crucial to remove the parchment before planting. This allows for faster and more uniform germination. It can be removed the parchment by hand or using a de-pulping machine.

2.3.3. Coffee Beans (Green seeds) soaking

Soaking coffee beans (seeds) in clean water for 48-72 hours with changing the water every 6-7 hours.

Furthermore, there are some vital additional factors to consider such as; seed selection, seed source where choosing seeds from reliable sources to ensure good quality and avoid potential diseases, and climate and location which the success of cultivation based on the specific climate and planting location.

Fig. 2: Different stages of coffee cherries and seeds for coffee seeds (*C. canephora*).

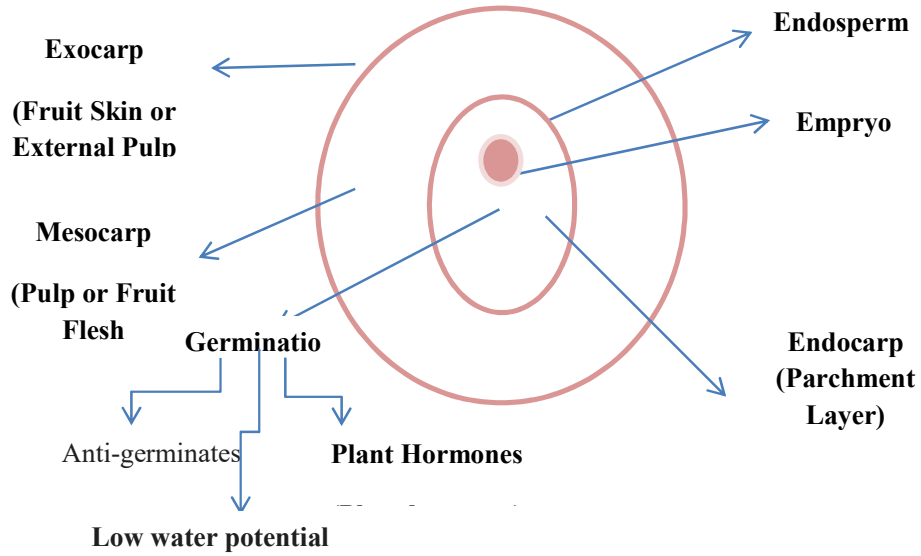

Fig. 3: Germination of coffee seeds is inhibited by the action of bioactive compounds located within the fruit flesh (pulp).

2.4. Treatments, Tools, and Experimental Design

2.4.1. Some of the materials used in this research are Robusta coffee seeds of "Nganda" cultivar, plastic growing trays (Beds) size 40 x 30 cm, PGPR, shading levels, planting medium, plastic, black color shade nets 63% Transmittance rate, water, and fungicide.

2.4.2. Sowing Date: Cherries were obtained at the end of January and after the soaking process and the whole preparations, sowing seeds was at the beginning of March.

2.4.3. To measure the shade level, Light Meter (HANNA, HI8564) model was exploited. It measures the amount of light reaching a specific spot. Then readings were taken in full sun and under the shade (level), and then divide the shade reading by the full sun reading to get a percentage shade value.

After the process of soaking seeds and beans and preparing a good soil mixture, preparing the tools, completing the sowing process, and before making various treatments of adding bacteria or shade.

It is crucial to define a tunnel inside the greenhouse in order to provide the optimal and necessary temperature humidity degrees for germination as which we will address in advance through another research paper as it shown in Figure 3. Taking into account not to irrigate except once a month or when needed, as well as periodic inspection constantly without lifting the tunnel cover completely.

Fig: 4: The plastic tunnel inside the greenhouse to provide temperature and humidity for germination.

The treatments and combinations between them were as following:

T_1:	0 PGPR + 0% shading as a (control).	**T_5:**	60% shade + PGPR
T_2:	0% shade + PGPR	**T_6:**	40% shade + 0 PGPR
T_3:	40% shade + PGPR	**T_7:**	50% shade + 0 PGPR
T_4:	50% shade + PGPR	**T_8:**	60% shade + 0 PGPR

A liquid formulation of PGPR mixed with water and directly applied to the soil around the base of the plant.

We utilized a scientific approach using a split-plot design. Five treatments were implemented, each replicated three times. This resulted in 15 experimental units, with each unit containing five seeds for observation. A total of 75 seeds were monitored throughout the experiment.

2.5. Measurements

2.5.1. Vegetative and Root Growth Parameters

2.5.1.1. Seed Germination Percentage (%)

Count the number of germinated seeds in each replicate. Calculating germination percentage by the equation: Germination Percentage (%) = (Number of germinated seeds / Total number of seeds planted) x 100.

2.5.1.2. Seed Germination Time (Days):

2.5.1.3. Plant height (cm): Plant length was determined from the soil surface up to the petiole of the last emerged leaf with (cm).

2.5.1.4. Number of leaves / Plant: Number of green leaves was recorded for each plant.

2.5.1.5. Leaf area (m^2): Leaf area was chosen as the third leaf from the plant top and calculated by the equation as following (leaf area= length x width x 0.8) as described by Murry, (1960; Ahmed and Morsy, 1999).

2.5.1.6. Number of roots / Plant

Plants were carefully removed from their growing medium and gently rinsed with water to remove adhering soil particles, minimizing root damage. However, the roots of each plant were spread out in a single layer on the dish or tray. Individual roots were meticulously counted by hand.

2.5.1.7. Root length (cm)

Root samples were collected by carefully extracting soil cores with a diameter of 10 cm and a depth of 20 cm from the rhizosphere of each plant. Roots were gently washed with tap water to remove adhering soil particles carefully. Root length was measured using a tape. Straighten out any bends or curves as much as possible for accurate measurement.

