



T.R.
EGE UNIVERSITY
Graduate School of Applied and Natural Science



**STRATEGIES TOWARD ACCELERATING THE
VEGETATIVE GROWTH AND DEVELOPMENT OF
COFFEA ARABICA JUVENILES' PLANTS**

MSc. THESIS

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Department of Seed Science and Technology

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Department of Seed Science and Technology
Erasmus Mundus Master Program in Plant Breeding Second Cycle Programme

Izmir
2022

EGE UNIVERSITY
GRADUATE SCHOOL OF APPLIED AND NATURAL SCIENCE
DECLARATION

I hereby declare that this thesis study entitled “**Strategies toward accelerating the vegetative growth and development of *Coffea arabica* juveniles’ plants**” represents my own work which has been done after registration for the degree of MSc at EGE University and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma, or other qualifications. I also declare that all the information in this document has been obtained and presented in accordance with Ege University academic rules and regulations as well as ethical conduct. Lastly, I declare that, as required by these rules and conduct, I have fully cited and referenced all material and results which are not original to this work. Due to the confidential nature of some part of this thesis work the corresponding literature was also hidden.

19/07/2022

COZIEN Guilhem

ÖZET**COFFEA ARABICA YAVRU BİTKİLERİNİN VEJETATİF BÜYÜME VE GELİŞİMİNİ HIZLANDIRMAYA YÖNELİK STRATEJİLER**

COZIEN, Guilhem

Yüksek Lisans Tezi, Tohum Bilimi ve Teknolojisi

Danışman: Dr. Eftal DÜZYAMAN; Dr. Birsen ÇAKIR

Eş danışman: Dr. David BRETON; Dr. Anders KVARNHEDEN

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Yetiştirilen başlıca tropikal ürünlerden biri olan kahve türleri, kahve yetiştiricilerinin, üreticilerinin ve tüketicilerinin ihtiyaçlarını karşılamak için her zaman iyileştirmeye ihtiyaç duyar. Coffea arabica, geliştirilmesi zor bir türdür. Gerçekten de, bu çok yıllık ürün, uzun nesil süresi nedeniyle yeni bir çeşit oluşturmak için 15 ila 20 yıla ihtiyaç duyar. Bu sorunun üstesinden gelmek için yeni yaklaşımlar, çevresel faktörlerin manipülasyonu yoluyla ürünlerin nesil süresinin kısaltılmasına ve dolayısıyla ıslah programlarının süresinin kısaltılmasına olanak tanımaktadır. Bu tez araştırması, sera ve in vitro koşullar altında C. arabica ağacının gelişim modelini hedefleyen farklı protokollerin potansiyelini keşfetmek için gerçekleştirilmiştir.

Bu araştırmanın ana kısmı, kahve ağacı gelişimini modüle etmek için sera koşullarında deneyler tasarlamak, başlatmak ve izlemektir. Literatür taramasıyla, kahve ağacı gelişiminde yer alan üç ana bileşeni izole ettik. Bu bibliyografya çalışmasını, Nestlé'nin Bitki Bilimi araştırma merkezinde yapılabilecek denemeleri tasarlamak için önemli bir organizasyon çalışması izledi (bütçe, bitki materyali, insan kaynakları, sera alanı, vb.) Dört deneysel deneme başlatıldı. Özel hedeflerine bağlı olarak, bazıları staj sırasında tamamlanırken, diğerleri 2 yıllık bir süre için başlatıldı. Nestlé'nin kahve koleksiyonunda bulunan ve farklı genetik geçmişleri ve pazar ihtiyaçlarını karşılamaya yönelik ilgileri nedeniyle seçilen altı Arabica çeşidinden 300'den fazla genç C. arabica bitkisi bu deneysel çalışmada

kullanıldı. Test edilen çevresel uygulamalar arasında, dal uzaması veya yaprak şekli değişikliği gibi fenotipik değişikliklerle gösterildiği gibi ağacın erken gelişimini etkileyen koşulları belirledik. Ayrıca, ağacın başlangıçtaki hidrik durumunun, ağacın çevresel değişikliklere verdiği tepki üzerinde bir etkisi olduğu gözlemlenmiştir. Genç bitkilerin ilk vejetatif gelişimi çevresel faktörlerden etkilenmiş, bu da ağaç gelişimini modüle etmenin olası bir yolunu ortaya koymuştur. Üreme aşaması staj dönemi boyunca değerlendirilmemiştir ancak toplam iki yıllık bir süre boyunca değerlendirilecektir. Çevresel koşulların değişmesi de in vitro yetiştirilen kahve bitkilerinde bazı büyüme değişikliklerine yol açmıştır. Ancak, test edilen uygulama, rutin büyüme koşullarımıza kıyasla vejetatif büyüme üzerinde olumsuz bir etkiye sahip olma eğilimindeydi. Kahvenin in vitro büyüme modelini yönlendirmeye yönelik bu başarısız girişim, yeni büyüme koşulları ayarlarını test etme ihtiyacını güçlendirdi.

Anahtar Kelimeler: Coffea arabica, bitki gelişimi, fenotip, çevre koşulları, fizyoloji, bahçecilik

ABSTRACT**STRATEGIES TOWARD ACCELERATING THE VEGETATIVE
GROWTH AND DEVELOPMENT OF *COFFEA ARABICA* JUVENILES'
PLANTS**

Cozien, Guilhem

Msc Thesis, Seed Science and Technology

Supervisor: Dr Eftal DUZYAMAN; Dr Birsen CAKIR

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As one of the main tropical crops cultivated, coffee species always need improvement to meet the coffee growers, manufacturers and consumers needs. *Coffea arabica* is a difficult species to improve. Indeed, this perennial crop needs between 15 and 20 years to create a new variety due to the long generation time. To tackle this issue, new approaches allow reduction of generation time of crops, through the manipulation of environmental factors shortening in turn the length of the breeding programs. This thesis research was carried out to explore the potential of different protocols targeting the *C. arabica* tree developmental pattern under greenhouse and *in vitro* conditions.

The central part of this research was to design, launch and monitor experiments in greenhouse conditions to modulate the coffee tree development. Through the literature review, we isolated three main components involved in the coffee tree development. This bibliography study was followed by an important organizational work to design trials that could be done in the Nestlé's Plant Science research center (budget, plant material, human resources, greenhouse space, etc.). Four experimental trials were launched. Depending on their specific goals, some were completed during the internship whereas others were initiated for a 2-year period. More than 300 juvenile *C. arabica* plants from six Arabica varieties present in Nestlé's coffee collection and chosen for their different genetic background and interest to meet the market needs, were used in this experimental

work. Among the environmental treatments tested we identified conditions that influenced the tree's early development as illustrated by phenotypic modifications like branch elongation or leaf shape modification. It was also observed that the initial hydric status of the tree had an impact on the tree response to environmental modifications. Initial vegetative development of juvenile plants was impacted by environmental factors, suggesting a possible way to modulate the tree development. The reproductive phase was not assessed during the internship period but will be evaluated over a total period of two years. Change of environmental conditions also lead to some growth modifications on coffee plants grown *in vitro*. However, the tested treatment tended to have a negative impact on the vegetative growth compared to our routine growth conditions. This unsuccessful attempt to steer the coffee growth pattern *in vitro* reinforced the need to test new growth condition settings.

Keywords: *Coffea arabica*, plant development, phenotype, environmental conditions, physiology, horticulture

PREFACE

Coffea genus, belonging to the *Rubiaceae* family, originates from Africa, and was recently extended to 124 species (2022). Among them, three are economically important for the fruit's ability to be transformed into coffee: *Coffea arabica*, *Coffea canephora* (Robusta) and to a much lower degree *Coffea liberica*. They are one of the most important cash crops in the world and to meet the increasing demand, 125 million people participate in the coffee industry. However, due to climate change, coffee production is predicted to be highly affected in the following decades. That is why it became a necessity to increase the efforts put into varietal improvement research, even if it is a lengthy and costly process ranging from 15 to 20 years due to the long generation time and field trials.

One of the possible solutions to tackle this problem is the use of speed breeding techniques. Through the usage of specific parameters like climate, it is possible to accelerate the plant development. This approach has been proven to be effective in many cultivated crops and need to be investigated and adapted for *Coffea* species. This is a challenging project for a Master thesis but a good opportunity to test the knowledge I gained during the Master and further develop my skills.

Through this thesis, we developed four protocols of speed breeding for the major cultivated species, *Coffea arabica*. Those protocols are still under evaluation but present promising results for the future. We emphasized the potential of photoperiod and chemical applications to accelerate the plant development as well as the flowering. It also seems promising for some modifications of the tree's architecture. We also started to explore the potential of new *in vitro* approaches for speed breeding and breeding purposes in coffee species. This field of research is unexplored anywhere else at our current knowledge.

İZMİR

19/07/2022

COZIEN Guilhem

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1. INTRODUCTION

1.1 Plant Research Center of Nestle at Tours, France

Nestle as a worldwide company invests more than 1.7 billion CHF in research and development every year in multiple research centers. The Plant Science Research Unit located in the Loire Valley in Tours (France) is part of the Nestlé Institute of Agricultural Sciences, one of the institutes of Nestlé Research located in Lausanne, Switzerland. Founded in the 1980s, the center focuses on building knowledge on strategic crops and improving the products quality while trying to favor sustainable farming. Recently new activities linked to plant-based nutrition, functional ingredients and packaging were added to the center. But the main activity of the center remains concentrated on coffee and cocoa species. Through research on varietal improvement, agronomy and plant production, the center ensures a constant improvement of the plant material and production methods. Moreover, through the collaboration with more than 100 universities worldwide and scientific publications released every year, the Nestlé research is at the forefront of the research for coffee and cocoa worldwide.

1.2 Research Background

Coffea genus, belonging to the *Rubiaceae* family, originates from Africa, and was recently extended to 124 species (2022) (Krishnan et al., 2015). Among them, three are economically important for the fruit's transformation into coffee: *Coffea arabica*, *Coffea canephora* (Robusta) and to a much lower degree *Coffea liberica* (Ferreira et al., 2019). They respectively account for approximately 60%, 40% and less than 1% of the world production (Guyot et al., 2020). They are one of the most important cash crops in the world, with a market generating more than US\$ 26 billion (Moat et al., 2017) and consumption increasing every year to reach 10 billion kg in 2021/2022 (statista.com). To meet this demand, 125 million people participate in the industry, with coffee trees grown in over 80 countries and covering 10.2 million hectares of land in the tropical and subtropical regions (Mishra et al., 2019). However, due to climate change, coffee production is predicted to be highly affected in the following decades (Da Matta et al., 2019).

That is why it became a necessity to increase the efforts put into varietal improvement research, even if it is a lengthy and costly process (Berthaud et al.,1988).

1.3 Thesis Goal

To meet the demand for cultivar improvements, there is a need to accelerate the breeding programs. One of the possible solutions to tackle this problem is the use of speed breeding techniques (Watson et al.,2018) This research approach, aiming to accelerate the breeding programs has been proven to be effective in many cultivated crops. For example, it allowed to achieve six generations for spring wheat (*Triticum aestivum*) and pea (*Pisum sativum*) per year, while in canola (*Brassica napus*) it is possible to generate four generations per year (Watson et al.,2018). Numerous approaches exist in speed breeding among which two are mainly investigated. The first one is focused on plant lifecycle acceleration through the usage of climatic parameters or chemical applications. The second approach is based on an early selection by accessing the plant characteristics before full development. This approach is based on genomic techniques with the usage of markers, genomic selection, or predictive models (Esposito et al.,2019). Even if has not been well studied yet on perennial crops and especially on *Coffea* genus, Speed Breeding approach could be a major improvement for coffee breeding programs. Therefore, in this thesis project we will investigate the use of speed breeding approaches, in greenhouse conditions, for the most economically important species of the *Coffea* genus, *Coffea arabica* (Moat et al, 2017) To reach this goal, multiple tasks were pursued:

- A literature review to identify the endogenous and environmental factors that are related with *Coffea arabica* flowering process.
- Selection of the most promising parameters for our research goal.
- Deepened literature review of those factors, their previous uses on other crops, on the *Coffea* genus and when applicable for speed breeding purposes.
- Design of the speed breeding protocols and trials.
- Trial installation and management (phenotyping, data analysis, etc.).
- Statistical analysis on the trials and adjustment of the research strategy when needed.
- Production of posters and presentations as a medium to spread the knowledge developed in this thesis work to all the stakeholders.

2. LITERATURE REVIEW

2.1 *Coffea Arabica* Biology and Lifecycle

Coffea arabica, originating from the high ground of Ethiopia is a natural allotetraploid ($2n = 4x = 44$) descending from a hybridization event between *Coffea canephora* and *Coffea eugenioides* (Carvalho, 1988; Scalabrin et al., 2020). It is a self-pollinating species with a diversity strongly affected by human interference (Figure 2.1). Indeed, due to dissemination events, *C. arabica* possesses nowadays one of the lowest genetic diversities among the major cash crops. Modern coffee cultivars are derived from only two based populations which were spread in the 18th century, Typica and Bourbon (Apel and Sweeten, 2010).

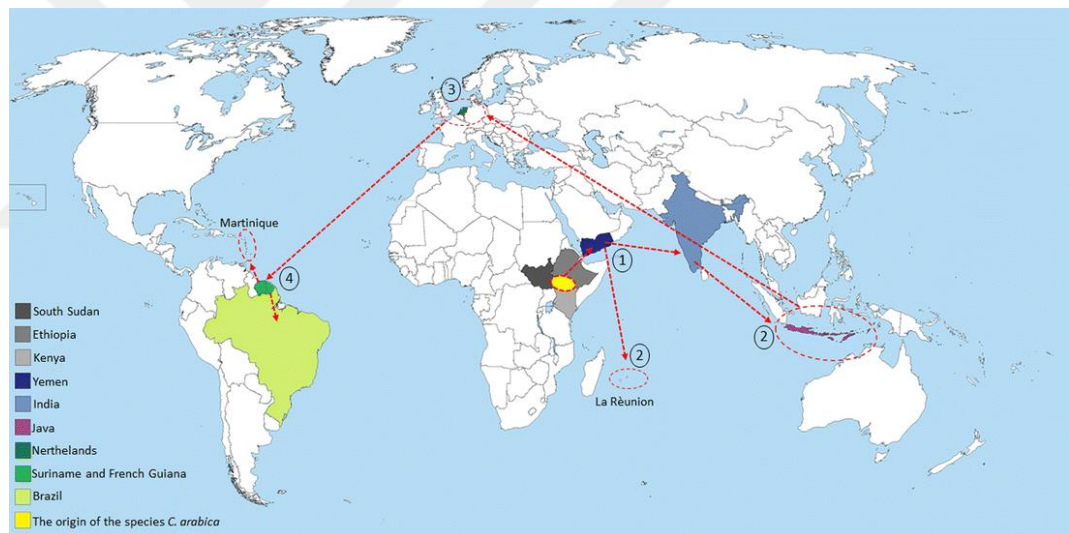


Figure 2.1. Source (2) Origin and dissemination throughout the world of *Coffea arabica* L. Yellow circle: origin of cultivated *C. arabica* L. *C. arabica* introduction into Yemen as early as 575AD (13). Coffee plant distribution to Réunion islands and taken from India to Java (Indonesia) (14-15). From Java, coffee was introduced in Europe (Amsterdam) in 1710 (13). (4) From Europe, coffee was taken to South America (Suriname) in 1718. From there it was introduced in Martinique island (1720 or 1723) and Brazil via French Guiana (1727) (16). From South America coffee was spread around the world. Note: colors indicate only the countries and not specific coffee growing regions within the countries.

C. arabica is a dicotyledon evergreen tree that reaches 8 to 10 m in height when left unpruned. It grows best at altitudes between 1300 and 1800 m above sea level with rainfall ranging from 1500 to 2500 mm. It can thrive under temperature

of 15 to 30°C Each node produces two opposite leaves and therefore has two-leaf axils residing on opposite sides of the node. The branching architecture is called dimorphic as there are two kinds of branches. Plagiotropic branches that grow horizontally compared to the main axis of the tree, while vertical shoots are commonly called orthotropic branch. Those branches are formed from two types of buds, the head of series buds that will develop into plagiotropic branches and the serial buds that will produce other orthotropic shoots commonly called “suckers”(Ferreira et al., 2019; Mishra, 2019; Nakamura et al.,1992).

