EFFECT OF SOAKING AND VARIED TIME EXPOSURE TO DIFFERENT MEDIUM BY

Citrus tangerina

BY

Emmanuel Chukwuemeka ANEKWE

LSC2007189

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

JANUARY, 2025.

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A PROJECT SUBMITTED TO THE DEPARTRMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCE, UNIVERSITY OF BENIN, IN PARTIAL FUFILLMENT OF THE REQUIREMENTS OF THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.SC.) IN PLANT BIOLOGY AND BIOTECHNOLOGY.

JANUARY, 2025.

CERTIFICATION

This is to certify that ANEKWE CHUKWUEMEKA EMMANUEL with matriculation number LSC2007189 of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin carried out this project work in partial fulfilment of the requirements of the award of the degree of Bachelor of Science (B.Sc.) in Plant Biology and Biotechnology.

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Prof. D.E Vwioko Date:

(Head of Department)

DEDICATION

I dedicate this project to God Almighty, for His love, grace, strength and provision to successfully complete this project. It was by his grace.

I also dedicate this project to my parents Mr. Fredrick Chukwukwolum Anekwe and Mrs.Nonye Uchenna Anekwe for their support, prayers, and words of encouragement.

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With utmost gratitude, I want to thank God Almighty for the successful completion of this project.

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I hereby use this medium to express my profound gratitude to my mother Mrs. Nonye Uchenna Anekwe, my close friends and my course mates. Thank you all for your support.

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ABSTRACT

Seed dormancy and slow germination rates are common challenges in the propagation of Citrus tangerina, limiting its agricultural productivity and commercial cultivation. This study examines the effects of soaking seeds in different mediums—distilled water, nitric acid (HNO_3), and salt solution (NaCl)—with varying exposure times (6, 12, and 24 hours) to determine their influence on germination performance and early seedling growth. The objective is to identify an effective pre-treatment method to enhance germination, improve seedling vigor, and assess salinity tolerance.

A completely randomized design (CRD) was employed, where treated seeds were monitored for germination percentage, mean germination time, seedling vigor index, root-shoot length, and overall seedling health. The results indicated that HNO_3 significantly improved germination rates and seedling vigor, likely due to its ability to soften the seed coat and break dormancy. Seeds soaked in distilled water exhibited moderate germination improvement, supporting its role in hydrating and activating metabolic processes. In contrast, NaCl treatment negatively impacted germination and seedling growth, with prolonged exposure (24 hours) leading to reduced vigor, suggesting that Citrus tangerina may be sensitive to saline conditions.

These findings provide valuable insights into optimal seed pre-treatment methods for Citrus tangerina, benefiting citrus nurseries and large-scale plantations by improving propagation success. Additionally, the study contributes to understanding seed dormancy mechanisms, stress tolerance, and germination enhancement techniques. Future research should explore the biochemical and physiological responses of seeds to these treatments to further optimize germination protocols and assess long-term seedling development.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND INFORMATION

Tangerine or *Citrus tangerina* is a small oblate, thin-skinned, bright orange, easy-to-peel type of mandarin indigenous to China and Southeast Asia. Tangerine cultivation slowly spread westwards along trade routes as far as the Mediterranean where, in the 1800s, acquired the name tangerine due to the export of this sweet mandarin from the port of Tangiers in Morocco (Maciel *et al*, 2023).

Mandarins originated in China and southeast Asia. The names “tangerine” and “mandarin” have often been used synonymously. The term “tangerine” was first used in the nineteenth century to describe mandarins with deep orange-red external colour. The mandarin/tangerine citrus group is very diverse, and attempts have been made to assign members into different categories and species. *i* (satsumas), *Citrus deliciosa* (Mediterranean mandarin), *Citrus nobilis* (King mandarin) and *Citrus reticulata* (common mandarins) are known world-wide, but only *Citrus reticulata* and associated hybrids are of economic importance in the U.S. (Saunt, 2000).