2.6. Data Analysis

We assigned experimental treatments randomly using a blocked design as described by Snedecor and Cochran, (1980). After performing a one-way analysis of variance (ANOVA) we compared the average results (means) of each treatment using the least significant difference (LSD) test at a 5% significance level ($p < 0.05$).

3. Results and Discussion

3.1. Physical Characteristics

3.1.1. Seed Germination Percentage (%)

For Seed germination and time, coffee seeds treated shade conditions and boosted soil with PGPR instead of soaking the seed in the dark then treat PGPR in the soil to shorten time and get benefited from it.

Results on coffee (*C. canephora*) seed germination percentage showed that both the individual effects of shade levels and the combined effects of dark and adding plant growth-promoting

rhizobacteria (PGPR) significantly influenced the germination rate which these findings are presented (Figure 5A). However, the combinations of (60%) shade and PGPR resulted in significantly higher germination (98.20%) as T6 than the un-shaded treatments. While significantly lower germination was recorded for un-shaded conditions without PGPR as a control treatment T1 indicated to a significant difference compared with the other in value (52.4%). Meanwhile, treatments without shade yielded similar percentages with no statistically significant variations.

Coffee seed germination, marked by radicle emergence, began around day five after imbibition. By day ten, half of the seeds had germinated. Even under the stress of darkness (an abiotic stress), achieving nearly complete germination still only took about fifteen days, as expected for high-quality seeds. This rapid germination under stress further supports the notion of high seed quality. Moreover, the gene encoding the enzyme endo-β-mannanase, located within the micropylar endosperm, exhibited high expression levels and a strong degree of similarity (homology) to known genes. This suggests a potentially important role for this enzyme in seed development, cloned by Silva *et al*. (2019). This result agrees with suggestion given by Arura and Yun, (2023) study indicates that germination in darkness is higher than in light. Additionally, exogenous gibberellic acid and abscisic acid inhibit germination, while kinetin reverses this inhibitory effect for *Coffee arabica* L. cv. Mundo Novo. As indicated by Resende *et al*. (2009), they investigated how light, gibberellins (plant growth hormones), and paclobutrazol (a growth inhibitor) influence coffee Arabica seed germination. They proved that high values of germination velocity rate were observed in dark. Furthermore, Coffee seeds treated with gibberellins exhibited both a slower rate of germination and a lower overall germination percentage in both conditions (light, dark). Conversely, while the gibberellin inhibitor also slowed germination, it did not negate the influence of light on germination rate. They elaborate that seeds in the dark have a higher germination speed index compared to those in the light, perhaps because in the dark the synthesis of gibberellins is reduced. In this case as our findings, the synthesis of endo-b-mannanase is also reduced, which leads to a decrease in mannose levels. Washa, (2015) was in line with proves of that, seed germination was found to be significantly higher in the dark condition than in light condition. On the other hand, Ali *et al*. (2013) recorded that soaking coffee seeds in EM solution did not significantly hasten emergence, but forest soil combined with a 4.5-hour soaking in EM solution produced vigorous seedlings for transplanting. However, adding it combined with the soil (75:25 ratio) resulted in the highest seedling emergence (76.47%).

3.1.2. Seed Germination Time (Days after Sowing)

The analysis of germination duration in days after seed sowing of tested *C. canephora* variety detected significant differences (Figure 5B). T6 as 60% shade and supported with PGPR had the shortest time to complete the full germination stages in (13 days). The longest time to complete germination was remarked for the T1 as a control treatment (47 days). Notably, the other treatments T6, T7, and T8 with or without one of the two factors shade and PGPR Germinated slightly slower compared to the others, but the differences were not statistically significant (Figure 5B).

A study by Resende *et al.* (2009) they explored that the role of light, gibberellins, and paclobutrazol in coffee seed germination. Interestingly, their research showed that seeds germinated much quicker in the absence of light. Potential explanations for these observations could include that Seeds in darkness may conserve energy by avoiding unnecessary metabolic processes until they encounter favorable conditions. Additionally, Different plant species exhibit varying responses to light and darkness during germination. In this context, some research has shown that germination time increased with decreasing dark conditions (Hamouzová *et al*., 2024). In contrast the investigation of (Waters *et al*., 2017) which proved that, unlike typical seeds, these "non-orthodox" ones begin germination even within the coffee cherry. This asynchronous process, meaning germination timing varies, is influenced by how the beans are processed after harvest. A cascade of metabolic reactions occurs during this early germination, ultimately shaping the final composition of carbohydrates, proteins, and lipids in the mature coffee beans.