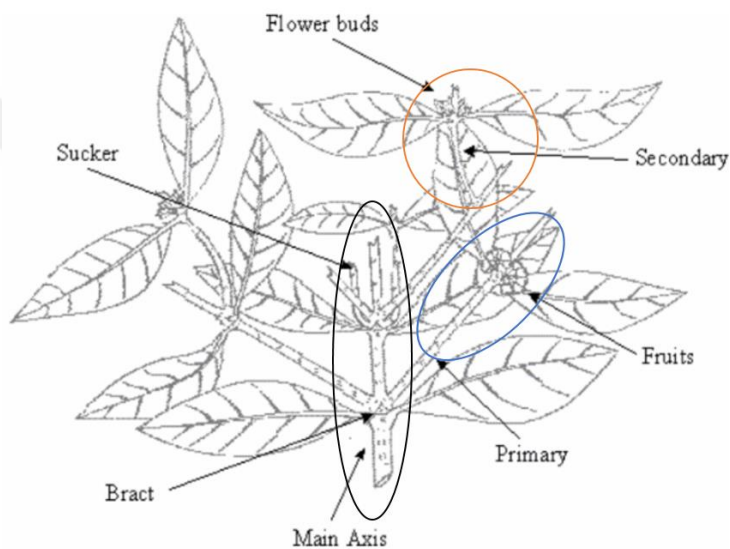


Figure 2.2.Schematic representation of *Arabica* shoot system. Orthotropic shoots are represented here by the main axis (black circle). Plagiotropic branches correspond to the primary branches (blue circle) and can keep growing with secondary branches (orange circle). One sucker can be seen growing from the main axis node.

After 3-4 years of growth, the first flowers will start to develop from the plagiotropic nodes. Coffee plants flowering involves two distinct processes, bud initiation and floral bud opening, also known as anthesis. Bud initiation will be triggered by multiple endogenous and exogenous factors. This initiation will induce the transformation of undifferentiated buds into floral ones. Those buds will grow and reach 4 to 6 mm before entering a dormancy period. This period will be broken during the anthesis. This second process, also controlled by multiple parameters, is mainly affected by rainfall. A dry period followed by rewatering will act as a signal for the dormant buds to open. The flowers will reach their full development 8 to 10 days after the re-watering (Alvim, 1960;

Mes,1957; Reddy,1979). The time between the bud initiation and the floral opening is in general comprised between 4 to 5 months. Those buds when opening produces fragrant inflorescences that will grow in clusters in the axils of coffee leaves. They will vary in number on the leaf axil from the primary and secondary branches.



Figure 2.3. Schematic representation of a plagiotropic branch with flowers and fruit's structure (left; retrieved from wikimedia); Picture of an adult tree ongoing a flowering and ripening at the same time. (Right; retrieved from the world of flowering plants website).

Under normal conditions, the style is receptive when the bud opens and remains receptive for three to four days depending on climatic conditions (Reddy, 1979; - Melese and Kolech, 2021; Walyaro and Van Der Vossen, 1977; Marias et al., 2017). About 6 to 8 weeks after coffee flowers are fertilized, cell division will occur, and the coffee fruit will remain as a pinhead for a climate-dependent period. The fruits will take between 4 and 8 months to ripen and reach full maturity. During this ripening, another flowering event can appear, and it is possible to see flowers and fruits at the same time, i.e., (Figure 2.3). It is a peculiarity of Arabica which possesses an uneven flowering and ripening.

2.2 Parameters Involved in Flowering Control

The flowering mechanism in *Coffea arabica* like in all other species is regulated by multiple factors. It has been subject to many studies, however, as a tree species, studies are taking a lot of time and we are still missing knowledge on a lot of mechanisms involved in flowering control. Most of the literature

investigated the anthesis but even if they are rarer, studies on flowering initiation also identified some of the parameters that could be involved. Among those factors we can highlight, temperature, photoperiod, water deficit and phytohormones.

2.2.1 Temperature

Coffea arabica is usually grown in temperatures ranging from 18-23°C. Those temperatures allow the plant to grow successfully in the wild and have a direct role of control in multiple plant mechanisms (DaMatta et al.,2019) Indeed, major differences in flowering can be observed depending on the temperature.

In general, warm temperatures tend to be associated with an activation of the floral meristems while, cold temperature is associated with bud latency. In the case of extreme temperature multiple negative effects have been documented, i.e., abnormal structure due to elevated temperature during a long period of time, flowers and fruits losses in case of intense heat period (Martins et al.,2016). Molecular and transcription mechanisms were also unraveled in previous studies. Indeed, along with light (section 2.2.2) temperature affects important flowering transcription factors like *CONSTANS*, *FLC* and *FT* (Fankhauser and Chory, 1997).

2.2.2 Photoperiod and light quality

Light and photoperiod are essential parameters of plant development. Mandatory for photosynthesis, it has a direct impact on the cultural management of coffee (Morais et al., 2003).That is why light effects on Arabica development were evaluated thoughtfully. It was identified by many scientists that Arabica is as a short-day plant and that the light quantity will affect the plant anatomy (Anderson et al.,1995; Anderson et al.,1988; Morais et al.,2004) and physiology (Andrés, and Coupland, 2012). Trees that are grown under full sun conditions tend to have more intense flowering events compared to trees grown under shade while light signals, transformed into chemical and molecular signaling, are directly involved with flowering control (Corbesier et al.,2007; Fowler et

al.,1999). Among those molecular compounds, the transcription factor FT seems to promote the conversion of the shoot apical meristem (SAM) into inflorescence meristem (Fowler et al.,1999; Shim and Imaizumi, 2015; Peña Quiñones et al.,2011).

Regarding the photoperiod, major effects were identified regarding the number of flowering events per year (López et al.,2021). Indeed, depending on the region of growth, the photoperiod is not the same. For example, in Brazil, the photoperiod is varying along the year, and one main flowering event per year can be found, with a vegetative growth the rest of the year. Oppositely, countries closer to equator that present limited variations of photoperiod along the year like Costa Rica, Ethiopia and some regions of Colombia will present multiple flowering events each year (López et al.,2021; Crisosto et al.,1992).

2.2.3 Irrigation and water stimuli

Water availability was identified in multiple studies as an important parameter linked to the flowering of *C. arabica*. Indeed, even if the threshold is still controversial (severity and length of the stress), Drinnan and Menzel, 1994; Kahn,1986). it is admitted that floral buds need to be submitted to a water stress, to have the competency to answer to flowering signals and to open (Alvim, 1960; Mes,1957). Rainfalls that will follow those dry periods seems to act as a flowering signal and trees that did not meet the hydric stress requirements face a stop in the bud growth and if this break period is too long, the buds will start to decline without opening (Gómez-Cadenas et al.,1996)

2.2.4 Phytohormones

The last parameter that can be found to affect plant development in all species is the presence of phytohormones. Indeed, those molecules are completely intertwined with the plant mechanisms and were found to control the development (Browning,1973). Their production will be dependent on the interaction between genes and environmental factors like the three previous parameters we mentioned.

Concerning *Coffea* flowering, three of those hormones are mainly involved, gibberellic acid (GAs), abscisic acids (ABA), and ethylene.

Gibberellin was found to have a synergetic effect with the rewatering period to trigger anthesis, while the latency of the floral buds seems to be linked to a balance between ABA and Gas, (Gómez-Cadenas et al.,1996) Finally, ethylene was recently observed to have important effects on the anthesis and might have some effects on the floral initiation (Adams and Langton, 2005).

2.3 Choice of Factors and Protocol Development

For Nestlé, it is a strategic goal to accelerate the release of improved *Arabica* varieties. Indeed, facing climate change it has never been so important to accelerate plant improvement (DaMatta et al.,2019). However, there is a lack of knowledge on speed breeding approaches for *C. arabica*. Especially on young trees in greenhouse conditions due to a focus given to adult trees in field conditions. Therefore, this thesis work will serve to select some of the most promising parameters, easily controllable in greenhouse conditions and evaluate them. The first axis of research will evaluate the potential of plant growth regulators to initiate flowering and affect vegetative growth. The second main axis will aim at evaluating the photoperiod effects (Adams and Langton, 2005). Lastly, we will investigate the *in vitro* breeding techniques that provided interesting results in other species (Teixeira da Silva et al.,2014)

2.4 Growth Regulator Application Trial (Product and Sources That Allow to Identify Them Are Confidential)

2.4.1 Context

Phytohormones influence many developmental processes-in plants. They are endogenously synthesized by the plants and have a major role. Flowering, as one of the main developmental steps, is precisely controlled by some of those hormones among which gibberellin, ABA, and ethylene seem to have the greater impact (Crisosto et al.,1992; Ságio et al.,2014; Silva VA et al.,2018; Lima et

al.,2021). Many examples highlight the importance of those hormones for *Coffea arabica* flowering. Indeed, a dry period (necessary for the floral bud competency to flower) will be associated with a decrease in the production of gibberellins and ethylene, while it will provoke the accumulation of ABA (Lima et al.,2021). This accumulation is correlated with a downregulation of *ACO* genes (Achard et al.,2007). Inducing the accumulation of ACC (ethylene precursor), (Lima et al.,2021). The decrease in gibberellins is associated with the accumulation of DELLA protein (Negative flowering regulator). This accumulation inhibits the production of two transcription factors *LFY* and *SOC1* and thus the flowering (Ó'Maoiléidigh et al 2013; Gómez-Cadenaset al.,1996).

Those dry periods that induce many changes in the hormonal content, are followed by important rainfall periods. They induce many changes in the plant. Gibberellins start to accumulate rapidly while ABA content is reduced. The balance GA/ABA, involved in the bud latency control (Gómez-Cadenaset al.,1996; Achard et al.,2007). Is broken. At the same time, the rewatering seems to trigger the transportation of ACC from the roots to the shoots and induce an ethylene burst. This phenomenon, observed in other species like tomato (*Solanum lycopersicum*) or wheat (*T. aestivum*), could be part of the control pathway of floral anthesis (Crisosto et al.,1992; Dodd et al., 2006; Saxena et al.,2019) We chose here to explore two potential growth regulators for applications on the trees.

2.4.2 Expected outcome of the trial

Through this trial, we explore the possibility of product X and Z application to influence flowering initiation and anthesis. We have high expectations toward those products to accelerate the plant development and affect its architecture.

2.5 Photoperiod Trial

2.5.1 Context

We presented previously the importance that speed breeding tools could have to accelerate *Coffea* variety improvement and the dependency on

greenhouses to reproduce environmental conditions need for the plant growth. Among the parameters that can easily be adapted for greenhouses, light is one of the most obvious (artificial lighting). Photoperiod and light have been proven to impact most if not all the plant main mechanisms, while being efficient for speed breeding purposes (Watson et al.,2018;Samantara et al.,2022; Cannell, 1975). It interacts with the microclimate of plants, having indirect effects on the temperature, humidity, light quality, and quantity, while affecting photosynthesis, plant development (Teixeira da Silva et al.,2014) and flowering (Camargo,1985). Multiple consequences of light changes on plants were reported in the literature. Some examples highlight the impact on phenotypic traits like size, nodes, and buds' number, (Cannell, 1985). While a reduction in the daylength can drastically reduce the carbon assimilation rate and affect the flowering time (Cannell, 1985; Ferrão et al.,2019; e Silva and Tenente, 2004). Photoperiod is also responsible for major phenotypic differences regarding flowering events between the producing countries; (see section 2.2.2). All those major effects on *Arabica* development highlight the need to explore this parameter.

2.5.2 Expected outcome of the trial

Based on the unpublished trial, we have high expectation of the condition D to enhance the vegetative growth compared to the other conditions.

2.6 *In Vitro* Flowering - First Trial

2.6.1 Context

Coffea arabica breeding research like all other crops is facing limitations on the research possibilities due to multiple factors. Among them, we can highlight the transportation, the research conditions, the rules on plant material exchange and protection (Matsumoto and Lopez, 2014;Georget et al.,2017). All those limiting factors create a situation where the breeding programs are limited but there is a tool that we could use in parallel to greenhouse culture to produce flowers. It is by passing through *in vitro* conditions, where *Coffea arabica* is known to grow well.

2.6.2 Interest of this trial

Cryoconservation, micropropagation, and even somatic embryogenesis are *in vitro* techniques widely used in *Arabica* research (Etienne et al.,2018; Murthy et al.,2012).Therefore, it could be interesting to explore the possibility of *in vitro*, for a breeding purpose like in orchids or other species (Adams and Langton, 2005;Etienne et al.,2018; Murthy et al.,2012).

Being sterile, the boxes could be transported easily compared to trees grown in soil. Through propagation and the limited space needed for the boxes the number of plants could be increased at a smaller cost compared to greenhouses. It would also be possible to match the blooming period of the *in vitro* plants with the trees in the greenhouse or in the field. To our knowledge trials for this goal are not published and no studies could be found in the literature regarding this topic. A reason that could explain this gap is the total absence of examples of *Coffea arabica* flowers appearing in *in vitro* conditions. At the same time, *Coffea arabica* research remains a domain with few researchers compared to other main crops and major private companies with secret projects. This absence of flowers is the main limiting factor for now in using *in vitro* plants for breeding purposes. Therefore, our aim will be to pave the way for this research by exploring some parameters that could trigger flowering. We will try firstly to trigger in young plantlets the development of plagiotropic branches from which flowers can grow.

2.6.3 *In vitro* flowering knowledge

We previously said that multiple factors are influencing the flowering control. Among them the easiest to affect in *in vitro* conditions are light spectrum, photoperiod, medium composition, room temperature and humidity. As a first approach to produce flowers *in vitro*, we wanted to test parameters that are easily measurable and accessible in a short period of time. Among those parameters, photoperiod can be easily influenced through the LED system while having proven effects on the plants, while medium composition can be controlled by affecting the recipe. That is why we settled on those two parameters.

2.6.4 Expected outcome of this trial

In this original experiment we are mainly predicting the results. We expect the explants to be affected differently depending on the medium composition and the photoperiod.

2.7 *In Vitro* Flowering Second Trial

2.7.1 Context

This experiment was designed 3 months after the launch of the *in vitro* flowering first trial. The results were not meeting the expectations at that point (see section 4.3). We chose here to evaluate another type of plant material.

2.7.2 Expected outcome of this trial

This trial was designed to take place during my stay. The length and development speed of those explants as well as the possibility of flowering is complicated to predict.

3. Material and methods

3.1 Growth Regulator Application Trial

3.1.1 Plant material

In this experiment, we used one-year old *Coffea arabica* trees of two different genotypes, GPFA 117 and GPFA 119. Those two genotypes correspond to the same genetic group. Indeed, they are both hybrids of traditional varieties. They have been selected for this trial, due to their availability and their interesting phenotypic characteristics. They were planted in 4l pot with a specific substrate for *Coffea* species (Table 3.1).

Table 3.1. *Coffea spp* specific substrate composition, Premier Tech commercial preparation, (Ref: 411231.)

Component	Percentage
Coco Fine	33%
Orgatech 0/10	33%
Pouzzolane 0/3	13%
Sand 0/2	10%
Medium Coco Fiber	5%
Clay	5%

3.1.2 First trial design

We chose at the beginning of the experiment to explore the effects of two compounds, products X and Y. Those two compounds seem indeed to have promising effects on adult trees (confidential). Regarding the experimental conditions our trial will investigate five conditions, (four experimental/one control). Product X has already been widely studied on many species, that is why we chose to evaluate one concentration (confidential). Contrary to product X, the product Y effects have been mainly studied on fruit conservation and it is still lacking on flowering (confidential). We thus chose to evaluate three different concentrations to assess its effects. The second goal of this trial being the

evaluation of the hydric status of the trees on the growth regulators potency, we added a level of complexity to our trial by using different irrigation levels during the first month of trial. We wanted here to induce differences in hydric status now of the first application. Based on data from the research center on water stress, we chose to evaluate three irrigation levels. Through those two parameters (Treatments x irrigation) we designed a total of fifteen experimental groups.

3.1.3 Change in experimental design

The experiment was launched in April 2022 with different irrigation levels. The trees were ready to be sprayed with the products but due to a delay in the delivery of product Y, we had to change it to another one at the last moment to fit the thesis timeline. Our attention turned toward a product that interacts with the same mechanisms, product Z. The usage has already been proven on ornamentals to hasten the flowering (confidential).

3.1.4 Experimental design

Our experimental design was established beginning of April 2022 in one of the greenhouses of the Nestle Research Center at Tours, France. This experiment will have a duration of at least one year until February 2023 (possible modification depending on the results).

In this trial, irrigation regimen and growth regulator applications are the two factors evaluated. Their combination creates a total of fifteen experimental groups.

The chemical applications consist of two plant growth regulators that will be sprayed on coffee leaves at different concentrations once every month. The control conditions (treatment A) consist in plants receiving sprayed water. Treatments B to D consist in a range of concentration of product Z. The last treatment, E, consists in the application of one concentration of product X.