Tangerine farming in Nigeria holds a relatively recent history that has evolved over the past century. This journey highlights the resilience of both the fruit and the farmers who have cultivated it. Tangerines, also known as mandarin oranges, were introduced to Nigeria during the era of British colonial rule. The British brought various citrus varieties, including tangerines, to Nigeria for cultivation. These early efforts laid the foundation for tangerine farming in the country. Tangerine cultivation began on a modest scale in southern Nigeria, particularly in states like Lagos, Ogun, and Oyo. The favorable climate in these regions provided an ideal environment for citrus production, and tangerine orchards started to take root. As awareness of the economic potential of tangerines grew, farming practices expanded. Farmers recognized the demand for this citrus fruit, both domestically and in international markets. The expansion of tangerine orchards contributed to the diversification of Nigeria's agricultural sector. Over time, various tangerine varieties were introduced and cultivated in Nigeria. Among them, the "Dancy" and "Ponkan" varieties gained popularity for their sweet and juicy characteristics. These varieties became staples in the Nigerian tangerine market. However, Tangerine farming faced challenges that included pests, diseases, and a lack of modern farming techniques. Issues such as citrus canker and citrus greening disease affected crop yields and fruit quality, posing significant hurdles for farmers. Recognizing the potential of tangerine farming, the Nigerian government initiated programs to promote citrus cultivation, including tangerines. These programs included research, extension services, and the distribution of improved citrus varieties to farmers. Nigeria's tangerine industry saw growth in export opportunities, particularly to European and Middle Eastern markets. Exporters worked diligently to meet international quality standards, opening doors to global trade and increasing the economic potential of tangerine farming. In recent years, there has been a concerted effort to modernize tangerine farming practices in Nigeria. This includes the adoption of improved irrigation methods, pest control measures, and post-harvest handling techniques to enhance both productivity and quality. Tangerine farming contributes significantly to rural livelihoods and Nigeria's agricultural economy. It provides income for smallholder farmers and supports local processing industries, such as tangerine juice production. Despite progress, challenges like citrus greening disease, market fluctuations, and infrastructure limitations continue to affect tangerine farming in Nigeria. Overcoming these challenges remains a priority for the industry's growth and sustainability (Abdulmalik, 2023).

Tangerine chemical composition is dependent on maturity level, storage, horticultural, and climate conditions, all of which impact the chemical profile of the fruit. Nonetheless, tangerines possess low amounts of proteins and lipids, with carbohydrates (namely, sucrose, glucose, and fructose) being the most prevalent macronutrient. Currently, tangerine production plays a crucial role in the agri-food industry, with global production being expected to increase by 2.0 MMT by 2021/22, reaching a groundbreaking record of 37.2 MMT (Maciel *et al*, 2023).

Although synonymously used, tangerines and mandarins are not necessarily the same, with the latter encompassing tangerines, clementines, and satsumas. The phytotomy of tangerines consists of an exocarp which entails the epidermis and flavedo (containing oil sacks that produce aromatic oils), the albedo (a sponge-like layer that is a source of flavanones), and the vascular bundles. Segments containing the juice sacs and seeds, with septa running along these segments converging onto the central axis of the tangerine, comprise the endocarp (Maciel *et al*, 2023).

The major problem of *Citrus tangerina* is the exhibition of dormancy by the seeds which inhibits or reduces germination. This issue does not only affect agricultural productivity but also limits the plant’s commercial potential. Overcoming this challenges requires strategies that enhances seed germination and seedling growth, ensuring more reliable and efficient propagation methods for farmers and growers.

Several studies has explored various methods such as bending, lopping and ringing in order to force the growth of an inserted bud have been in use for many years on citrus and other crops. Results were erratic, however, and topping became common practice. This works well after the bud has passed through a rest period (winter or drought), but results are less satisfactory in the humid tropics.