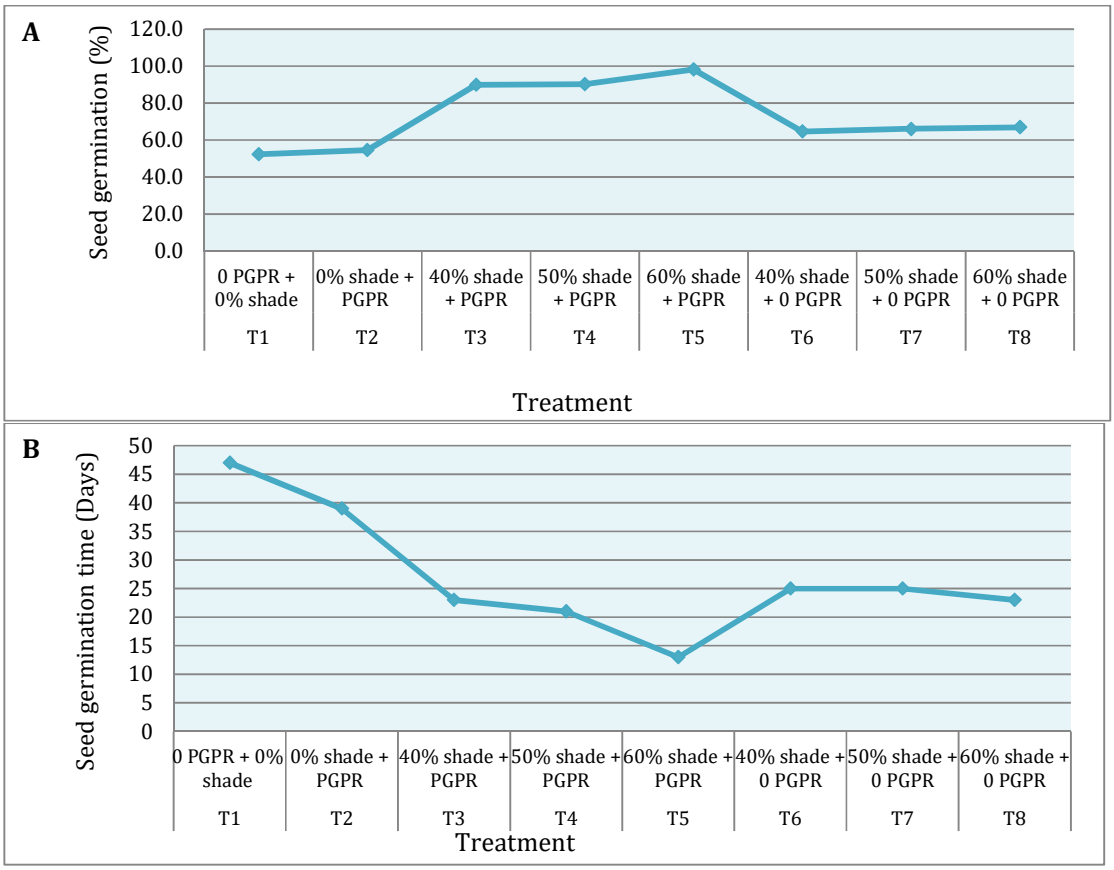

Fig. 5: A, B. Percentage and time in days of *C. canephora* seeds germination stages of development in seedlings of under different shade levels with or without adding PGPR.

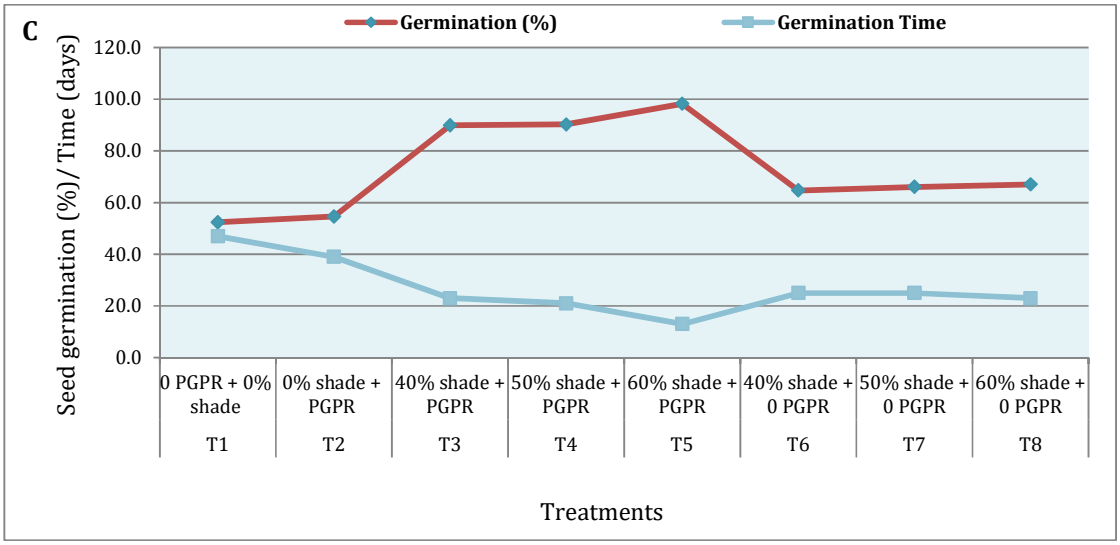

Fig. 5: C. The interaction between seed germination percentage and time in days of *C. canephora* seeds under different shade levels with or without adding PGPR.

Figure 5C revels that the Interactive effects of shade PGPR had a significant and strong influence on both seed germination percentage and duration together. it's worth emphasizing that the mean germination duration emphasized a decline with the increasing of the light conditions and with the absence of PGPR. Likewise, germination rate increased with increasing shade level in the support conditions of PGPR.

Fig. 6: Different stages of seed germination for coffee seeds (*C. canephora*) under the plastic tunnel covered with black color shade nets (0, 40, 50, and 60%).

3.2. Plant Height (cm)

Coffee seedling growth, as shown in Figure 7, benefited from shade. Seedlings under treatments T5 and T8 which correspond to 60% shading with PGR and 50% shading without PGR respectively have led to the most significant increase in plant height. Whopping readings were obtained of T5 & T8, 24.50 and 23.40 cm respectively at 8 weeks after planting. In contrast, un-shaded seedlings grew the least, reaching only 10.43 cm. In the initial stages (between the second and third week), shading did not markedly influence the stature of the seedlings. Nevertheless, by the culmination and the end of the study (week 8), shading had a pronounced and substantial effect on the seedlings elongation.

The highest value for T5 and T8 indicate that these specific conditions were more conducive to plant growth compared to the other treatment combinations. This could be due to a variety of factors, including the optimal balance of light intensity and hormonal stimulation that promotes cell elongation and division, leading to increased plant height.