To fulfill the goal of creating a water stress during the first month of trial before the first applications, the irrigation level is also controlled in this experiment. Three different irrigation regimens were chosen (Table 3.2,3.3).

Table 3.2. Growth regulator application trial, treatment details.

Treatment code	Commercial reference	Concentration	Active substance	Plant number per treatment	Quantity of products delivered per plant
A	Control		None (water)	36	
B	Z	+		36	
C	Z	++		36	
D	Z	+++		36	
E	X			36	

Table 3.3. Growth regulator application trial, irrigation regimens details.

Treatment	Day of irrigation	Number of irrigations per day	Duration of irrigation per membranes	Quantity of water given per irrigation	Total of water given per day
Control	Continuous	2			+++
Experimental 1	Continuous	2			++
Experimental 2	Continuous	2			+

Those different irrigation regimens were applied during the first month of the trial to create a water stress. After the first treatment all plants irrigation was replaced by the stress-free condition.

Those plants were then placed following a split plot design with a randomized distribution in three different blocks. Each treatment group was formed of four plants per block (two of each genotype). To limit the border effects and the risks of contamination between treatments, border plants were placed to surround each group. A total of 180 plants, 90 of each variety, were evaluated in this experiment and more than 200 were used as borders (Figure 3.1).



Figure 3.1. Growth regulator application trial map and picture of this trial.

3.1.5 Preparation and Application of the different treatments

3.1.5.1 Preparation of the slurries protocol for each application events **(Confidential)**

Treatment A (control): 4 l of water was added in a 4 l sprayer (Libertis model, Technoma).

3.1.5.2 Application of the slurries

Plants were taken out of the design and placed on a shelf tunnel. The respecting slurries of 4 l were sprayed onto the 36 plants evenly which were left for 1 hour on the shelf to allow the products to penetrate the plant. Then the tunnel was removed from the shelf and the plants were left to dry in the open air. This drying period was important to limit the possible contamination between treatments when putting the plants back in the design or during the experiment.



Figure 3.2. Picture of the hormonal application

3.1.6 Greenhouse climatic strategy and fertilization

The hormonal trial was performed in greenhouse conditions with controlled climatic conditions. The climatic strategy for this trial is the one in the following table. Regarding the fertilization strategy, we will support the vegetative development all through the experiment by supplementing the irrigation water with 0.7 g/l of a nutrient solution (20 N-20 P-20 K).



Figure 3.3. Picture of the Dosatron mechanism (left; retrieved from Dosatron website) and the sprayer used for this experiment (right; retrieved from jardinetmaison.fr).

Table 3.4. Growth regulator application trial, climatic strategy details.

Climate	Day	Night
Photoperiod	Natural (Tours, France)	Natural (Tours, France)
Heating	22 °C	18 °C
Aeration	23 °C	20 °C
Misting (RH%)	70%	70%
Cooling temperature	>28 °C	>24 °C

3.1.7 Pest management

To limit the presence of insects in the greenhouse, multiple strategies have been put in place. We added nematodes, *Steinernema feltiae*, to the soil to limit the larvae development. A powder of nematodes larvae, from the brand Entonem (50 million larvae for 25 g) was dissolved in water to reach a concentration of 0.375 g/l. 20ml of this solution was added in every pot. We also used yellow sticky traps (Biobest) to catch flying insects. Some occasional treatments were done when the quantity of insects was going past a certain threshold. Preventive measures (protective clothes, footbath, access restriction) were also added.

3.1.8 Measurements

To evaluate the treatments and irrigation effects, the trees were evaluated for the following phenotypic traits:

- Plant height (from collar to terminal apex) in cm
- Internodes on the main orthotropic axis number
- Plagiotropic branches and leaves number
- Plagiotropic branches total length
- Plagiotropic total nodes
- Undifferentiated and floral bud's number
- Flowers and fruits number

Those phenotypic trait measurements will be used to produce calculated parameters like the plant growth speed. At the same time, to evaluate the impact of the different irrigation regimens multiple measurements were done on the

leaves water potential, and soil water potential. The real climatic conditions were also monitored to ensure the good strategy implementation and integrate the climatic conditions into the data analysis of the phenotypic observation.

3.1.8.1 Leaves water potential measurement

The Schölander bomb (Tyree and Hammel, 1972). is a commonly used tool in plant physiology to measure the water status. It represents the binding state of water within the plant tissue.

Principle: a detached leaf of the evaluated plants main axis is enclosed in a compartment of the machine: the chamber. Only the sectioned part of the petiole protrudes from the chamber. It is this part that must be observed during a measurement. The pressure in the chamber will be increased, thus putting pressure on the plant, until water appears on the surface of the section. The pressure corresponding to the moment when water appears on the surface of the section corresponds to the equilibrium pressure, i.e., the pressure in the conductive vessels of the plant.

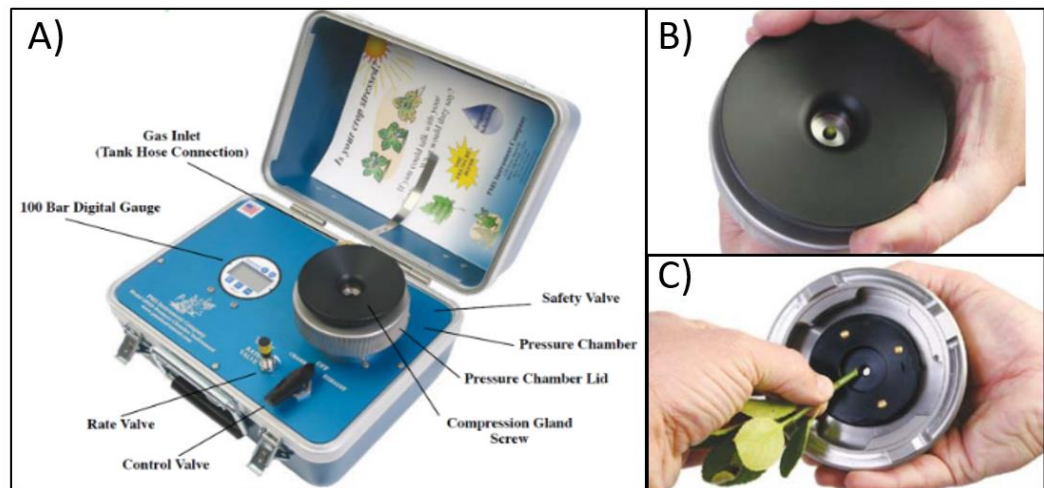


Figure 3.4. a) Schölander bomb measurement tool, b) Protruding leaf section from the air lid, c) Plant position in the air lid. Pictures taken from the Nestlé's internal protocol o the Schölander bomb usage.

In our case, we explored two measurement methods to see the water stress impact on the plant leaf potential. The first measurement protocol was not

satisfactory due to the rapid loss of water potential between the leaf sampling and the measurements. Just a few minutes of difference had an important impact. Thus, we tried another method for the second measurement to improve the accuracy of the measurement

3.1.8.2 Retained method for the measurement

Plants were organized in groups of four depending on the treatments. To increase the measurement efficiency, those groups were sampled two times. The leaves of one genotype were sampled first and placed in an aluminum envelope to limit the water status losses. They were then measured directly in a time span of two minutes after the sampling. Once the first genotype was done, the same method was used for the second genotype. The leaf sampled on each plant were on the bottom part of the main axis.



Figure 3.5. Pictures of the scholander bomb and the leaf retrieval method in an aluminum foil.

3.1.8.3 Soil hydric status measurement

Irrimeters (Chard, 2002). are a commonly used tool in plant physiology to measure soil moisture. The sensor from the brand Watermark absorbs the moisture in the soil and displays a value between 0 and 200 without unit on the electronic device indicating a very high moisture level and the total absence of moisture in the soil, respectively. As the operational status of the probes varied, large differences in values for the same substrate were observed during the material quality control. Therefore, to characterize as well as possible the impact of the different irrigation regimens, each tree soil moisture was measured with the irrometer system and by weighting the pot.

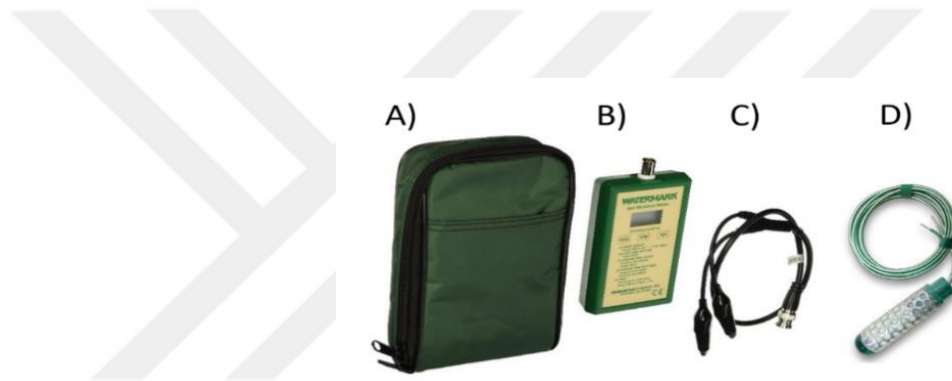


Figure 3.6. Irrimeter picture with a) protection bag, b) electronic device, c) cables, d) sensor (retrieved from Watermark website).

3.2 Photoperiod Trial

3.2.1 Experimental design and plant material

In our case, our trial will evaluate the effect of four different photoperiods on three different varieties of *Coffea arabica*.

Those four conditions were selected based on an unpublished trial. This trial showed the efficiency of one specific photoperiod (D). To prove the effect of this photoperiod we added three other conditions, one control (A; natural photoperiod) and two artificial photoperiods (B; C)

The preparation of the trial started in March 2022 with the construction of black boxes to isolate the different photoperiod conditions. Three black boxes (4 x 4 x 3 m) were built inside a greenhouse between March and May 2022. They are equipped with a central LED (Confidential information over LED brand and specifications) to ensure the growth of the plants. A ventilation system was installed to ensure good air movement inside the boxes and limit the heating effect of the boxes. The space in the greenhouse was attributed to a control condition in natural photoperiod.



Figure 3.7. Homemade black boxes for the photoperiod trial (external view, left); Picture of the plants under the C condition the 10/06/2022 (T0; middle); picture of the plants under the C condition the 11/07/2022 (T1; right).

Regarding the plant material, a total of 100 6-month-old plants were placed the 10/06/2022 in the greenhouse (25 plants per condition). Those 100 plants grown from seeds belong to 3 Arabica varieties and are positioned in the trial following the trial map in figure 3.8 Those three varieties, GPFA70, GPFA124 and Typica correspond to different genetic groups of *Coffea arabica* species. Those genetic groups correspond respectively to an improved Catimor variety, a hybrid Ethiopian x Typica and a traditional variety. Through those three varieties we will be able to evaluate the main genetic group reaction of Arabica under specific photoperiod treatments. Due to the light heterogeneity in the boxes, only the nine central trees will be considered when the trees grow older (Figure 3.8-3,9). We are using here Typica trees as border plants to protect the central trees

SECOND TRIAL MAP

CONDITION 1					CONDITION 2					PLANT NUMBER		GENOTYPE	
97	98	99	100	85	41	42	43	44	45	1-12	GPFA 70		
96	1	23	25	86	40	12	36	15	46	13-24	GPFA 124		
95	22	27	2	87	39	35	14	11	47	25-36	TYPICA		
94	26	3	24	88	38	13	10	34	48	36-100	TYPICA BORDER		
93	92	91	90	89	37	52	51	50	49		EXPERIMENTAL TREES		
CONDITION 3					CONDITION 4								
73	74	75	76	77	57	58	59	60	61	BORDER TREES			
72	7	19	31	78	56	16	4	28	62				
71	32	8	20	79	55	29	18	5	63				
70	21	33	9	80	54	30	6	17	64				
69	84	83	82	81	53	68	67	66	65				

Figure 3.8. Photoperiod trial map.

In this trial, four different photoperiods will be evaluated. A control reproducing the greenhouse conditions with the natural photoperiod of Tours, France (Latitude: 47°22'59'' N Longitude 0°40'59'' E). This control (condition A) will be supplemented with LED lighting when the light intensity is too weak to ensure normal plant development. The LED will be turned on when the light sensor does not catch 250 Watt/m² between 8 am and 8 pm. The three other photoperiod conditions will be evaluated inside the black boxes.

3.2.2 LED lighting

The LEDs position can be adapted using a cable system to match the plant growth. In June 2022, they have been placed at 2.5 m high and will be put higher with the plant growth to allow the best lighting possible. The modelization of the photon flux delivered by the LED lamp is given in figure 3.9 (according to LED provider).

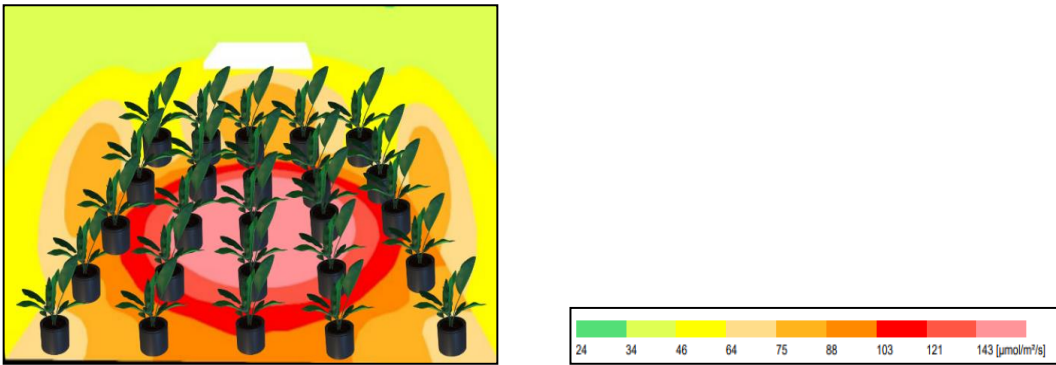


Figure 3.9. Map of the lighting measurement in the black boxes with the positioning of the plants, false color rendering, indicate a light heterogeneity between the center and the borders (left; source: LED provider).

3.2.3 Substrate composition and Pest management

All the 100 plants were repotted on 10/06/2022 in 1 l pot with a specific substrate for Coffea plant culture (see table 3.1). Nematode's larvae powder from the brand Entonem was added to the medium to limit the quantity of flies in the greenhouse. This powder was dissolved in water to a concentration of 0.375 g/l and 5 ml were added in each pot. Other control methods like in the first experiment were also done in this trial.

3.2.4 Greenhouse climatic strategy, irrigation, and fertilization

The photoperiod trial was performed in greenhouse conditions with a controlled climate. The strategy for this trial is the same as the one in the growth regulator application trial (Table 3.1). The irrigation here is set to fit the plant growth, it means that the quantity of water given will increase to match the trees needs along the experiment. Regarding fertilization, it is delivered to support vegetative development during the beginning of the experiment. A change in the fertilization strategy to support the flowering can happen later in the experiment (after the thesis ends) when the plants are sufficiently developed. The fertilization strategy of the photoperiod was the same as the growth regulator application trial.

3.2.5 Measurements

Identical measurement to the growth regulator application trial (section 3.1.5) are done here except the irrigation and hydric status that will not be monitored.

3.3 *In Vitro* First Trial

3.3.1 Experimental design and plant material

In this trial we chose to test two different photoperiods. The control photoperiod used in the culture growing rooms and an experimental one. Regarding the medium composition, the control medium was compared with a modified medium. Regarding the plant material, we chose to evaluate a variety of *Coffea arabica* already available in *in vitro* conditions, coded GPFA116. The plantlets were produced through somatic embryogenesis (Murthy et al., 2012; Ramanayake et al., 2006; Rout and Das, 1994). Through the two evaluated parameters, we created four experimental conditions.

Arabica plantlets produced by somatic embryogenesis from the GPFA116 genotype were transferred on fresh culture medium into plastic boxes (12 x 5 cm micro box Dutcher, RA60 PSKR STER). Transferred plantlets were cut and the roots were removed to start over with fresh and green material. These 38 boxes were prepared with two different media and six plantlets were placed in each of those boxes for a total of 228 plantlets. Due to unforeseen events, one of the boxes was lost and therefore, 37 boxes of 6 plantlets were placed in their different growing conditions. following the instructions of Figure 3.10.



Figure 3.10. Picture of the experimental growing conditions, ERATIS growing chamber (left) and growing culture room (right).

3.3.2 Plant medium composition

For this experiment, two different types of media were produced in the laboratory. A total of 20 boxes of control medium and 18 boxes of the modified medium were prepared.