A bending method which proved successful in Surinam was later abandoned, but was adopted in Indonesia and the Philippines (Samson, 1986).

However, limited studies has been done in evaluating the effects of pre-treatment methods in *Citrus tangerina* seeds in relation to varying chemical solution to influence seed germination and seedling growth. This neglect presents an opportunity to investigate how pre-treatment methods interact to affect the germination success of *Citrus tangerina* seeds.

Pre-treatment methods like mechanical scarification, soaking of seeds in water, subjecting them to heat or cold, exposure to mechanical or chemical agents can help overcome dormancy by altering the seed coat or improving the internal physiological conditions (Bewley *et al*., 2013). Seed pre-treatment methods are essential in promoting germination especially in plant species with hard coat or dormant seeds.

These treatment increases water uptake and stimulate enzymatic activities which contributes to a faster germination and a more consistent germination. This study aims to explore the use of varying time soaking as a pre-treatment method in enhancing the germination of *Citrus tangerina* seeds. The type of soil *Citrus tangerina* seeds are sown in also plays a vital role in the germination rate and seedling growth. Different soil types offers varying level of nutrients, moisture, and aeration, all of which affects seedling growth (Uddin *et al*., 2021) (Khan *et al*., 2019).

Mandarins are stored at 5 to 8 °C (41.0 to 46.4 °F) with 95% RH for periods up to 4 weeks. Chilling injury can occur in storage if temperatures fall < 5 °C (41 °F). Storage duration depends on variety, maturity and decay control. Thiabendazole (TBZ) can be incorporated into fruit coatings and used to control postharvest decays during storage (Peterson *et al*., 2006).

1.2 Justification and Significance of the Study

The germination and early growth of plants are significantly influenced by pre-treatment methods, particularly soaking seeds in various solutions. Citrus tangerina, commonly known as tangerine, is a commercially important citrus species with applications in fruit production, juice extraction, and medicinal uses (Iqbal et al., 2020). However, its seed dormancy and slow germination rate pose challenges to large-scale cultivation.

Pre-soaking treatments using water, nitric acid (HNO_3), and salt solutions are commonly employed to enhance germination by softening the seed coat, breaking dormancy, and improving water absorption (Baskin *and* Baskin, 2014). The selection of these treatments is based on their potential to optimize germination rates and seedling vigor in Citrus tangerina. Water serves as a control to determine the natural germination potential, while HNO_3 mimics the natural scarification process that seeds undergo in acidic soil conditions (Dhoran *and* Gudadhe, 2012). Salt solutions are included to evaluate the species' tolerance to salinity stress, which is critical for assessing its adaptability to different soil conditions (Munns *and* Tester, 2008).

Thus, this study is justified as it seeks to provide an effective pre-soaking method that can enhance the agricultural and horticultural productivity of Citrus tangerina by improving seed germination and early seedling development.

1.2.1 Significance of the Study

This research holds practical and scientific importance in the following ways:

i. Enhanced Germination and Seedling Growth – By identifying the most effective soaking treatment, this study can contribute to improved seed viability and early growth, leading to higher crop yields (Bewley et al., 2013).

ii. Agricultural and Horticultural Applications – Farmers and horticulturists can use the findings to optimize seed treatment protocols for Citrus tangerina, improving germination success rates in nurseries and plantations (Hartmann et al., 2018).

iii. Understanding Seed Coat Dormancy Mechanisms – Investigating the role of different soaking solutions provides insights into the physiological and biochemical processes governing dormancy and germination in citrus seeds (Koornneef et al., 2002).

iv. Salinity Tolerance Assessment – Given the increasing salinization of agricultural lands, evaluating the effects of salt solutions on seed germination can help determine the species' resilience to saline environments (Munns *and* Tester, 2008).

v. Contribution to Citrus Research – The study adds to the existing body of knowledge on citrus seed physiology, particularly the impact of chemical treatments on dormancy release and seedling vigor, which may have implications for breeding and conservation efforts (Albrecht et al., 2012).