Suggests of (Sembiring *et al*., 2023) they demonstrated that the application of different growth regulators did not significantly impact the height of Sigarar Utang variety Arabica coffee seedlings (*Coffea arabica* L.) at eight weeks after planting. However, the type of shade provided had a significant effect on seedling height at the same time point.

The results of (Baliza *et al*., 2012) illustrated that gradual shading levels (35%, 50%, and 65% shade) promoted increased plant height compared to the control group. Conversely, plant diameter and number of plagiotropic branches (lateral branches growing horizontally) exhibited a significant decrease only under the most shaded treatment (90% shade, equivalent to 10% solar radiation). They elaborate that, higher soil moisture of shaded system increases water and nutrient uptake over time. This is in the agreement of our present work. A trend towards thinner diameters and fewer branches was observed at lower shade levels as well. These findings partially align with the observed physiological characteristics, particularly during the rainy season. This suggests that enhanced photosynthetic activity due to increased light availability (at lower shade levels) likely contributes to the observed increase in plant height (Melo *et al*., 2008). Kevin Piato *et al*., (2020) were in contrast in their study which revealed that shade positively impacted the growth of older Robusta coffee trees (average age: 16 years). However, the effect of shade on younger trees was either negligible or detrimental. These findings highlight the crucial roles of both clonal variety and tree age in shade response. Notably, a knowledge gap exists regarding the specific effects of shade on coffee plants in relation to both coffee tree age and shade tree age, as well as potential interactive effects between these factors. Further in-depth research is necessary to elucidate the mechanisms by which shade trees influence Robusta coffee growth and productivity. Otherwise, (Torres *et al*., 2019) evaluated plant height, number of branches, and extraradical mycelium of arbuscular mycorrhizal fungi (AMF). The results showed that applying plant growth-promoting rhizobacteria (PGPR) significantly enhanced all these parameters. These findings suggest that combining an appropriate AMF consortium with a low amount of organic fertilizer could be a sustainable management strategy to improve coffee plant growth and performance.

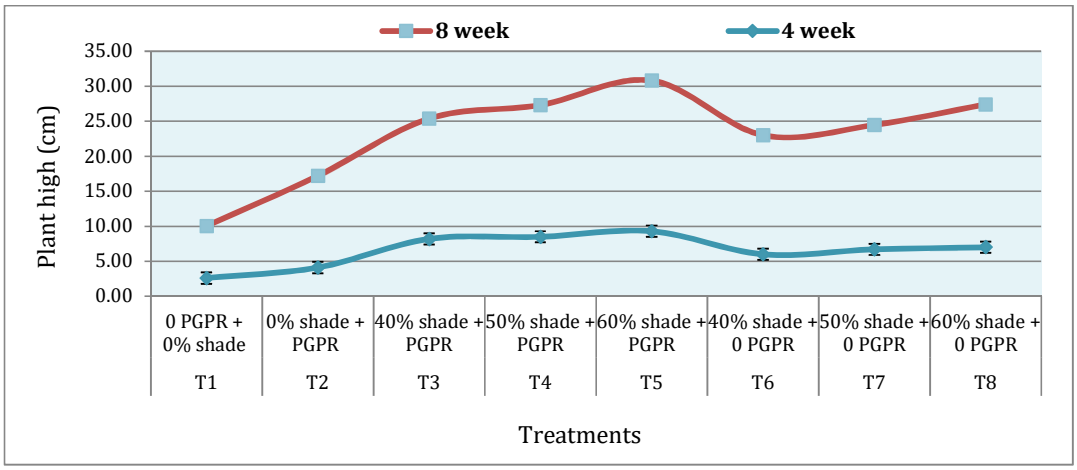

Fig. 7: Plant height of *C. canephora* seedlings under different shade levels with or without adding PGPR.

Fig. 8: Different stages of plant high for coffee seedlings (*C. canephora*) under the plastic tunnel covered with black color shade nets (0, 40, 50, and 60%).

3.3. Leaves Number/Plant

Variance analysis indicated that neither the application of PGPR nor the shading methods used had any significant differences between each other in the first stage of plants (week 4) except some negative changes with the T6 and T5 with no significant difference 2leaf/ plant for both. Essentially, PGPR treatments and shading techniques contributed to slight increase in the leaf numbers of the coffee seedlings. Importantly, the rapid leaves number observed in this study was particularly significant in the late growth stage in week 8 of the experiment with T6 in value (10.06 leaf /plant) Figure 9A. Furthermore, number of leaves might also be influenced by the light with or without PGPR which recorded the lowest values in 4.50 leaf with T1 (control), and 4.80 for light conditions synergetic with PGPR. This occurs because of shading which push seeds to etiolated, so the plant becomes higher as shown by Alridiwirsah *et al*., (2015). Arisandi *et al*. (2015) added further suggest that young coffee plants require more shade than mature, fruiting trees.

3.4. Leaves Area (m^2)

Applying different growth regulators didn't significantly impact the leaf area of coffee seeds during the first four weeks. It's likely that the young coffee seedlings needed more time to fully absorb and utilize the growth regulators, leading to no observable effect on leaf growth during this initial period. Meanwhile, at the stage of week 8, the seedlings grown under 60% shade with PGPR supported soil reached the maximum values 6.26m^2. This contrasts with un-shaded plants without PGPR treatment, which showed the lowest recorded value Figure 9B.

Consequently, understanding the interplay between light and other factors is crucial for plant growth, thus (Sembiring *et al*., 2023) resulted for an opposite theory than our findings where they indicated that, neither the application of various growth regulators nor the type of shade significantly affected the number of leaves produced by coffee seeds. Moreover, there are other factors that influence

it. It has been proposed by Gardner *et al*. (1991) proposed that variation in the number and size of leaves is a product of both the plant's genetic makeup (genotype) and the surrounding environment. However, our findings revealed an interaction between shade and PGPR treatments, as confirmed by statistical analysis (ANOVA). This adds nuance to previous research, suggesting that the influence of shade on coffee plants might be modulated by the presence of plant growth-promoting rhizobacteria.