3.3.3 Climatic conditions

To ensure the best growth of *Coffea arabica* in *in vitro* plantlets, Nestlé have identified the best environmental conditions. Three parameters are important here, photoperiod that we tested in this experiment, temperature and relative humidity following the information's in Table 3.5.

Table 3.5. Climatic strategy details for the orthotropic trial.

Treatments	Growing environment	Temperature °C	Relative humidity %
A	Growth room	24	Not under control in the room
B	Growing room	24	Not under control in the room
C	Eratis growing chamber	24	60%
D	Eratis growing chamber	24	60%

3.3.4 Measurements and analysis

Individual plant phenotype was assessed monthly to follow the rooting and mortality percentage. Pictures of all the boxes from a top view were done using a phone camera (Wiko; Power up). They were taken every month to follow the development of the leaf area using the open-source software ImageJ (1.51k, National Institutes of Health, USA). The total projected green leaf area and dead leaf area were monitored with this analysis. Climatic data (temperature, relative humidity) were recorded to check the implementation of the settings and to be included in the phenotypic data analysis

3.4 *In Vitro* Second Trial

3.4.1 Experimental design and plant material

For this experiment, a preliminary step was needed, material preparation. Indeed, a high contamination risk exists when taking explants directly from plants in non-sterile conditions (endophytic bacteria, fungi). Therefore, we sampled explants of 2 arabica genotypes (GPFA117/119) and passed them through a sterilization protocol. Once sterilized, the explants were added in tubes with a control medium to allow the first growth of those plantlets. Contaminated tubes were removed along the development (Figure 3.11).



Figure 3.11. Picture of the in vitro tubes in the growing room with plagiotropic explants.

3.4.2 Measurements

(Identical to the orthotropic experiment, see section 3.C.4)

3.5 Climate Data Monitoring

All climatic data in this thesis were collected using the temperature and relative humidity data logger HOBO U23 Pro V2 from the brand ONSET (Figure 3.12).



Figure 3.12. Picture of the HOBO data logger (retrieved from ONSET website).

3.6 Results analysis and statistics

Data collected through all the experiments will be analyzed. Descriptive statistic (mean, standard deviation) will first be done to highlight tendencies on the different parameters. Those data's will be then evaluated through statistical analysis with the software Minitab (v. 18.1). A parametric test, ANOVA (Cuevas et al.,2004) will be done, when possible, on the quantitative variables, followed by mean comparisons tests. Tests will be done to verify if we can apply the ANOVA test or not. Regarding the normality we are using the Kolmogorov-Smirnov test on the residuals (Berger and Zhou, 2014). while for the equal variances test, we are using the Levene's Test (Glass, 1966).

Nonparametric tests, Kruskal-Wallis and Chi-square (Tallarida and Murray, 1987;Kruskal and Wallis, 1953). will be done when ANOVA is not possible or when qualitative parameters are evaluated. A Minitab macro is used to do multiple Kruskal-Wallis comparisons in those cases (retrieved from Minitab website)

4. RESULTS

4.1 Growth Regulator Application

4.1.1 Climatic data monitoring

Climatic conditions have important impacts on the development of *Coffea arabica* in in vitro conditions. Therefore, it is important to monitor that the experimental conditions we want are the ones we have in reality (Figure 4.1.,4.2).

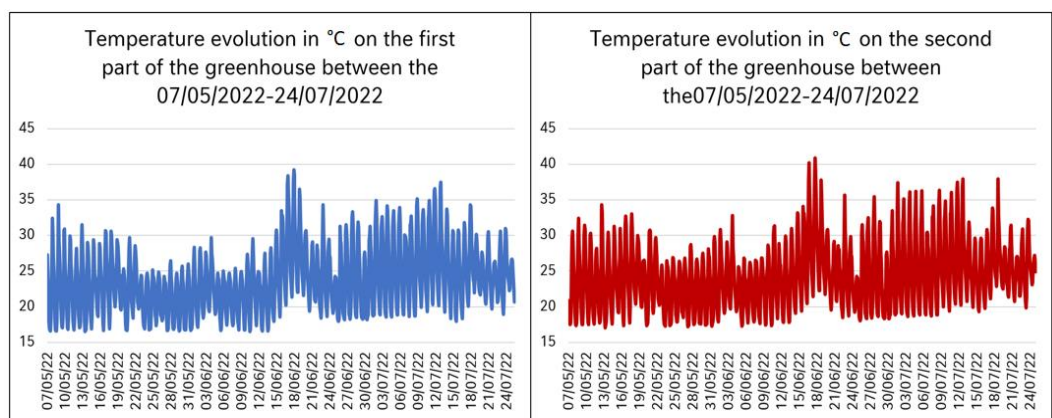


Figure 4.1. Temperature in °C evolution between the 07/04/2022 and 24/07/2022 depending on the data logger position in the greenhouse. First part of the greenhouse in blue (left); second part of the greenhouse in red (right).

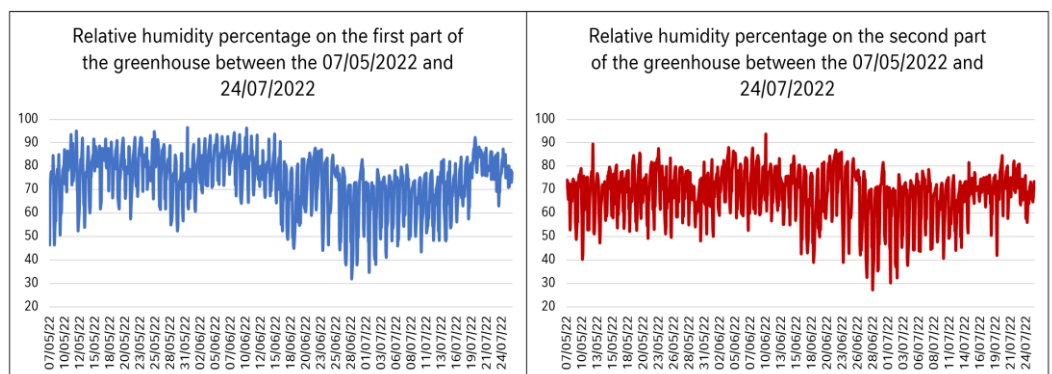


Figure 4.2. Relative humidity percentage evolution between the 07/04/2022 and 24/07/2022 depending on the data logger position in the greenhouse. First part of the greenhouse in blue (left); second part of the greenhouse in red (right).

Through the monitoring of the climate, we can see that the two parts of the greenhouse are similar in conditions. The second part of the greenhouse shows a bigger range of change in humidity and temperature compared to the first part.

4.1.2 Hydric status measurements result after the first month.

Through the different parameters measured, we observed that we successfully created a difference in water status between the plants. Those differences remain small, and no symptoms of water stress could be observed phenotypically.

4.1.2.1 Soil hydric potential measurement using irrometers

The soil water status was measured using the irrometers sensors. Those sensors indicate values without units ranging from 0 (well irrigated soil) to 200 (total absence of water in the soil). Those measurements allowed us to observe a difference between the irrigation regimens. Indeed, pots irrigated with the control irrigation had in average a score of 1.31-1.50 for both measurements. While pots irrigated with the first experimental condition showed a small hydric potential reduction, evolving through the measurement. An average of 6.42 at the first measurement increasing to 14 .00 one week later. A stronger effect is visible for the pots irrigated with the second irrigation regimen, with an average of 38.07 at the first measurement increasing to 64.14 during the second. Those values indicate a medium stress for the soil water potential. While comparing the values for both genotypes of this experiment, it is possible to highlight that there is a significant difference in the soil hydric potential depending on the genotype. Indeed, at the same measurement dates, the soil hydric potential of GPFA119 was always smaller than the one from GPFA117 (Table 4.1.-4.2).

Table 4.1. Irrometers measurements on the pots depending on the irrigation regimens.

Irrigation regimens	09/05/2022	18/05/2022
Control	1.31	1.50
Experimental 1	6.42	14.00
Experimental 2	38.07	64.14

Table 4.2. Irrometers measurements depending on the interaction irrigation regimens and genotypes.

Interaction variety x irrigation regimens	09/05/2022	18/05/2022
119 Control	2.00	2.00
119 Experimental 1	7.375	19.375
119 Experimental 2	52.4	91.4
117 Control	0.83	1.20
117 Experimental 1	4.50	3.25
117 Experimental 2	30.11	49.00

4.1.2.2 Schölander bomb, measurement of the leaf water potential

To validate the irrigation regimens impact on the plants, we measured the leaf water potential. The Schölander bomb indicates the pressure necessary to apply on the leaves to push the water outside. The value is negative and when they are closer to 0, they indicate a good water leaf potential while when the water content in the leaf's decreases, the pressure necessary will increase and thus the value given by the device will decrease. The first phenotyping with the Schölander bomb was not accurate enough and therefore we chose to exploit only the 16/05/2022 results. Through the measurement, we observed that significant differences arise between the three different regimens.

Irrigating the trees with experimental two regimen induced a difference in the leaf hydric potential of -0.20 MPa and -0.34 MPa with the control and experimental condition 1. Regarding the difference between the control and the experimental irrigation 1, we can highlight a difference of 0.14 MPa. The value of

approximately -0.80 MPa for the well irrigated plants is a sign of better hydration compared to the -1.20 MPa of the irrigation 2.

No significant difference on the leaf hydric status could be noted between the two genotypes of the experiment.

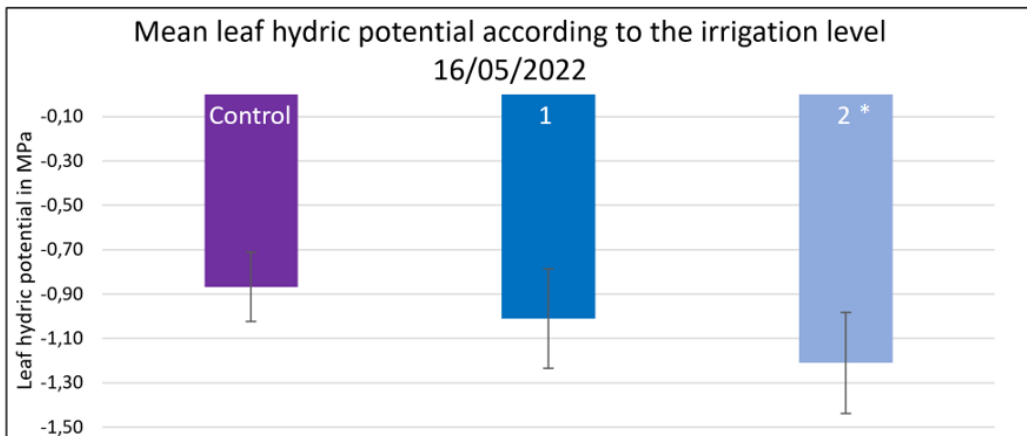


Figure 4.3. Mean leaf hydric potential of the trees depending on the irrigation regimens they received. The control regimen being a non-limiting irrigation while irrigation 1 and 2 are inferior to the plant needs inducing stress. Irrigation 1 being superior to irrigation 2 in quantity of water delivered per day. Measures come from leaves of the main orthotropic axis. Asterisks (*) indicate statistically significant differences compared to the control (Kruskal-Wallis test). Vertical bars represent standard deviation.

4.1.2.3 Pot weight control

Through the different weighting, we observed that there are differences between the pot weight along the month (Figure 4.4).

Indeed, at 19 days and 28 days after the start of the trial we saw that the pot weight was decreasing for the two stress irrigation regimens, and it remained stable for the control. The water needs of the plants were not met for those two conditions and the soil slowly started to dry under the plant uptakes. The control irrigation seems to have allowed a sufficient water uptake for the plant while maintaining a good moisture of the substrate. After the first treatment all pot irrigations were changed to match the control. The last measurement (31/05) allowed us to see that we successfully rehydrated all the pot.

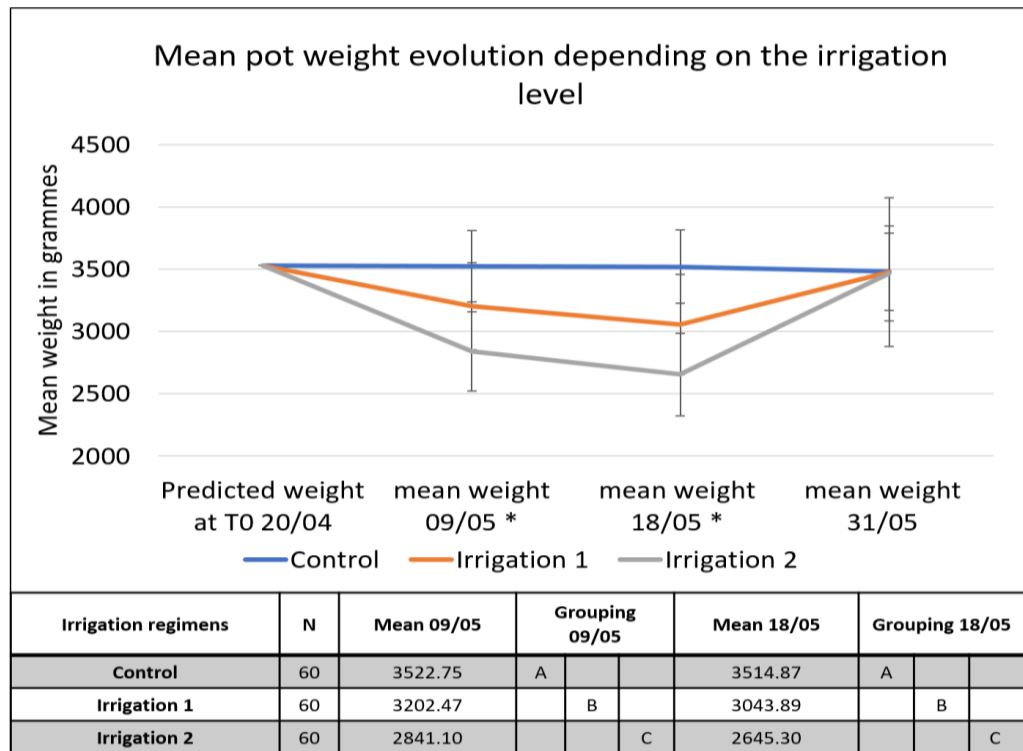


Figure 4.4. Mean pot weight evolution along the first month of trial depending on the irrigation regimens of the pot. Three different irrigation regimens are applied with the control being non limiting for the plant growth while irrigation 1 and 2 are delivering a limited amount of water per day. Asterisks (*) indicate statistically significant differences, Pairwise Tukey mean comparison test. Vertical bars represent standard deviation. Significantly different groups do not share a common letter in the grouping column.

Those significant differences on the pot weight are due to multiple parameters, irrigation levels, genotype, and the interaction between both irrigations. Irrigations regimens induced significant differences, that increased the more time passed (Table 4.3). We can see that there were significant differences between the three levels of irrigation with the maximum weight for the control irrigation at both measurements. A difference of 320 g with irrigation 1 (3202 g) and of 621 g with irrigation 2 (2841 g) at the first measurement was seen compared to the control. While differences of 471 g with irrigation 1 (3044 g) and of 870 g with irrigation 2 (2645 g) were visible during the second measurement.

Apart from the irrigation effects, we could see here a similar effect of the genotypes than the one for the irrometer measurements. Pots with GPFA119 were always lighter than the ones with GPFA117 (Table 4.4).

Table 4.3. Weight measurement of the pot depending on the genotype. Significantly different groups do not share a common letter in the grouping column. Pairwise Tukey mean comparison test.

Genotype	N	Mean in g 09/05	Grouping			Mean in g 18/05	Grouping		
117	90	3407.06	A			3308.46	A		
119	90	2970.49		B		2847.16		B	

Table 4.4. Weight measurement of the pot depending on the interaction between irrigation regimens and genotypes. Significantly different groups do not share a common letter in the grouping column. Pairwise Tukey mean comparison test.

Irrigation*genotype	N	Mean in g 09/05	Grouping				Mean in g 18/05	Grouping			
Control 117	30	3686.83	A				3683.73	A			
Irrigation 1-117	30	3437.99		B			3316.49		B		
Control 119	30	3351.27		B			3349.97		B		
Irrigation 2-117	30	3084.63			C		2911.12			C	
Irrigation 1-119	30	2957.00			C		2782.97			C	
Irrigation 2-119	30	2590.68				D	2392.20				D

4.1.3 Effect of the growth regulator application on the plant development

Through the different measurements that were done every month on the trees, some effects of the growth regulators can be highlighted, while the different irrigation regimens of the first month had a lasting effect on the plant growth.