1.3 Citrus tangerina

Citrus tangerina, commonly known as tangerine, is a species in the Citrus reticulata group within the Rutaceae family. It is a variety of mandarin, recognized for its sweet, tangy flavor, and easy-to-peel skin. Tangerines are widely cultivated across tropical and subtropical regions, with China being the largest producer. The fruit's high popularity is attributed to its refreshing taste, nutritional benefits, and ease of consumption.

Tangerines are believed to have originated in Southeast Asia, with early cultivation records dating back to China and Japan. The fruit is closely related to other mandarin varieties, and it was spread to the Mediterranean and eventually to the Americas through trade routes in the 19th century. Today, tangerines are grown extensively in countries like China, the United States, Spain, and Brazil (FAO, 2021).

The tree requires a warm climate, abundant sunlight, and well-drained soil for optimal growth. It thrives in subtropical and tropical environments, but it is sensitive to frost and extreme cold temperatures.

Tangerines are a vital crop in the global citrus industry, particularly in juice production and as fresh fruit for consumption. The global production of tangerines has increased significantly, reaching over 42 million tonnes in 2021 (FAO, 2021). In addition to their fresh fruit market, tangerines are also processed into juices, marmalades, and essential oils, which are used in cosmetics and pharmaceuticals due to their beneficial properties.

Tangerines hold cultural significance in many regions, especially in East Asia, where they are associated with prosperity and good fortune. During the Chinese New Year, tangerines are often gifted as symbols of wealth and happiness.

1.3.1 Taxonomy and classification of *citrus tangerina*

The genus *Citrus tangerina*, which belongs to the Rutaceae family, consists of different forms such as trees, shrubs, and herbs in the world. Citrus is the most cultivated and traded fruit variety in the world as a garden plant and one of the most important commercial fruit crops grown on all continents of the world (Turan and Mammadov, 2021). Historically, tangerines have been classified as a distinct species under the name *Citrus tangerina* (Tanaka, 1954). However, recent phylogenetic studies have suggested that they are better classified as a subspecies or variety of Citrus reticulata, which includes various mandarin oranges (Scora, 1975). The differentiation between tangerines and other mandarins is largely morphological, with tangerines generally possessing a thinner peel and being smaller in size compared to other varieties of mandarin oranges. *Citrus tangerina* is generally classified within the species Citrus reticulata, which includes mandarins. Taxonomic studies have often debated the classification of tangerines due to hybridization with other citrus species. Citrus taxonomy is complicated because of the frequent hybridization events that occur between species, leading to the current understanding that tangerines are a type of mandarin (Curk *et al*., 2016).

The taxonomic classification of *Citrus tangerina* (commonly known as tangerine) is as follows:

Kingdom: Plantae

Subkingdom: Viridiplantae

Infra-kingdom: Streptophyta

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Sapindales

Family: Rutaceae

Genus: *Citrus*

Species: *Citrus reticulata*

Variety/Subspecies: *Citrus tangerina*

Citrus tangerina is often treated as a subspecies or cultivar of Citrus reticulata due to their close genetic relationship and shared characteristics (Gmitter *et al*., 2012).

There is significant hybridization among citrus species, which complicates the taxonomy. Modern genetic studies have reclassified many varieties based on molecular evidence (Scora, 1975).