Across both nursery locations, plants grown under 80% and 50% shade exhibited the largest leaf area at the end of the nursery period, with no statistically significant difference between these two shade levels. Conversely, plants grown in full sun (0% shade) had the smallest leaves at the Yantzaza site. Interestingly, in Yantzaza, leaf area under 0% shade was not significantly different from that observed under 30% shade. Plants grown under higher shade levels exhibited greater photosynthetic efficiency. Conversely, seedlings exposed to full sunlight displayed the lowest efficiency (Encalada Córdova *et al*., 2016). This suggests that increased radiation levels may promote the development of smaller plants, potentially due to a shift in resource allocation away from photosynthesis towards growth and development (Arcila, 2008). Dias -Moreira *et al.* (2019) were in line with our results where they investigated the effects of Arbuscular Mycorrhizal Fungi (AMF) on the development of coffee seedlings of the coffee. Specifically, they used three AMF species: Rhizophagus clarus, Claroideoglomus etunicatum, and Dentiscutata heterogama. Their findings revealed a significant increase in both leaf area and plant height in seedlings inoculated with AMF compared to the control group.

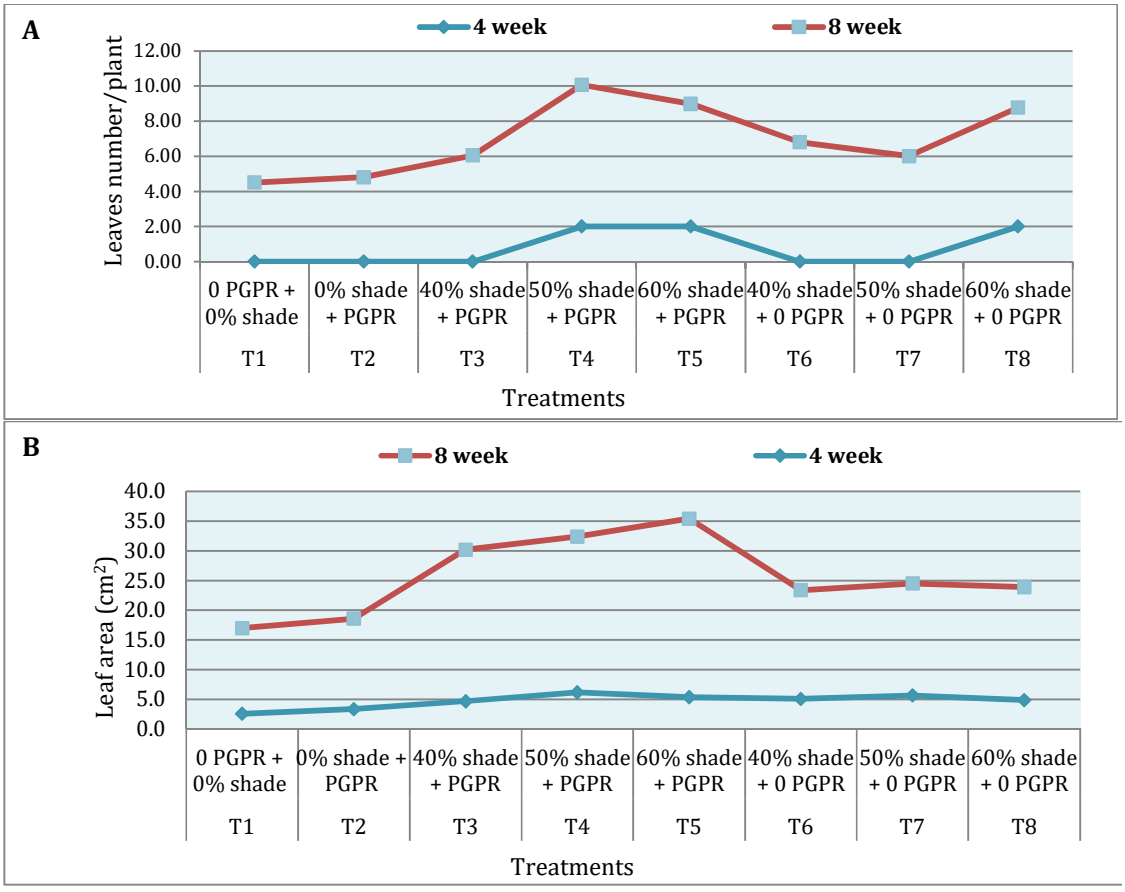

Fig. 9: A, B. Characterization of the number of leaves and leaf area in plants of *C. canephora*, coming from seeds previously germinated after 4 & 8 week under different level of shade with or without PGPR.