4.1.3.1 Effect on the trees average height

Average height was significantly affected by the treatments and the irrigation regimens. It is easy to notice on Figure 4.5 that the treatments influenced the growth. Indeed, during the first month of trial where only the irrigation was controlled and no treatment were done, the growth pattern is similar for all the trees (Figure 4.6). However, at T1 (first treatment), we can see that the

growth pattern tendency changed completely with the product X inducing an important height growth while the product Z induced an opposite effect, compared to the control. Regarding the genotype effect on the height, no significant differences is visible.

Treatment E induced the tallest trees with an average of 39.7 cm while treatment C induced the smallest trees with an average of 31.4 cm. Trees treated with treatments A, B and D showed medium size that cannot be significantly distinguished. The different irrigations at the beginning of the trial have a lingering effect on the development with the stressful irrigation regimens inducing smaller trees compared to the control irrigation (Figure 4.7).

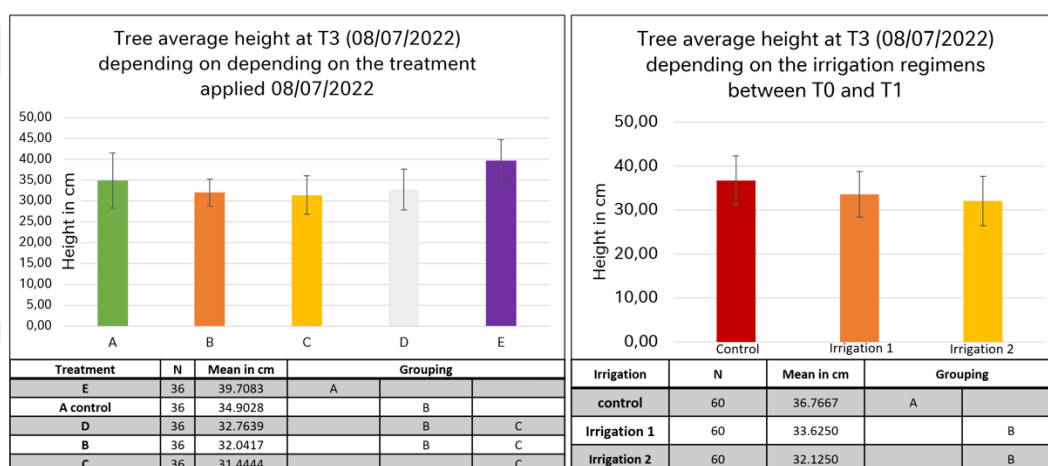


Figure 4.5. Trees height depending on the treatment (left) and the irrigation regimens (right). 5 treatments with the control condition following the guidelines of cultivation in greenhouse from Nestlé. The 4 other conditions represent the product X for the condition E and 3 different concentrations of the product Z in condition B,C,D. 3 different irrigation regimens with a control irrigation non limiting for the plant growth and 2 irrigation regimens with a reduced amount of water causing a stress to the plants. Groups that do not share a common letter are significantly different, Pairwise Tukey mean comparison test. Vertical bars represent standard deviation.

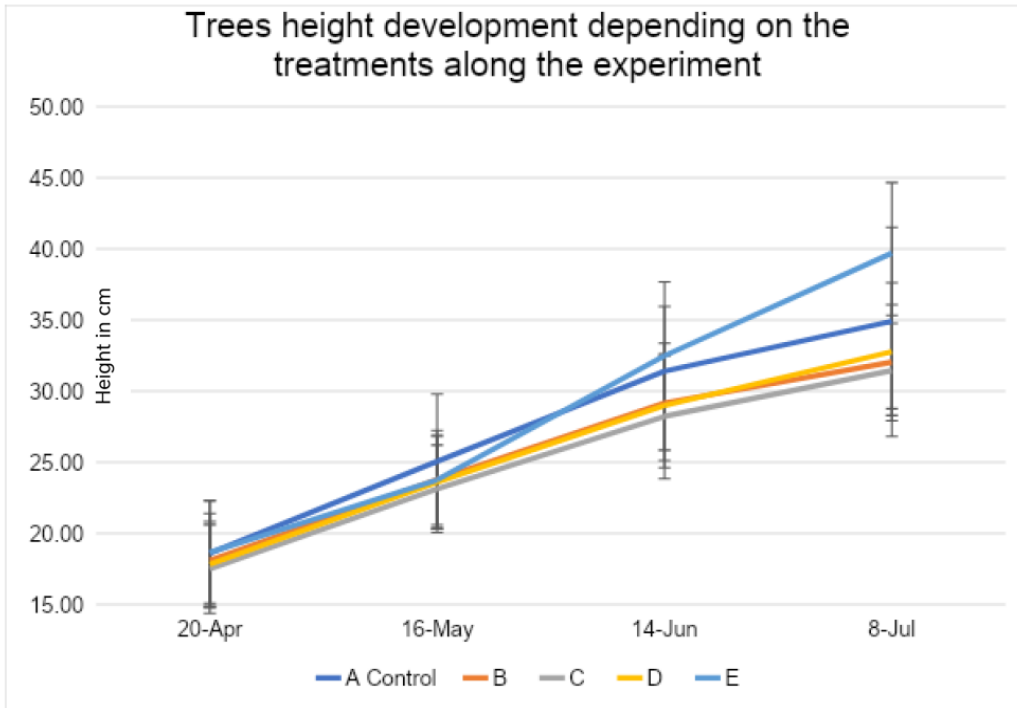


Figure 4.6. Trees height evolution during the 3 first months of the experiment depending on the treatments. 5 treatments with the control condition following the guidelines of cultivation in greenhouse from Nestlé. The 4 other conditions represent the product X for the condition E and 3 different concentrations of the product Z in condition B,C,D. 3 different irrigation regimens with a control irrigation non limiting for the plant growth and 2 irrigation regimens with a reduced amount of water causing a stress to the plants. Vertical bars represent standard deviation.

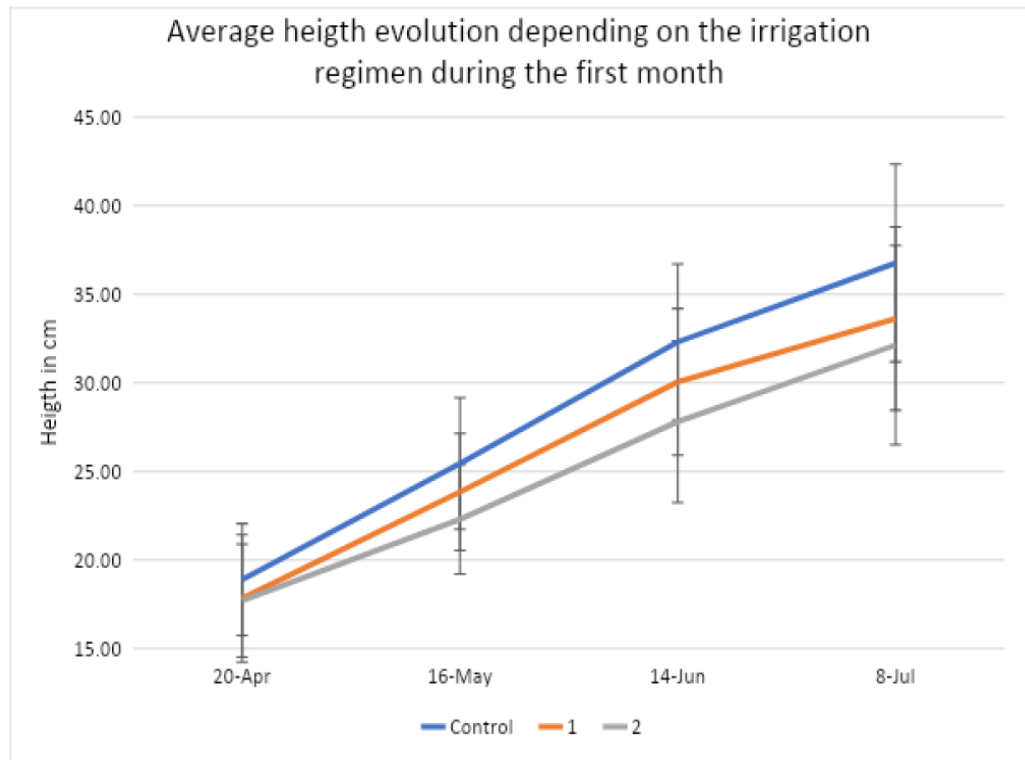


Figure 4.7. Trees height evolution during the 3 first months of the experiment depending on the irrigation regimens of the first month. 3 different irrigation regimens with a control irrigation non limiting for the plant growth and 2 irrigation regimens with a reduced amount of water causing a stress to the plants. Vertical bars represent standard deviation.

4.1.3.2 Effect on the plagiotropic branches number

On this growth parameter, the only noticeable difference in the trial is due to the variety effect. Indeed, through a Kruskal-Wallis test, we observed that there was a significant difference between GPFA119, presenting in average 9.76 plagiotropic branches, and GPFA117, presenting in average 6.32 branches.

This difference was noticeable all along the experiment and especially at T0, with most of the GPFA119 possessing 4 plagiotropic branches while GPFA117 trees mostly had 0. The treatments did not have a significant effect on the growth (supplementary data).

4.1.3.3 Effect on the main axis node number

The main axis nodes number was significantly affected by the treatments and the irrigation. While the genotype did not have a significant effect on the main axis node number. Here, this difference is of only one internode. Thus, it is difficult to really estimate the impact of the growing conditions on the trees. Treatment E presents a higher number of nodes compared to control A (Table 4.5-4.6).

The genotype did not have a significant effect on the main axis nodes number growth. Graph of the growth depending on the treatment in the supplementary data.

Table 4.5. Main axis node number depending on the treatment applied (08/07/2022). Significant differences between treatments is represented by an asterisks *. Kruskal-Wallis test.

Treatments	Main axis node number *	Standard deviation
A (Control)	10.34	2.12
B	10.28	1.16
C	10.14	1.33
D	10.75	1.20
E	11.06	1.24

Table 4.6. Main axis node number depending on the irrigation regimen applied during the first month (08/07/2022). Significant differences between treatments is represented by an asterisks *. Kruskal-Wallis test.

Irrigation	Main axis node number *	Standard deviation
Control	10.82	1.30
1	10.55	1.25
2	10.17	1.78

4.1.3.4 Effect on the total length of the plagiotropic branches

ANOVA test performed on the average total plagiotropic length revealed that it was significantly affected by the treatments and the variety. The irrigation regimens of the first month also had significant effects.

Treatment E induced the longest plagiotropic branches length per tree with an average of 157.2 cm while treatment D induced the smallest with an average of 95.764 cm per tree. Trees treated with treatments A, B and D showed medium size that cannot be significantly distinguished (Figure 4.8).

Regarding the irrigation effects, trees irrigated with irrigation 1 have approximately the same average total length than the trees irrigated with the control irrigation. However, the trees that received the second experimental irrigation got their growth slowed down compared to the two other irrigations. Finally, the genotypes evaluated also had an important influence on the total length of the plagiotropic branches. Indeed, GPFA119 trees present an average total length 70 cm more important than the GPFA117 trees (Figure 4.9-4.10).

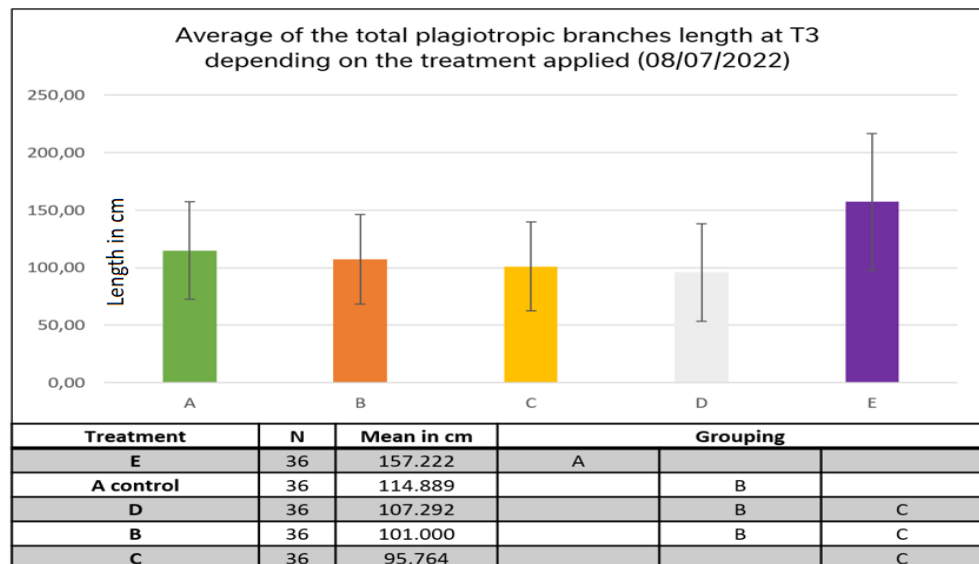


Figure 4.8. Average trees total plagiotropic branches length depending on the treatment applied on them. 5 treatments with the control condition following the guidelines of cultivation in greenhouse from Nestlé. The 4 other conditions represent the product X for the condition E and 3 different concentrations of the product Z in condition B,C,D Groups that do not share a common letter are significantly different, Pairwise Tukey mean comparison test. Vertical bars represent standard deviation.

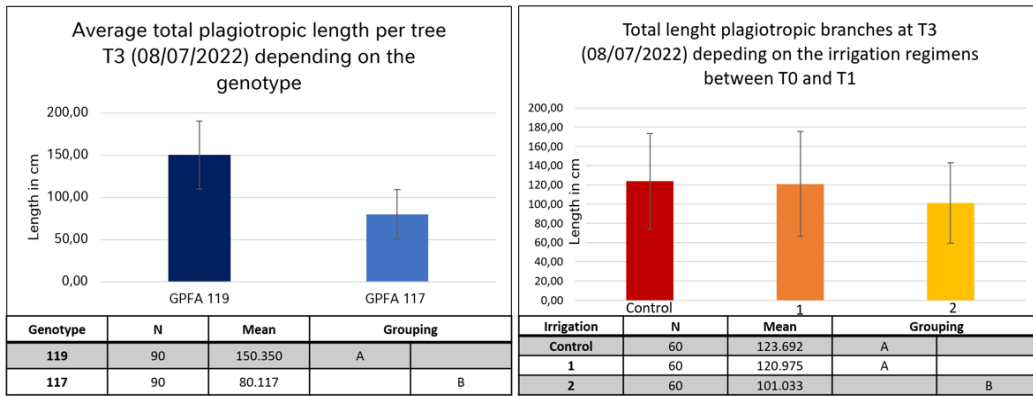


Figure 4.9. Average total plagiotropic branches length per tree depending on the genotype (left) and the irrigation regimens (right). Groups that do not share a common letter are significantly different, pairwise Tukey mean comparison test. Vertical bars represent standard deviation.

Regarding the evolution of the total plagiotropic branches length, the treatments influenced the growth (Figure 4.10). Indeed, during the first month of trial where only the irrigation was controlled and no treatment was done, the growth pattern was similar. However, at T1, we can see that the growth pattern tendency changed completely with the product X inducing an important growth while the product Z induced a slight reduction in growth compared to the control (Figure 4.11).

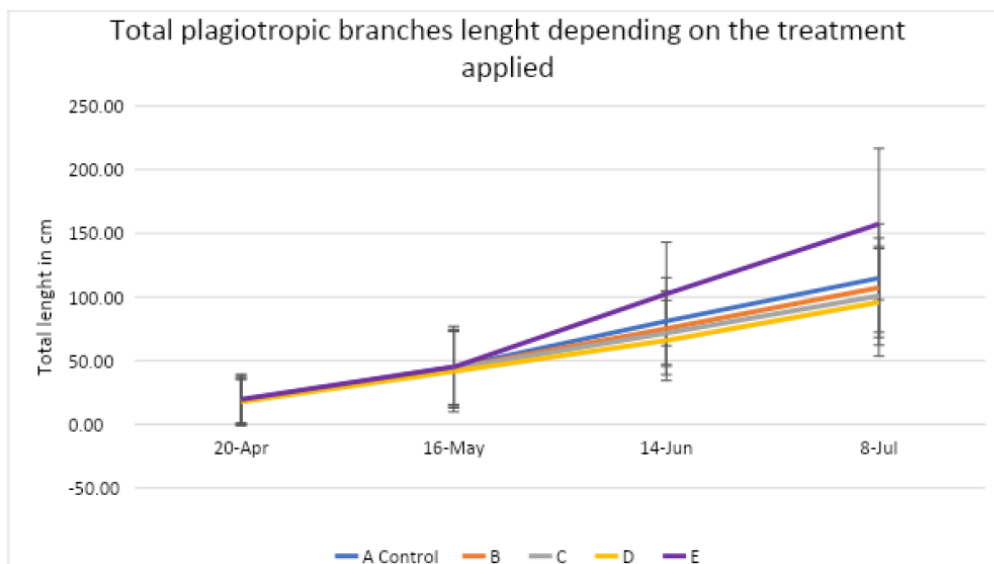


Figure 4.10. Trees average total plagiotropic length per tree evolution during the first 3 months of the experiment depending on the treatments. 5 treatments with the control condition following the guidelines of cultivation in greenhouse from Nestlé. The 4 other conditions represent the product X for the condition E and 3 different concentrations of the product Z in condition B,C,D. Vertical bars represent standard deviation.