Citrus tangerina (Tangerine is sometimes classified as a subspecies or variety of Citrus reticulata, but *Citrus tangerina* is often used as a synonym). In modern taxonomic treatments, tangerines are frequently considered part of Citrus reticulata, which encompasses many mandarin types. Genetic research highlights the hybrid origin of tangerines, which have characteristics of both mandarins and other citrus types. Molecular markers and DNA barcoding have helped clarify their phylogeny, suggesting complex relationships between Citrus reticulata, Citrus sinensis (sweet oranges), and Citrus paradisi (grapefruit) (Wu *et al*., 2018). It is grown especially in tropical and subtropical regions and some countries of the Mediterranean Basin such as Greece, Italy, Spain, Tunisia and Turkey, as well as important citrus producers, in regions with Mediterranean climate such as Australia, California, Florida, and South Africa (Zou *et al*., 2016; Amutha *et al*., 2017; Vitale *et al*., 2021). Although most researchers say that the homeland of citrus is South East Asia, it is claimed that the origin of citrus fruits is not known with certainty (Okwu, 2008).

1.3.2 Morphology of *Citrus tangerina*

Citrus tangerina, commonly known as tangerine, is a small, evergreen tree or shrub from the Rutaceae family. The tree produces sweet, aromatic fruits and is widely cultivated for both its nutritional and economic value.it typically grows as a small Medium-sized tree, reaching heights up to 4–6 meters, with a dense, rounded canopy.Brown trunk, with a smooth to slightly rough texture. Branches often have small thorns. Leaves are simple, evergreen, and alternately arranged. Having Lanceolate or oblong shape, with a length of 5–8 cm and a width of 2–4 cm. Texture of leaves are usually Leathery and glossy green with slightly serrated edges. Petioles are Short, winged, characteristic of the citrus genus. Flowers are Small, white, and fragrant, measuring 2–3 cm in diameter. Having Five, waxy, and thick petals. With Numerous stamens, surrounding a single pistil. Bisexual in nature, capable of self-pollination, though cross-pollination enhances fruit quality. Flowers are borne singly or in small clusters (cymes). Fruits are globular to slightly oblate, 5–10 cm in diameter. With bright orangethin peel, easy to separate from the flesh, with a pebbled texture. Flesh is divided into 8–12 juicy, orange-colored segments. Sweet-tangy in flavor, with high juice content. Having oval seeds, small, and white to pale green inside. Seedless varieties are also common. Having Shallow, fibrous root system. Suited for water absorption in well-drained soils, typical of tropical and subtropical climates. Moderate growth rate, thrives in full sunlight, and requires regular pruning to maintain shape and productivity. (Scora, R. W. (1975); Gmitter, F. G., *et al*. (2012); FAO. (2021); Etebu, E., and Nwauzoma, A. B. (2014).)

1.3.3 Distribution

Citrus tangerina (family: Rutaceae) is a small to medium-sized fruit tree presumed to be native to southern China. It spread to Japan, Korea, India, Brazil, and the temperate citrus growing regions in the US at least 2000 years ago. Its fruit is minneola, also known as tangelo, and hybrids with other species produce clementine and satsuma. Tangerine scion cultivars were first introduced to Brazil from the US in 1914. Other introductions occurred, primarily from Japan and Europe during the 1920s and 1930s, resulting in an extensive collection of tangerine genetic resources in Brazil. The productive and high-quality cultivars from Japan and Spain were reliably identified for decades (Aparecida Castilho Maro *et al*., 2018).

Recent advances in DNA sequencing technologies have made genome-scale polymorphism analysis easy and efficient. With a focus on tangerine, fruit trees with high economic or cultural importance, and good genetic resources, 15 tangerine cultivars in Brazil, Japan, and Spain were sequenced individually using single-molecule real-time sequencing complemented with Illumina sequencing. Based on 115,000 highly confident genome-wide SNPs, tangerine cultivars were phylogenetically analyzed, and important findings regarding the tangerine global distribution pattern in Brazil, Japan, and Spain were revealed (A. Figueira *et al*., 2020).