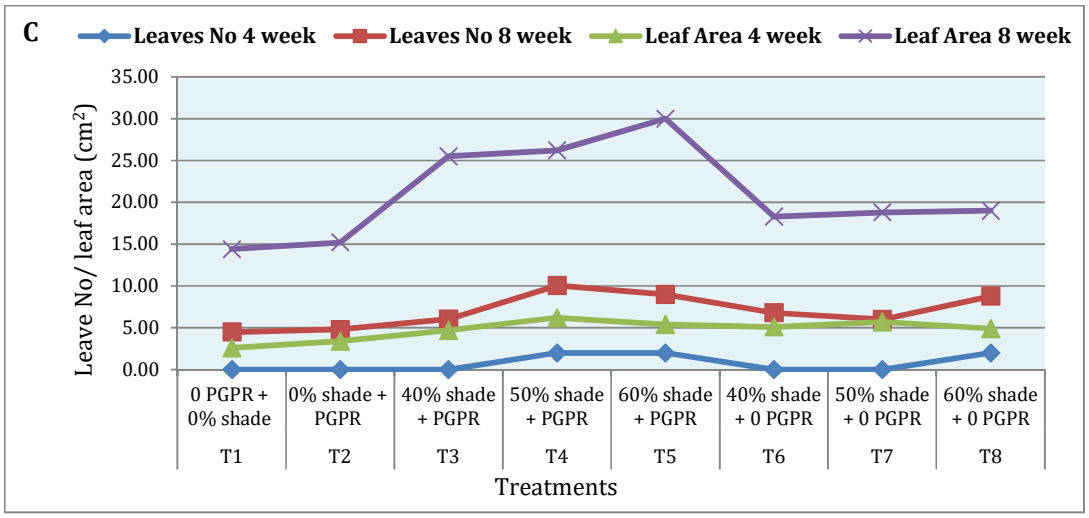

Fig. 9: C. The interaction between Leaves number/plant and leaf area of *C. canephora* seedlings under different shade levels with or without adding PGPR.

Figure 9C highlights a significant and interactive effect of shade and PGPR application on both leaf number and leaf area. Notably, this interaction was more pronounced for leaf area at week 8 of the study. While leaf number also showed a significant response, this effect became more evident after week 6.

Fig. 10: Different stages of leaves number and area for coffee seedlings (*C. canephora*) under the plastic tunnel covered with black color shade nets (0, 40, 50, and 60%).

3.5. Roots Number/Plant

Coffee plants were subjected to varying levels of shade individually or combined with PGPR, where root density significantly differed among treatment groups. At the first 4 weeks of the study there was a significant difference of treatments impact reached to the fluency at week 6. At week 8, the Coffee plants grown under 60% and moderate shade at 50% especially those within PGPR treatment exhibited higher root density compared to those in full sun. Figure 11A, Figure 12. While root density remained high, there was a diminishing trend beyond a certain shade threshold.

3.6. Root Length (cm)

At the first 4 weeks of the study there was no significant difference of treatments other than the control. Coffee plants under moderate shade had longer roots compared to full sun. Root length was optimal under 60% shade but decreased under light shade and un-shaded conditions. Additionally, PGPR effectively colonized the rhizosphere due to PGPR-treated plants showed higher root numbers and length for Nitrogen-fixing which contributed to root growth.

However, use of the plant growth-promoting microorganisms (PGPM) in coffee plants and the influence appeared significantly in increasing the root mass and generally improved the performance of coffee plants according to Urgiles-Gómez *et al*. (2021). In other line but at the same direction (Sembiring *et al*., 2023) they observed that the increase in the root-to-canopy ratio coincides with an elevation in carbohydrate content within the stems and roots of coffee seedlings. This rise in

carbohydrates suggests that the light intensity received by the seedlings was optimal for efficient photosynthesis. This aligns with Alvi *et al*. (2018), which highlights that the accumulation of dry matter (including roots) reflects a plant's ability to capture sunlight through photosynthesis and its interaction with other environmental factors.

Figure 11C reveals that a significant interaction between shade and PGPR application, influencing both root number and root length. Interestingly, this interaction had a stronger effect on root length, with the most pronounced differences observed at week 8 of the study. In contrast, while root number also showed a significant response to the combined treatment, this effect became more evident earlier, starting from week 4.