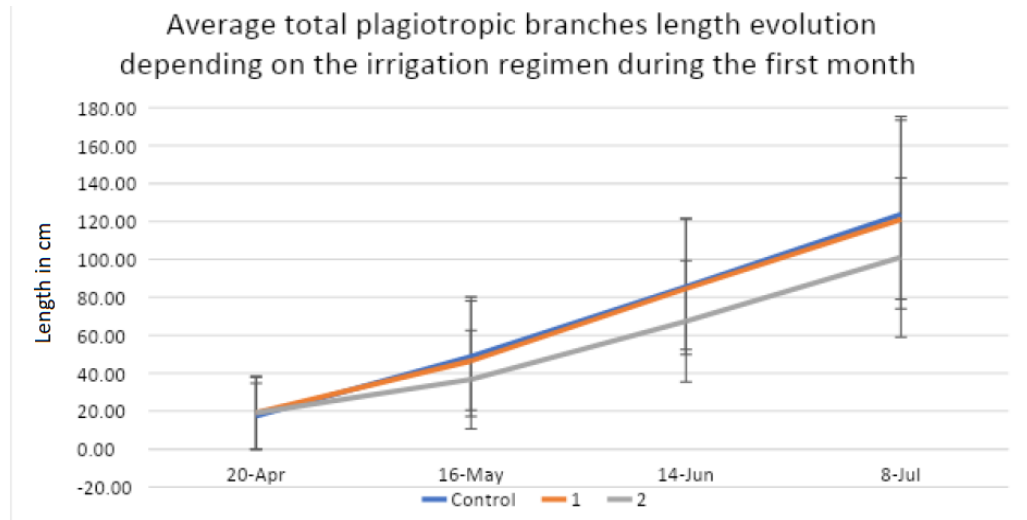


Figure 4.11. Trees average total plagiotropic length evolution during the first 3 months of the experiment depending on the irrigation regimens of the first trial month. 3 different irrigation regimens with a control irrigation non limiting for the plant growth and 2 irrigation regimens with a reduced amount of water causing a stress to the plants. Vertical bars represent standard deviation.

4.1.3.5 Effect on the total node number on plagiotropic branches

The total node number was significantly influenced by the treatments, the genotype, and the irrigation regimens. Treatment E induced a significant difference with all other groups (Figure 4.8). We can highlight here that depending on the treatment that was applied on the plants, a significant difference was found between treatment E and other treatments. Indeed, with a mean node number of 31.5, treatment E induced the development of approximately 15% more nodes compared to other treatments like we can see in Figure 4.12. Here, the different irrigations at the beginning of the trial influenced the development. We can see that the trees irrigated with irrigation 2 are approximately at the same average total plagiotropic nodes number than the trees irrigated with the control irrigation (Figure 4.13). However, the trees that got the irrigation 2 got their growth slowed down compared to the 2 other irrigations. Finally, the genotypes evaluated also had an important influence on the total plagiotropic node number. Indeed, GPFA119 trees present more than twice the number of plagiotropic nodes than the GPFA117 ones.

Regarding the evolution of the total plagiotropic node number, the treatments influenced the growth (Figure 4.14). Indeed, during the first month of trial where only the irrigation was controlled and no treatment was done, the growth pattern is similar. However, at T1, we can see that the growth pattern tendency changed completely with the product X inducing an important growth while the product Z does not seem to affect the growth for now. It is possible to see compared to the control that the product X treatment influenced the plant development.

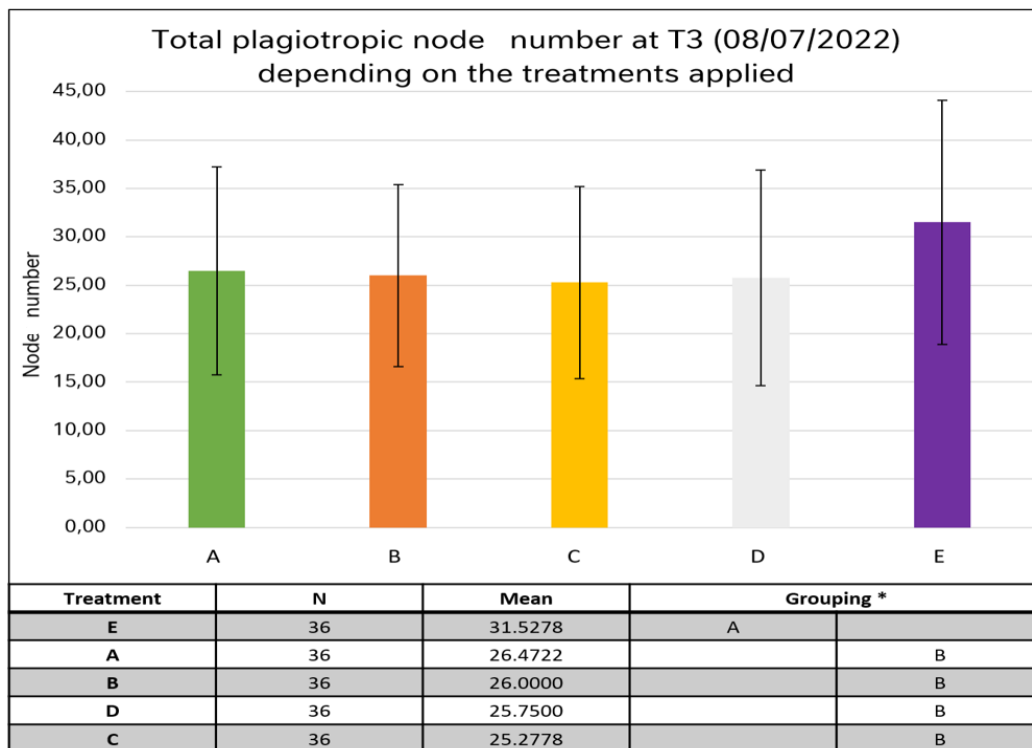


Figure 4.12. Average node number per tree depending on the treatment applied on the trees. 5 treatments with the control condition following the guidelines of cultivation in greenhouse from Nestlé. The 4 other conditions represent the product X for the condition E and 3 different concentrations of the product Z in condition B,C,D. Means that do not share a letter are significantly different. Pairwise Tukey mean comparison test, Vertical bars represent standard deviation.

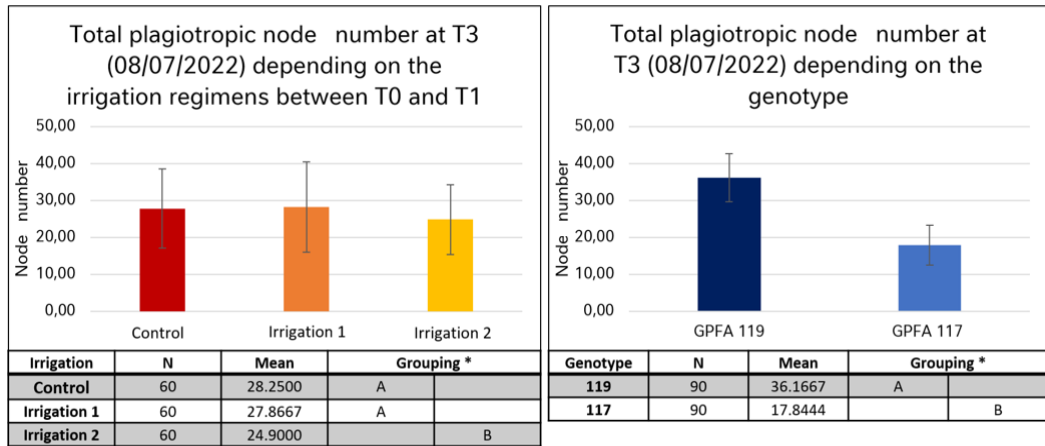


Figure 4.13. Influence of the irrigation regimens (left) and the genotype (right) on the plagiotropic nodes total number. 3 different irrigation regimens with a control irrigation non limiting for the plant growth and 2 irrigation regimens with a reduced amount of water causing a stress to the plants Means that do not share a letter are significantly different, pairwise Tukey mean comparison test. Vertical bars represent standard deviation.

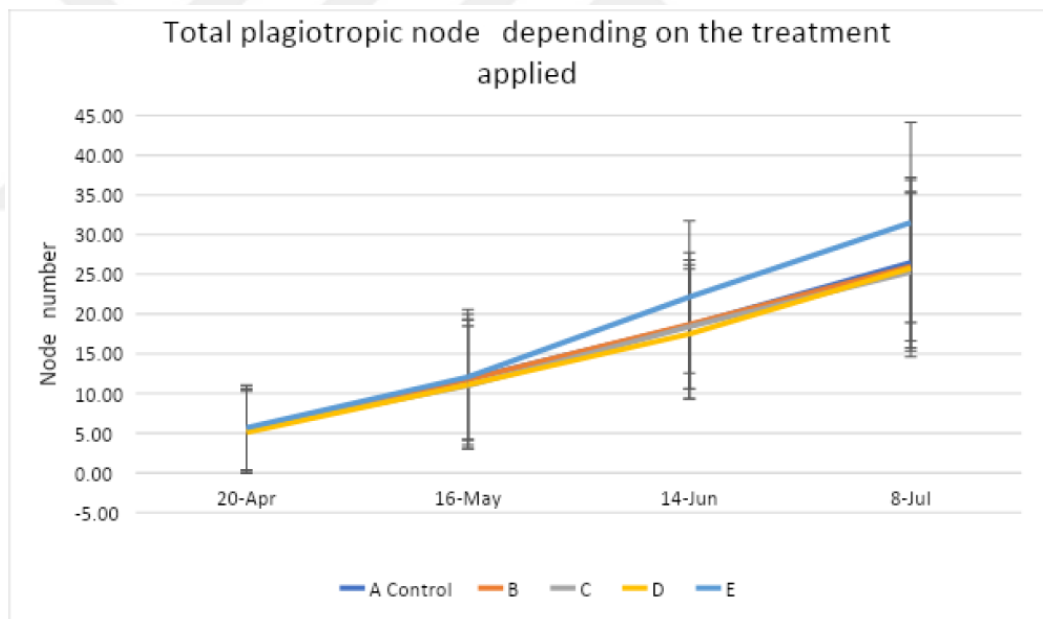


Figure 4.14. Average total node number on plagiotropic branches per tree during the first 3 months of the experiment depending on the treatments applied. 5 treatments with the control condition following the guidelines of cultivation in greenhouse from Nestlé. The 4 other conditions represent the product X for the condition E and 3 different concentrations of the product Z in condition B,C,D. Vertical bars represent standard deviation.

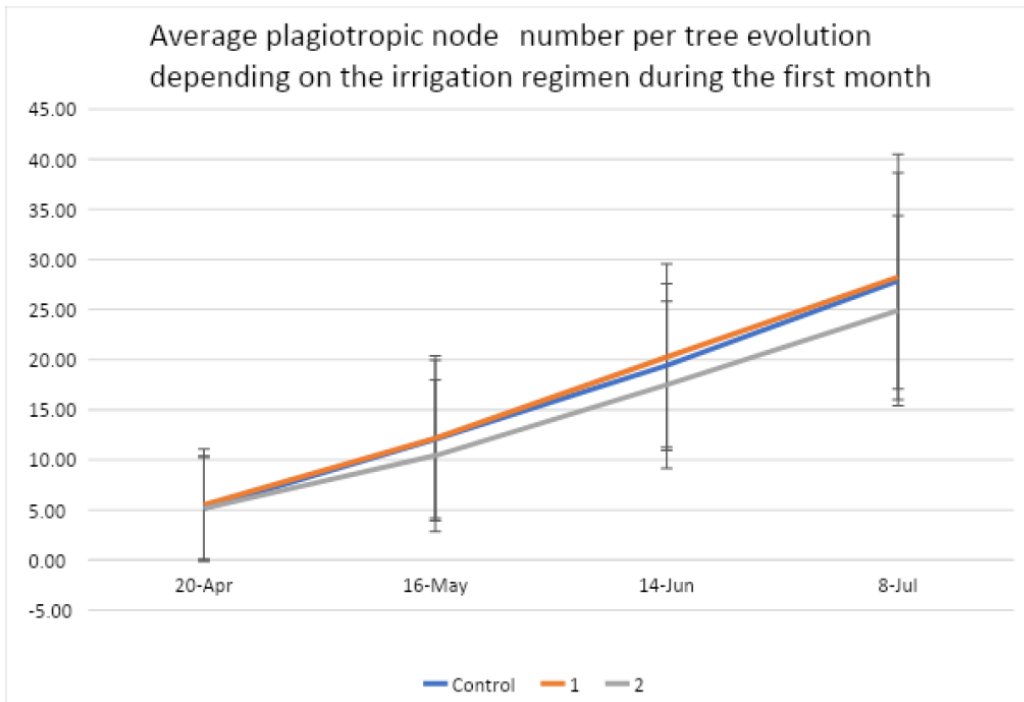


Figure 4.15. Average total node number on plagiotropic branches per tree during the first 3 months of the experiment depending on the irrigation regimens. 3 different irrigation regimens with a control irrigation non limiting for the plant growth and 2 irrigation regimens with a reduced amount of water causing a stress to the plants. Vertical bars represent standard deviation.

4.2 Photoperiod Trial

Our objective here was to initiate a long-term trial to compare photoperiod conditions on the coffee plant development. This trial was launched on June 10 and the first measures were collected after one month.

4.2.1 Climatic data monitoring

In order to better analyze differences between the conditions, we are monitoring the climate. Especially for this experiment where the different conditions are contained in homemade black boxes that impact the climatic conditions. The sensor for the B condition Black box did not work, and data could not be retrieved for the period 13/07-26/07.

We can see here that the boxes present slight variations of climate. Regarding the relative humidity, we can see that two black boxes have an inferior

relative humidity inside compared to the control conditions outside of a box. Regarding the temperature we can see that the variations are similar for all conditions, but their range is slightly inferior for two black boxes.

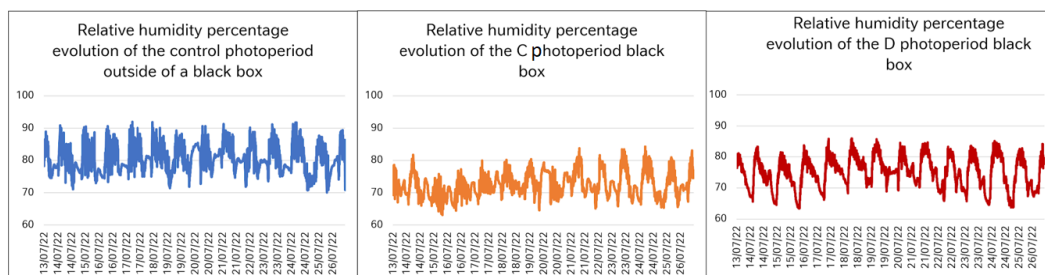


Figure 4.16. Relative humidity percentage evolution between the 13/07 and 26/07 depending on the black boxes. Control photoperiod (A; left); C photoperiod box (middle) and D photoperiod box (right).

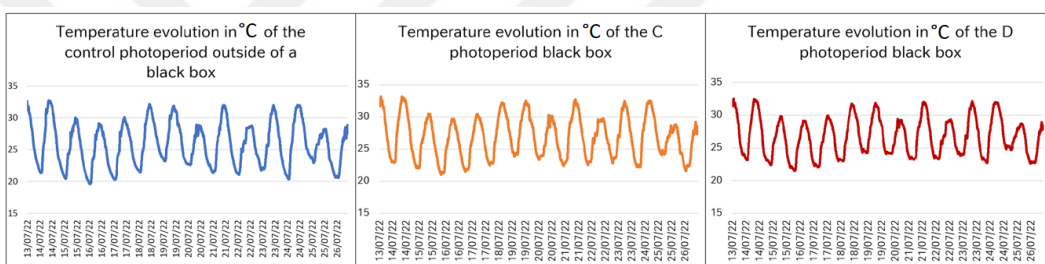


Figure 4.17. Temperature in °C evolution between the 13/07/2022 and 26/07/2022 depending on the black boxes. Control photoperiod (A; left); C photoperiod box (middle) and D photoperiod box (right).