1.3.4 Historical and Geographic Origins.

Tangerine (*Citrus tangerina*) is one of the important fruit crops worldwide. It is a group of citrus fruits characterized by a loose rind and easy separation from the segments. The cultivated tangerines include two major groups: the early ripening “mandarin” group and the late ripening “king” group. Tangerine fruits are highly appreciated by consumers for their sweet taste, juicy texture, and rich source of vitamins. Besides, tangerine trees can grow well even in cold and dry areas, making them suitable for cultivation in a variety of regions. As a result, tangerine fruits are continuously successful in being planted in new geographical regions. In particular, the Mediterranean region is one of the recently expanded planting areas of tangerine fruits. The cultivation of tangerine fruits in this region was observed as early as the 1800s. However, the exact timing and mode of introduction and spread of tangerine fruits in this region are still unclear. Understanding the historical spread of tangerine fruits in new regions could contribute to better management of citriculture under drastic environmental changes. In order to investigate the historical spread of tangerine fruits, historical documents describing the first observations and introductions of tangerine fruits in the Mediterranean region were collected and analyzed. In addition, the planting area of tangerine fruits in the Mediterranean region was examined based on recent literature and reports. The results suggest that tangerine fruits were introduced in the Mediterranean region multiple times through different pathways. The earliest introduction of tangerine fruits to the Mediterranean region was likely in Spain around 1804. After this event, tangerine fruits were spread widely in the Mediterranean region (Albert Wu *et al*., 2021). Later, tangerine fruits were introduced to many other countries in the Mediterranean region, such as Italy, France, and Morocco.

1.3.5. Mechanism of Seed Dispersal and Introduction of *Citrus tangerina*

1. Natural-Mediated Introduction

i. Animal Dispersal: The sweet and aromatic fruit attracts animals, including birds and mammals. Animals consume the fruit and excrete the seeds elsewhere, aiding in natural dispersal (Scora, 1975).

ii. Gravity (Barochory): Mature fruits naturally fall from the tree, allowing seeds to germinate near the parent plant if environmental conditions are favorable.

iii. Water Dispersal (Hydrochory): In regions near water bodies, seeds may be transported by flowing water, extending their dispersal range.

2. Human-Mediated Introduction

i. Agricultural Expansion: Humans have played a significant role in the global spread of *Citrus tangerina*. It was first domesticated in Southeast Asia and later introduced to the Mediterranean, Africa, and the Americas through trade and agricultural practices (Etebu and Nwauzoma, 2014).

ii. Commercial Cultivation: The fruit's high market demand for fresh consumption and processed products (juices, oils) has led to its deliberate cultivation across tropical and subtropical regions.

iii. Colonial Trade Routes: European colonization and trade in the 19th century facilitated the introduction of citrus varieties, including tangerines, to new regions like North and South America (Gmitter *et al*., 2012).

1.3.6 Habitat of *Citrus tangerina*

Citrus tangerina is a petite evergreen tree characterized by its lush, dark green leaves that possess a leathery texture and delightful fragrance. The tree produces flowers that range from white to a pale yellowish hue, emitting a pleasant aroma; these flowers can be found either alone or in charming clusters. The fruits of *Citrus tangerina* resemble those of Citrus reticulata, although they are typically smaller, featuring a pebbly surface and a thin rind that is notably aromatic. Each fruit contains between seven to ten wedges filled with juicy pulp, separated by a delicate, transparent, membranous skin. When hybridized with seed-bearing citrus varieties, these tangerines often yield an abundance of seeds. Originating from tropical or subtropical regions of Asia, the wild tangerine trees flourish in the forests of South China, where the cultivation of tangerines began. When observed by Papas, the tangerine exhibited its stature as a sizable tree adorned with alternate, petiolate leaves that are oblong-elliptic or lanceolate, exhibiting serrulate or sinuate edges, all bathed in a dark green hue. (Albert Wu *et al*., 2021) (Aparecida Castilho Maro *et al*., 2018).