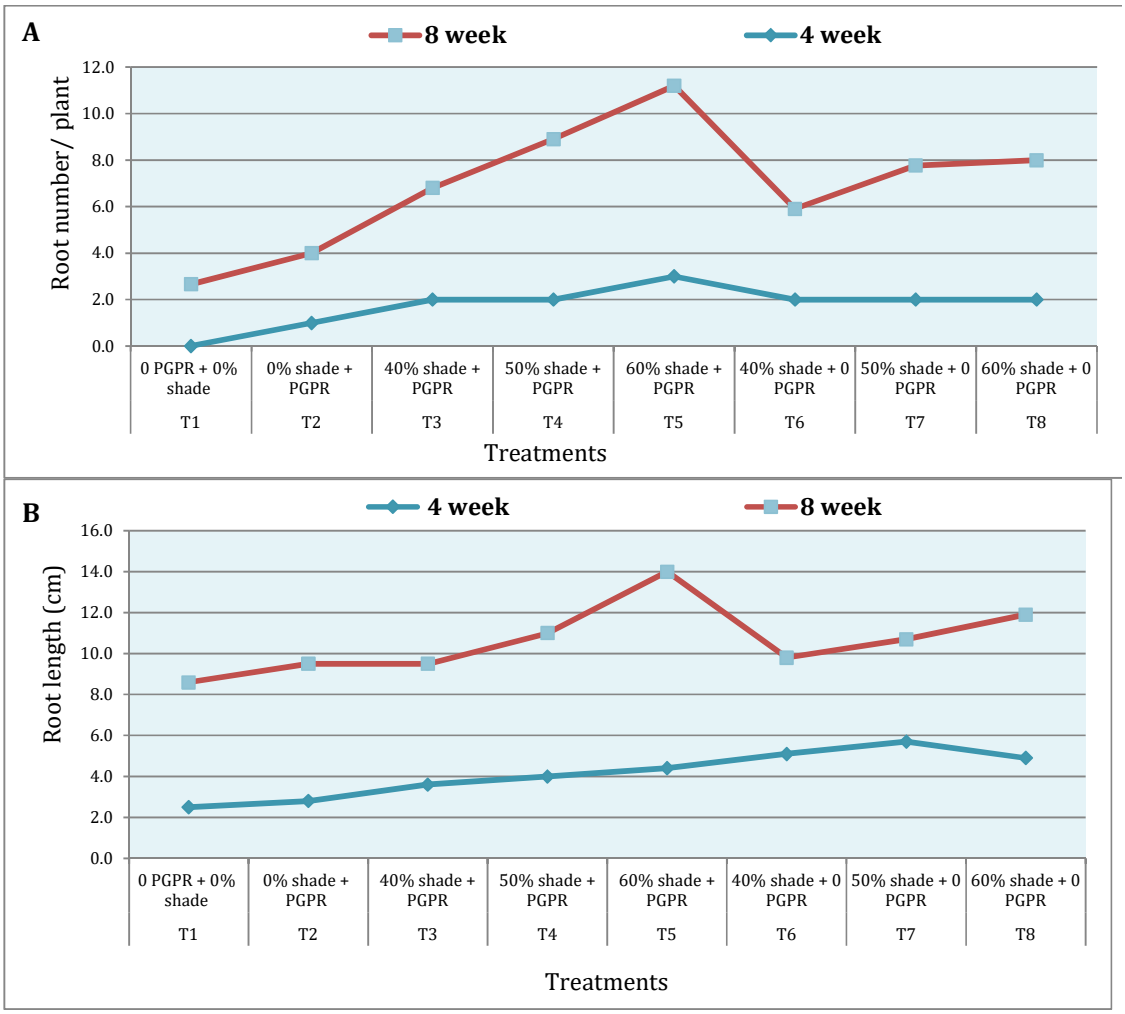

Fig. 11: A,B. Characterization of the number of leaf and leaf area in plants of *C. canephora*, coming from seeds previously germinated after 4 & 8 week under different level of shade with or without PGPR.

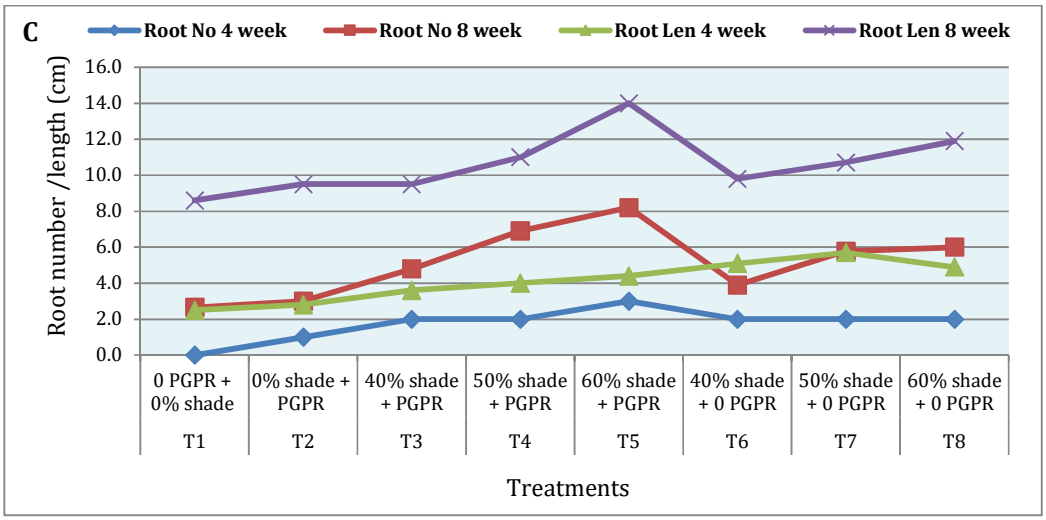

Fig. 11: C. Characterization of the number of leaf and leaf area in plants of *C. canephora,* coming from seeds previously germinated after 4 & 8 week under different level of shade with or without PGPR.

Fig. 12: Characterization of root numbers and length of germinated seeds of *C. canephora,* after 4 week under different level of shade with or without PGPR.

4. Conclusion

Our research demonstrates a significant interaction between PGPR application and shade levels on coffee plant growth. Notably, the combination of 60% shade and PGPR treatment significantly increased vegetative growth characteristics, including plant height, leaf number, leaf area, root number, and root length. Also, future studies will investigate the impact of this treatment combination on total leaf chlorophyll content, nitrogen content, carbohydrate levels, and caffeine concentration in the plants.

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Competing Interests

Authors have declared that no competing interests exist.

References

Arcila, J. 2008. Growth and Development of the Coffee Plant. In: Arcila, J., F. Farfan, A.M. Moreno, L.F. Salazar, and E. Hincapie. Coffee Production Systems in Colombia. Chinchina, Cenicafe. p. 21-60. (In Spanish).

Arora, A. S., and C.M. Yun, 2023. Dynamic spectrum lighting impact on plant morphology and cannabinoid profile of medical and recreational cannabis–A novel leapfrog strategy towards shaping the future of horticulture lighting. Industrial Crops and Products, 199: 116799.

Avila-Victor, C.M., V.M. Ordaz-Chaparro, E.D.J. Arjona-Suárez, L. Iracheta-Donjuan, F.C. Gómez-Merino, and A. Robledo-Paz, 2023. In vitro mass propagation of coffee plants (*Coffea arabica* L. var. Colombia) through indirect somatic embryogenesis. Plants, 12(6): 1237.

Davis, A.P., and C. Kiwuka, 2023. A review of the indigenous coffee resources of Uganda and their potential for coffee sector sustainability and development. Frontiers in Plant Science, 13: 1057317.

Dias Moreira, S., A. Cabral França, P.H. Grazziotti, F.D. Soares Leal, and E. de Barros Silva, 2019. Arbuscular mycorrhizal fungi and phosphorus doses on coffee growth under a non-sterile soil. Rev. Caatinga, 32: 72–80.