4.2.2 Observation at one month of growth

At this date no significant differences on the growth can be highlighted. Indeed, no photoperiod induced a major difference on the plants in general compared to the control (Figure 4.19). Furthermore, this observation can be extended to the different genotypes. Indeed, the different genotypes under the different photoperiod did not show any major differences in the parameters measured.

Interestingly, the Typica variety is since the beginning of the experiment a bit more developed in height and leaf number than the two others (Figure 4.18). While GPFA 124 trees were the smallest at the beginning of the experiment, they

are after one month of growth a bit taller than GPFA70 trees (Figure 4.17 and 4.19). GPFA 70 presented during this first month of growth the best leaf development.

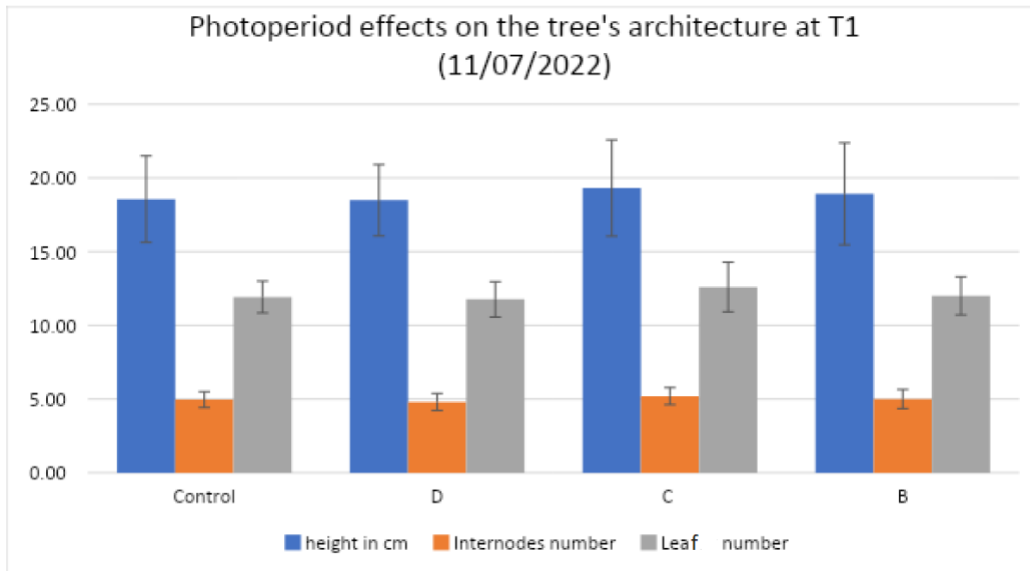


Figure 4.18. Trees architecture according to the photoperiod treatments after one month of growth. Vertical bars represent standard deviation. 4 photoperiod conditions were applied to the trees. The control photoperiod corresponds to the cultivation guidelines of Nestlé. Photoperiod B and C correspond to photoperiods with day longer than night while photoperiod D correspond to a photoperiod with night longer than day. Each bar represents a calculated mean of 25 measured values.

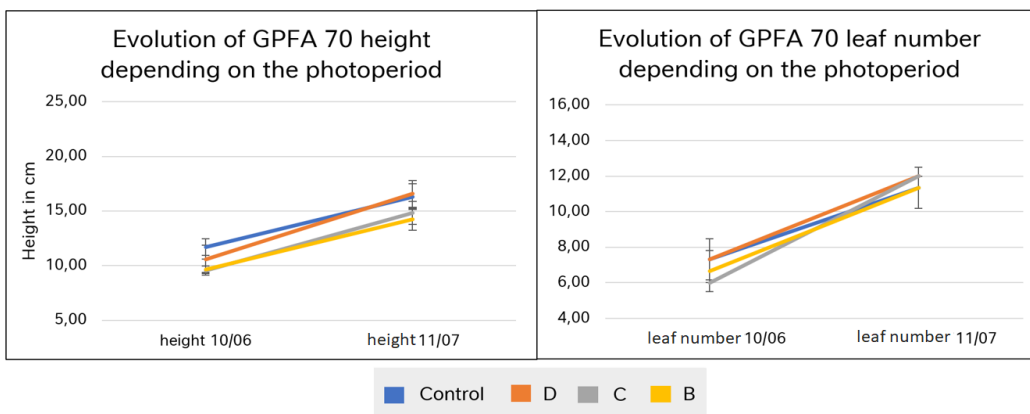


Figure 4.19. GPFA 70 average architecture evolution, during the first month, depending on the photoperiod. 4 photoperiod conditions were applied to the trees. The control photoperiod corresponds to the cultivation guidelines of Nestlé. Photoperiod B and C correspond to photoperiods with day longer than night while photoperiod D correspond to a photoperiod with night longer than day. Height (left) and leaf number (right). Vertical bars represent standard deviation

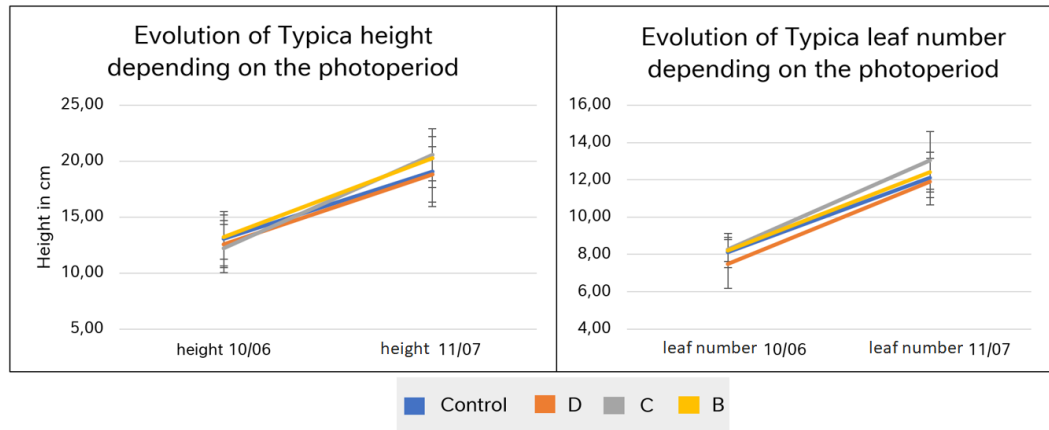


Figure 4.20. Typica average architecture evolution, during the first month, depending on the photoperiod. 4 photoperiod conditions were applied to the trees. The control photoperiod corresponds to the cultivation guidelines of Nestlé. Photoperiod B and C correspond to photoperiods with day longer than night while photoperiod D correspond to a photoperiod with night longer than day. Height (left) and leaf number (right). Vertical bars represent standard deviation.

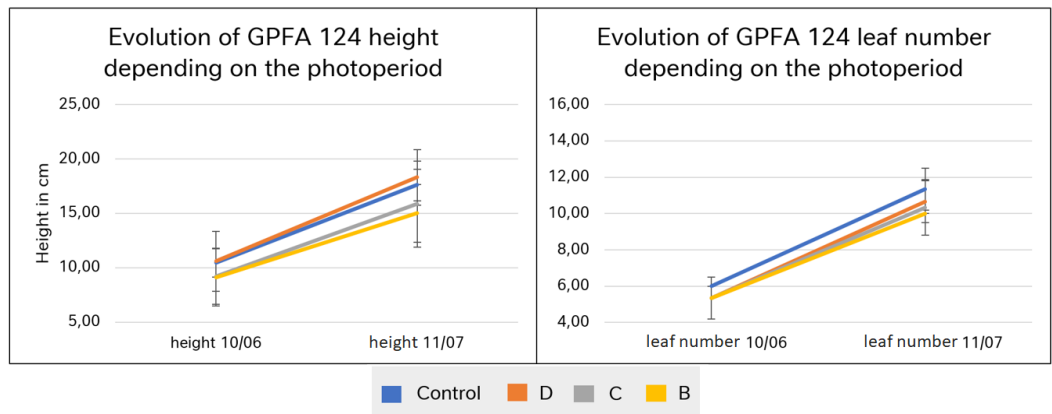


Figure 4.21. GPFA 124 average architecture evolution, during the first month, depending on the photoperiod. 4 photoperiod conditions were applied to the trees. The control photoperiod corresponds to the cultivation guidelines of Nestlé. Photoperiod B and C correspond to photoperiods with day longer than night while photoperiod D correspond to a photoperiod with night longer than day. Height (left) and leaf number (right). Vertical bars represent standard deviation.

4.3 *In Vitro* Flowering, First Trial

Our objective here was to explore the potential of different medium composition and photoperiods to trigger the development of plagiotropic branches on *in vitro* plantlets.

4.3.1 Climatic data monitoring

Real climatic data were monitored during the experiment to verify if the strategy was well applied and analyze the results of the experiment with those parameters in mind. Differences between the two rooms could be observed (Figure 4.22). A temperature difference of approximately 1 degree was found between the two conditions. While the relative humidity was stable in the Eratis chamber (outside of the moment when the chamber was opened). The control growing room in contrast had a fluctuating humidity, ranging from 20 to 40%. During the experiment, no branches initiations or floral buds could be observed in any of the four conditions. However, differences on mortality and rooting were observed.

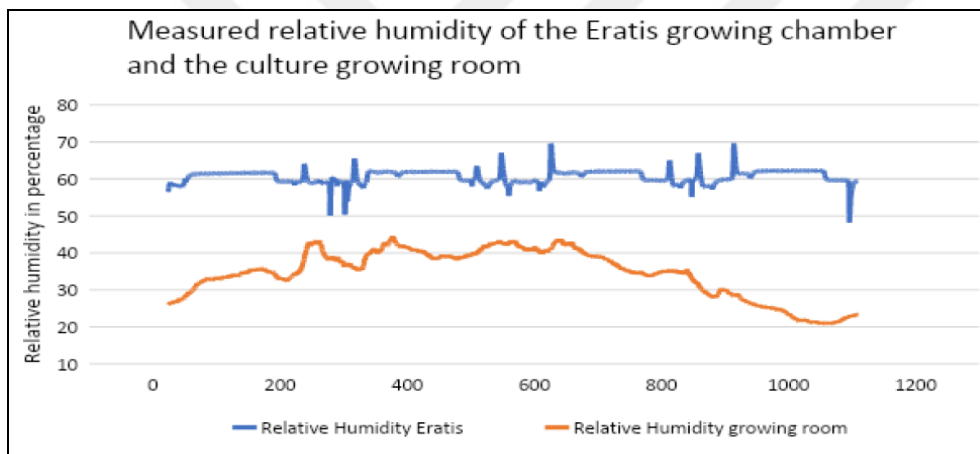


Figure 4.22. Temperature in °C in the Eratis growing chamber and the culture growing room.

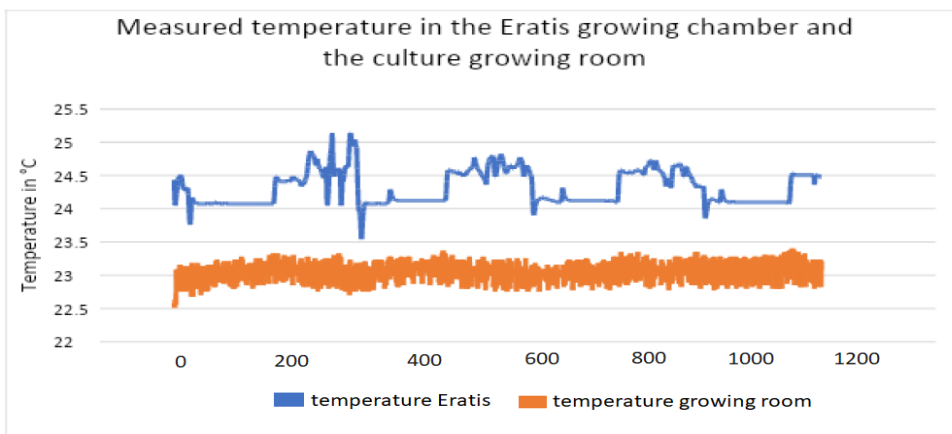


Figure 4.23. Measured temperature of the Eratis growing chamber and the culture growing room.

4.3.2 Growing conditions effects on the plantlets

Through this experiment, some effects due to the photoperiod were observed on the plantlets. On the 16 plants that died during this trial, 15 were grown on under the experimental photoperiod. It suggests an impact on the mortality rate of this photoperiod, Figure 4.24. The medium composition also had some consequences on the plant development. The rooting was significantly affected by the medium composition with a 50% reduction in the rooting rate on the modified medium compared to the control (Figure 4.24). Also, culture medium seems to have an impact on mortality but at this point of the trial the difference is not significant through the Chi square test. When following the evolution of those two parameters during the trial, we can distinguish that the modified medium induced a delayed rooting. Indeed, plantlet started to root only two months after the plantlet transfer on the growing medium (Figure 4.25).

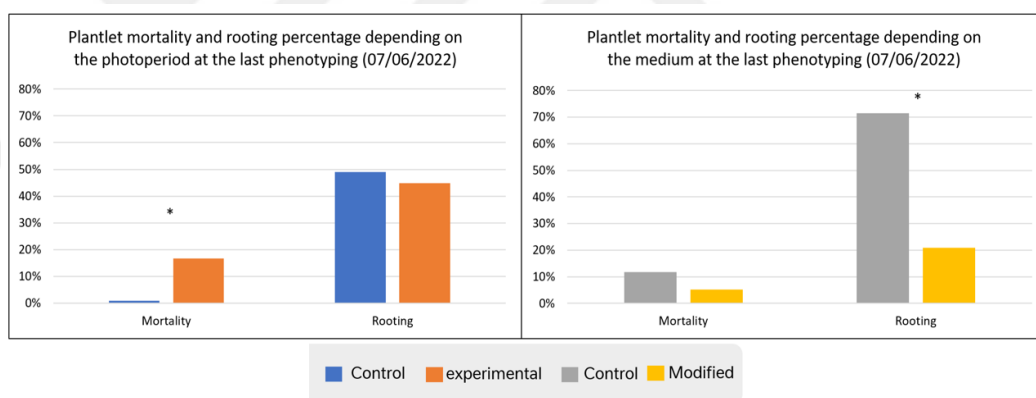


Figure 4.24. *In vitro* plantlet mortality and rooting depending on photoperiod (left) and medium composition (right) after 4 months of culture. Asterisks (*) indicate statistically significant differences using a chi square test. Qualitative variable.

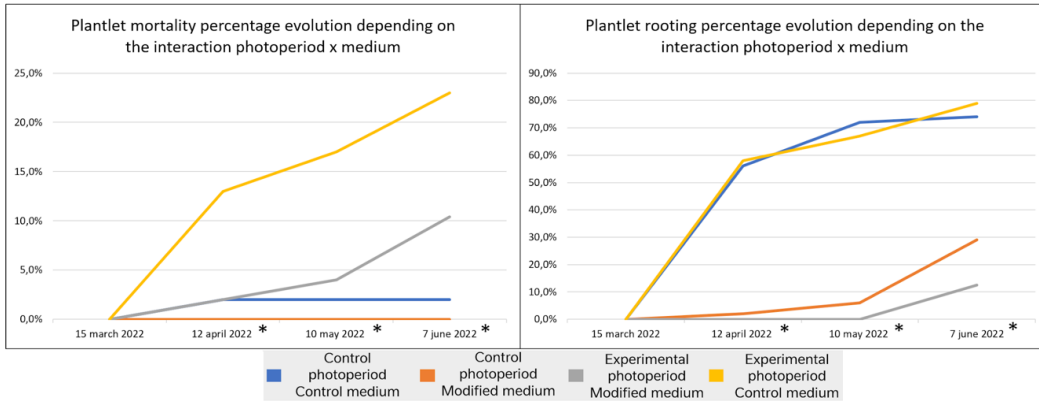


Figure 4.25. Plantlet mortality (left) and rooting (right) percentage evolution during the experiment according to the photoperiod treatment and culture medium composition. Asterisks (*) indicate statistically significant differences using a chi square test.

4.3.3 Analysis of green leaf area

In this trial, the projected leaf area from an upper view of the boxes was followed to estimate the plantlets growth. The results of the average areas per plantlet can be seen in Table 4.7. A Kruskal-Wallis test highlighted a significant effect of the photoperiod on the green leaf area (Table 4.7) While the medium did not significantly affect the plant growth. (Supplementary data). However, we observe a tendency of the modified medium to induce a reduction of the leaf development compared to the plantlet on the control medium (Figure 4.3.7). The relative growth of the different conditions compared with the control allow us to see that the interaction between photoperiod and medium had significant effects on the plant development.