Dubois, M., K.A. Gilles, J. Hamilton, R. Rebers, and F. Smith, 1956. Colorimetric method for determination of sugar and related substances. Analytical Chemistry, 28: 350.

Encalada Córdova, M., F. Soto Carreño, and D. Morales Guevara, 2016. Coffee (*Coffea arabica* L.) seedling growth with four shade levels under two soil and climate conditions of Ecuador. Food and Agriculture Organization (FAO)., 2023.

Gardner, F.P., R.B. Pearce and R.L. Mitchell, 1991. Physiology of cultivated plants (Jakarta: Translated by Susilo H, University of Indonesia Press) 428.

Guevara-Barrera, J.M., S. Magnitskiy, and H.E. Balaguera-López, 2024. Physiological evaluation of *Sonchus oleraceus* L. seeds with different pre-germinative treatments under high tropical latitudinal conditions. Revista Facultad Nacional de Agronomía Medellín, 77(1): 10541-10552.

Hamouzová, K., J. Šuk, S. Bhattacharya, J. Mikulka, V. Valičková, M. Kolářová, and J. Soukup, 2024. The effect of various factors (light, temperature, salt, and drought) on germination of *Bromus sterilis* L. Plant, Soil and Environment, 70, 2024 (5): 287–295 .

Kangile, J.R., R.M. Kadigi, C.P. Mgeni, B.P. Munishi, J. Kashaigili, and P.K. Munishi, 2021. The role of coffee production and trade on gender equity and livelihood improvement in Tanzania. Sustainability, 13(18): 10191.

Kevin, P., L. François , S. Cristian , C. Carlos , C. Darío , P. Jimmy and N. Lindsey, 2020.

Krishnan, S., 2022. Coffee: Genetic Diversity, Erosion, Conservation, and Utilization. Cash Crops: Genetic Diversity, Erosion, Conservation and Utilization, 55-80.

Melo, R. R., M. C. L. Cunha, F. Rodolfo Júnior, and D. M. Stangerlin. 2008. Crescimento inicial de mudas de *Enterolobium contorsiliquum* (Vell.) Morong sob diferentes níveis de luminosidade. Agrária 3: 138-144.

Nagata, M., and I. Yamashta, 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruits. J. Japan. Soc. Food Sci. Technol. (Nippon Shokuhin KogyoGakkaishi), 39(10): 925-928.

Official Methods of Analysis of AOAC International (Association of Official Analytical Chemists International). 2023.

Omran, E.E., 2012. Detection of land-use and surface temperature change at different resolutions. Journal of Geographic Information System, 4(3): 189-203. doi: 10.4236/jgis.2012.43024.

Panaligan, A.C., M.D. Baltazar, and G.J.D. Alejandro, 2021. Molecular authentication of commercially cultivated coffee (Coffea spp.) in the Philippines using DNA barcodes. International Journal of Agriculture & Biology, 25(1):227-230. DOI: 10.17957/IJAB/15.1660

Patay, E.B., L. Fritea, A. Antonescu, A. Antonescu, and L. Dobjanschi, 2017. *Coffea arabica*: a plant with rich content in caffeine. The question of caffeine, 27.

Rabbani, I., F. Armin, and R. Rasyid, 2021. A Review: The Use of Chromatographic Methods in Caffeine Analysis during 2000-2020. IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS), 16(1): 14-21.

Resende, M. de L., Silva T. T. de A., R. M. Guimarães and E. A. A. da.Silva, 2009. Influence of light and gibberellin on the germination velocity of coffee seeds (*Coffea arabica* L.). Coffee Science, 4(2): 149–154.

Sachs, J., K. Y. Cordes, J. Rising, P. Toledano and N. Maennling, 2019. "Ensuring Economic Viability and Sustainability of Coffee Production," Columbia Center on Sustainable Investment (October 2019).

Sembiring, L.J., R.I. Damanik, and C. Hanum, 2023, September). Growth of arabica coffee seeds (*Coffea arabica* L.) Sigarar Utang Variety as a response of treatment of various growth

regulators and types of shade. In IOP Conference Series: Earth and Environmental Science, 1241(1): 012031). IOP Publishing.

Silva, E.A.A.D., M.L. Acencio, L.A. Bovolenta, N. Lemke, A.D.M. Varani, J.P. Bravo, … and E.G.M. Lemos, 2019. Gene expression during the germination of coffee seed. Journal of Seed Science, 41:168-179.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th ed., The Iowa State Univ. Press. Amer., Iowa, U.S.A., 593.

Urgiles-Gómez, N., M.E. Avila-Salem, P. Loján, M. Encalada, L. Hurtado, S. Araujo and P. Cornejo, 2021. Plant growth-promoting microorganisms in coffee production: From isolation to field application. Agronomy, 11(8): 1531.

Urgiles-Gómez, N., M.E. Avila-Salem, P. Loján, M. Encalada, L. Hurtado, S. Araujo, ... and P. Cornejo, 2021. Plant growth-promoting microorganisms in coffee production: From isolation to field application. Agronomy, 11(8): 1531.

Vallejos-Torres, G.; L. Arevalo, I. Iliquin and y Reynaldo SOLIS, 2019 . Respuesta en Campo de Clones de Café a la Inoculación con Consorcios de Hongos Micorrízicos Arbusculares en la Región Amazonas, Perú. Inf. tecnol., 30 (73-84). http://dx.doi.org/10.4067/S0718-0764 201900060 0073.

Washa B.W., 2015. Potential of the Dark as a Factor affecting Seed Germination. International Journal of Science and Technology, 5:2.

Waters, D.M., E.K. Arendt, and A.V. Moroni, 2017. Overview on the mechanisms of coffee germination and fermentation and their significance for coffee and coffee beverage quality. Critical reviews in food science and nutrition, 57(2): 259-274.