Table 4.7. Growth of the green leaf area analyzed manually with image J. Total leaf area corresponds to the sum of green and dead leaf area, the total leaf area that the plant developed during its development.

Photoperiod	Medium	Green leaf area from an upper view picture in cm ² 15/03	Green leaf area in cm ² 07/06/2022	Dead leaf area in cm ² 07/06/2022	Full leaf area in cm ²
Control	Control	6.60	8.04	0.26	8.35
Control	Modified	7.69	8.69	0.24	8.93
Experimental	Modified	6.58	5.37	1.89	7.26
Experimental	Control	7.61	7.09	3.20	10.15
Photoperiod	Medium	Standard deviation green leaf area 15/03	Standard deviation green leaf area 07/06	Standard deviation dead leaf area 07/06	Standard deviation full leaf area
Control	Control	2.80	3.97	0.68	3.89
Control	Modified	4.03	4.50	0.66	4.47
Experimental	Modified	3.20	3.03	1.88	3.11
Experimental	Control	4.68	6.44	3.04	6.50

5. DISCUSSION

Speed breeding approaches are still mainly unexplored for perennial species. Here with this thesis work, we wanted to uncover the potential of growth regulators, photoperiod, and *in vitro* flowering for *C. arabica* speed breeding.

5.1 Growth Regulator Application

Through this experiment we applied different growth regulator treatments on the plants and expected to see developmental changes on the trees. At the same time, we wanted to explore the potency of those products depending on the hydric status of the plant. Indeed, underlying mechanisms of the plants under stress might produce molecular compounds that could interact with the applied growth regulators and trigger developmental process. A slight hydric stress was therefore foreseen to trigger those underlying mechanisms without significant-impact on the trees (visible stress symptoms).

5.1.1 Different irrigation regimens induced a water stress

Regarding our goal, we can say that we successfully created a stress based on the different measurements. Furthermore, this stress remained limited because no stress symptoms could be observed on the trees.

Among the measurements we did, we observed thanks to the irrometers that the plants irrigated with less water had an inferior soil hydric status. Irrometers are sensors that indicate the soil hydric status by showing a number between 0 and 200 without units. A soil well irrigated will present a value close to 0 while this number will get bigger the more the soil is dehydrated. In our case, soil that was irrigated with the control regimen had an average value of 1.5 the 18/05/2022, while plant irrigated with conditions 1 and 2 had a soil hydric status of 14 and 64,14 respectively Table 4.1.

This success in creating a water stress was also confirmed by weighting the pot. Indeed, we highlighted that depending on the irrigation regimens, the pots did

not have the same average weight. Pots irrigated with the control irrigation were the heaviest with an average of 3515 g (18/05/2022), while the pots irrigated with irrigation 1 and 2 weighted in average 3044 and 2645 g, respectively. Moreover, this difference did not appear instantly but slowly with a reduction in weight for the irrigation 1 and 2 over the weeks (Figure 4.2).

Leaf water potential measurement through Schölander bomb also confirmed this stress. In the literature, a well irrigated plant presents a leaf hydric potential around -1 MPa, while plants suffering from severe water stress can present a leaf hydric potential of -4.5 MPa . Here, we observed that the plants irrigated with the control irrigation every day present a leaf water potential of -0.87 MPa, while the plants irrigated with condition 1 and 2 present a leaf water potential of -1.01 and -1.21 MPa, respectively. The results indicate that the plants less irrigated present a smaller leaf water potential.

5.1.1.1 A variety effects on the water uptake

Apart from confirming that we created a water stress for the trees, those measurements allowed us to highlight the effect of plant development on the water needs of the trees. Indeed, at the beginning of the experiment, GPFA119 trees were taller and had a superior number of branches and nodes compared to GPFA117 (Supplementary data).

Those values indicate well why we can observe a difference during the pot weight measurement and irrometers (Table 4.2) depending on if we are looking at trees from the GPFA119 variety or if we look at the GPFA117 variety.

5.1.1.2 Return to a normal irrigation and soil water potential

We wanted to explore in this trial the effect of the applied product depending on the water status of the trees. However, our focus is still on the development of a speed breeding protocol. Water deficiency did not bring any developmental changes towards a faster flowering and conversely, has negative effects on the plant development by interacting with multiple mechanisms, among

which photosynthesis with a reduction in efficiency (Rahman et al.,1992).That is why we wanted to restore the water potential after the first application. Water is not supposed to be a limiting factor after the first month of experiment. We managed to restore the water status of the trees by putting all the plants on the control regimen of irrigation. Here, water was not a limiting factor anymore through the last pot weighing, (31/05/2022) as all the pot weights were measured around 3500 g even if they had a weaker irrigation during the first month.

5.1.1.3 Hydric stress effect on the plant development

Even if the stress that we put on the trees during the first month was limited and no visible symptoms could be observed, it had some effects on the plant development. Indeed, after two months of good irrigation, some significant lingering effects can be correlated with the different irrigation regimens. The trees that received the control irrigation per day and the ones that received only irrigation 1 and 2 during the first month have differences in some architectural parameters.

Trees that were irrigated with regimens 1 and 2 had their height reduced of 9% and 15% compared to the control (Figure 4.3). The total plagiotropic length and the nodes number are also still influenced by the different irrigation regimens that were applied during the first month. Trees that received the control irrigation and condition 1 present longer branches and more nodes compared to the trees that received irrigation 2, Figure 4.6 and 4.9.

5.1.1.4 Lack of interaction between tree hydric status and growth regulator

In this trial 2 periods can be highlighted for the data analysis of the interaction between irrigation and treatment. Indeed, at T1, the trees got treated for the first time and the irrigation regimens was switched back to the control irrigation for all the trees. At this date, the trees had different hydric potential like we previously said and therefore we will see the direct interaction between hydric

status and growth regulator potency. Here, we can conclude that hydric stress did not significantly affect the growth regulator effect on the trees,

Even if taken independently, both parameters have an influence on the plant development. The interaction does not bring any significant effect, or it is too superficial to measure in this experimental design (supplementary data). The second period consists in the rest of the experiment where all the trees when treated are supposed to have the same hydric potential (same control irrigation). In this period, we are evaluating the lingering effects of the hydric stress on the growth regulator efficiency. The same conclusion can be reached after three months of experiments. No interaction was found, while the treatments and the irrigation had significant effects on the trees separately.

At the moment of writing this report, all irrigation levels reacted similarly to the application of products X and Z. If an interaction exists, we cannot identify it with this experimental design or it will affect the trees at another developmental step, i.e., flowering, ripening, etc.

5.1.2 Treatment effects on the plant development

Regarding the main goal of our experiment to accelerate the plant development, some of the preliminary results already indicate that the applied molecules affect the plant development. However, no effect of the treatments on floral buds' induction can be highlighted after two months of treatments. It is important to keep in mind that those results are preliminary and that the control trees are expected to flower in February 2023, seven months in the future.

Even if no effect on flowering induction can be seen yet, some effects on the tree's architecture can be observed already.

5.1.2.1 Product X treatment effects on architecture (confidential)

Applying product X (treatment E) on the trees modified the tree's architecture. Indeed, height, average total branches length and average total nodes

number were increased compared to the control (Figure 4.4). Those effects are magnified for the branches length with some trees that present branches longer than their main axis, i.e., tree number 170 for example is 43 cm tall with one of its branches measuring 45.5 cm the 08/07/2022. The effects were strong enough to identify the trees treated with product X by eye. Therefore, at this point of the trial, applying this product seems to be promising to enhance the plant development and modify the tree structure toward aerated trees with long branches, Figure 5.1.



Figure 5.1. Pictures of tree number 28 (treatment A); 65 (treatment B); 92 (treatment C); 130 (treatment D) and 154 (treatment E). Pictures taken the 05/08/2022. The scale on the left side of each picture corresponds to 20 cm in total 2 graduation of 10 cm.

5.1.2.2 Product Z treatment effects on architecture

Here, the product Z treatment effects were easily visible because they induced opposite effect compared to the product X treatment. The trees treated with product Z had their height and total plagiotropic length reduced compared to the X treated ones. This reduction is also visible when compared to the control trees. This is in accordance with what was found in the literature. The 3 concentrations seem to induce a reduction in height and mean total length of the plagiotropic branches compared to the control, while the mean total nodes number stay similar (Figure 4.5). Here we can highlight that the different concentrations presented no significant differences between each other's at T3 for the plant architecture.

Z application seems promising to reduce the tree's space by reducing the plagiotropic internodes. This is an interesting point to increase the numbers of growing trees in the same space, Figure 5.1. Those differences should be easier to

see with time passing. While we expect to see more variation in the effects triggered by the 3 concentrations.

5.1.2.3 Variety effects

Finally, something important to notice here is the variety effect on the plant architecture and development. Like we could have expected with the starting material, morphological differences still exist between both varieties after three months of trials. Even if they present a similar height, GPFA 119 trees tend to produce more plagiotropic branches compared to the other varieties. This number of branches explain also why the GPFA119 trees have a greater average total length of branches and total nodes number compared to GPFA117 trees.

No significant difference was found on the interaction between treatments and variety. The treatments seem to affect similarly both varieties.

5.2 Photoperiod Trial

Through this trial we wanted to grow trees under different photoperiods to evaluate the potential for vegetative and speed breeding purposes. Indeed, plants are heavily influenced by photoperiod like we presented in the literature review section (Anderson et al.,1988; Melis, 1991; Morais et al.,2004)

At the moment to write the report, the trial has only been ongoing for one month and really scarce data are available to evaluate the potential of photoperiod as speed breeding parameter, especially regarding flowering. Indeed, this trial started with 6-month-old trees while the usual flowering time for Arabica trees is around 2-3 years old. Therefore, the focus of this thesis work on this experiment was to design, install and ensure the trial launch. Measurements to control the experimental conditions were done to ensure a good beginning for this 2-year experiment.

5.2.1 Primary results and important monitoring points

Through the first month of trial, no significant differences can be highlighted between the 4 groups of plants. When looking to the Figure 4.1 we can see that all the plants averages are similar. Only a variety effect can be outlined at this point. Typical trees are, since the beginning of the experiment, a bit more developed in height and leaf number than the two others

(Figure 4.3). Regarding the GPFA 124 trees, they were the smallest at the beginning of the experiment, but after 1 month of growth they are a bit taller than GPFA 70 trees (Figure 4.2 and 4.4). GPFA 70 presented during this first month of growth the best leaf development (Figure 4.4).

The important knowledge we gained during this first month of trial is related to the points we need to monitor closely for the rest of the experiment. The climatic conditions between the different black boxes are difficult to control like we can see in section 4.2.1. Indeed, the material we used seems to affect the temperature average with a reduction in variation compared to the control (4.2.2). It also affects the relative humidity (4.2.1), water projected from the fog system can only enter the boxes by the holes between plastic layers. It reduces the relative humidity in those boxes compared to the control which is directly under the fog system.

Furthermore, the photoperiod length could affect the relative humidity between boxes. Those climatic conditions are important to monitor closely if we want to precisely analyze the environmental effects on the tree's development. The sensor also needs to be controlled during the experiment to ensure to not lose data. The last interesting monitoring point discovered during this first trial month was the presence of a phenotypically abnormal tree in the border. Indeed, tree number 46 presents 3 leaves per nodes, it is trifoliated.



Figure 5.2. Pictures of the tree number 46, trifoliated per node. Picture taken from the top (left) and from the upper front (right; 79).

5.3 *In Vitro* Flowering First Trial

We wanted with this experiment to observe if it was possible to trigger the branching of *in vitro* plantlets and flowering buds.

Here the primary goal of this trial was not met. The different treatments chosen could not trigger branching and flowering. However, through this experiment different effects of the conditions can be highlighted.

5.3.1 Photoperiod change induced a mortality increase

Plants are plastic, they can adapt to their environment by influencing some of their structural characteristics, this process is called acclimation. In the case of light, highly conserved and well documented phenomenon exists to allow this acclimation. For example, the size of the Photosystem I and II can be modulated by light intensity, (Anderson et al.,1988) while the ratio between those two photosystems can be adapted depending on light quality and quantity (Melis, 1991; Morais et al.,2004)).

Our plant material in this experiment was generated by somatic embryogenesis and grown during multiple years in one, never changing, photoperiod conditions (control). Therefore, the plantlets were perfectly adapted

to those conditions. The sudden change in photoperiod could be associated with an important stress for the plantlets and in this experiment, we clearly observed the effect of that stress.

Transferring the plantlets grown under the control photoperiod, to the experimental photoperiod induced an important stress with an increase in the mortality rate compared to the control photoperiod and a developmental reduction. 15 of the plantlets grown under the experimental photoperiod died while only 1 plantlet did not survive under the control photoperiod. Their development was also affected by an important necrosis of the leaf area for all boxes grown in the experimental photoperiod. This leaf area necrosis ranges from 26% to 32% of the total leaf area, in average depending on the medium composition while only 3 % of the leaf area was dead for medium composition in the control photoperiod (Figure 4.2- 4.3. Table 4.1).

5.3.2 Medium composition induced a delay in rooting and a reduction in leaf development

As one of the major components of *in vitro* techniques, the medium composition can influence plant development. It can influence the plant capacity to survive *in vitro*; (Mukherjee et al.,2010; Shulgina et al.,2021) the type of cells that will develop (Samson et al.,2006) or the rooting (Rahman et al.,1992) In our case, the plantlets grown on the modified medium had their first roots appear after two months of trial. Furthermore, the percentage of plants that had roots after three months of trial was only reaching 30% (control photoperiod) and 10% (experimental photoperiod) of the total number of plants. While the plant growing on the control medium reached a rooting percentage of 70-80% for both conditions after 3 months of growth Figure 4.2- 4.3.

The medium composition also presented in our case a reduction in the plantlet development. Indeed, when we compare the leaf area development on Table 4.3.1 and Figure 4.6 we can see that the total leaf area was underdeveloped when the medium was modified compared to the control medium. The delay in rooting could be correlated with this reduction in plant growth.

5.3.3 Medium composition and photoperiod interaction affected the plant survivability

We highlighted above that photoperiod and medium composition are two important parameters for the success of an *in vitro* culture. In our case, the change in photoperiod induced an increase in the death rate of the plant and the medium composition induced a delay in the rooting and reduction in plant development.

But a third effect can be highlighted here, and it is due to the interaction of both parameters. When looking at Figure 4.2, we can see that the combination of photoperiod and medium composition are not showing the same evolution pattern. Indeed, the mortality rate is increasing fast for the treatment D. While the same photoperiod but with a different medium presents a delay in this mortality increase, treatment C. Mortality rate on the modified medium was three times lower compared to the control after two months of growth and still two times lower after three months. Those experimental results indicate here a tendency of modified medium in delaying the mortality of the plantlets.

two different developmental types seem to appear here depending on the medium. A fast growth of the roots for the control medium, with a good shoot development but associated with a higher mortality rate. While the modification in the medium composition led to a delayed rooting and a smaller shoot development and death rate compared to the control.

6. CONCLUSION AND RECOMMENDATIONS

At the moment to write this thesis report, the trials that I launched with the help of the production team and greenhouse team of the center are still at the beginning phase and observing flowering structure is still a long way to go. That is why an important work of monitoring is needed in the next months to have the most accurate observation of the parameter's effects on the tree's development. Here, based on the first months of experiment, a focus needs to be given on the climate to highlight differences between the conditions and their effects on the results (photoperiod trial). The efficiency of the growth regulator application along time and the risk of burns due to the products needs also to be closely observed (growth regulator trial).

But even if we are only at the beginning steps of those trials, we already managed to unravel some interesting effects of the control parameters tested.

Indeed, the two growth regulators that we are testing are clearly affecting the plant structure until this point. Product X application on *C. arabica* trees seems to enhance the plant development on practically all the parameters we followed (height, total plagiotropic length and total nodes number per tree). Regarding product Z, applying this product on the trees seems to reduce the height and the length of branches while maintaining the total number of nodes. If the effect of this growth regulator persists in the next months, they could be interesting to affect the plant architecture depending on the uses we want to do.

Concerning the *in vitro* trials, we opened here a new field of research, and the results could be used in the future as a base for the development of research trials on this topic.

Lastly, it is too early to make any conclusions on the different conditions of the photoperiod trial. But we have great expectations toward this parameter.

7. SUPPLEMENTARY DATA

Table 7.1. Average plant architecture of the 2 genotypes at the beginning of the growth regulator trial. Calculated average on 90 plants per genotypes.

Genotype	Mean height (cm)	Mean branch numbers
GPFA 119	19,467	4,656
GPFA 117	16,800	0,411

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