1.3 7. Genetic Studies of *Citrus tangerina*

Modern genetic research on Citrus species, including tangerines, has shed light on the complexities of their origins. Studies using molecular markers have demonstrated that tangerines, like most citrus fruits, are hybrid species. Many tangerines have a mixed heritage, often involving introgression from other Citrus species such as pomelo (Citrus maxima) and sweet orange (Citrus sinensis) (Nicolosi *et al*., 2000). These findings have led to a more unified view of citrus taxonomy, blurring the lines between distinct species and varieties (Wu *et al*., 2014). Breeding programs aimed at developing new tangerine varieties focus on enhancing fruit quality, disease resistance, and environmental resilience. Advances in molecular markers have accelerated these efforts (Gmitter and Hu, 1990). Additionally, new genetic engineering technologies like CRISPR/Cas9 are being applied to improve citrus crops, including tangerines (Dutt *et al*., 2021).

1.3.8 Physiology and Phenology Associations and Environmental Requirements

Understanding the physiology and phenology associations and environmental requirements is crucial for the introduction and success of new planting crops and species in fresh fruit export. Tangerine, *Citrus tangerina*, introduced in 1933, is a slow growing plant that becomes 6 to 8 years productive but remains 2.5 to 3 years non-productive after planting. The experiment was conducted to characterize tangerina sprouting (budding) and flowering (flowering) in productivity and non-productivity years and to find out the association with environmental parameters such as temperature, humidity and sunshine duration (Lin *et al*., 2016).

In a productive year, the highest sprouting (budding) or flowering (flowering) percentage was 76.8% (2nd March) and the lowest was 5.40% (8th May) with 14.37oC minimum temperature. In a non-productive year, the highest sprouting (budding) or flowering (flowering) percentage was 56.63% (27th March) and the lowest was 3.56% (4th May) with 15.26oC minimum temperature. This indicated that sprouting (budding) and flowering varies with time, and it was earlier in a productive year than in a non-productive year (GAMBETTA ROMASO *et al*., 2015). The experiment also revealed that flowering (flowering) is significantly correlated with minimum temperature, humidity and sunshine duration but budding (budding) do not show any significant correlation with environmental parameters.

1.3.9 Cultivation and Propagation

Citrus tangerina is widely cultivated in subtropical regions due to its adaptability to different soil types and climates. Agronomic studies focus on improving yield, pest management, and soil conditions. For instance, (Zekri and Obreza, 2018) discussed how optimal soil and nutrient management strategies have a significant impact on fruit yield and quality. Tangerines are cultivated in many tropical and subtropical regions worldwide. They require warm climates and are typically grown in regions such as the Mediterranean, South America, and the southeastern United States (Ferguson, 2004). Propagation is mainly by grafting, ensuring uniformity in fruit production. The ease of peelability and high sugar content makes them a desirable crop for both commercial and personal cultivation. Studies on soil requirements have shown that tangerines thrive in well-drained sandy loam soils, which prevent root diseases common in citrus plants (Jackson, 1999). Citrus crops, including *Citrus tangerina*, are prone to diseases like huanglongbing (citrus greening). This disease is spread by the Asian citrus psyllid (Diaphorina citri), which has caused significant damage to citrus production globally. Bové (2006) reviewed the devastation caused by this disease and outlined integrated pest management techniques.

1.3.10. Nutritional and Medicinal Properties

Tangerines are rich in essential nutrients, particularly vitamin C, dietary fiber, and several bioactive compounds. Studies emphasize the fruit's high content of flavonoids, phenolics, and carotenoids, which are linked to various health benefits, including antioxidant and anti-inflammatory effects (Patil *et al*., 2009). The phytochemical composition of *Citrus tangerina* has been widely studied. Tangerine peel contains a significant amount of flavonoids, including hesperidin, naringin, and polymethoxylated flavones, which have shown antioxidant properties and potential for reducing cholesterol levels (Lv *et al*., 2015). Tangerines are known for their rich content of essential nutrients, especially vitamin C, fiber, and flavonoids. These compounds contribute to their antioxidant properties, which have been linked to potential health benefits, such as improved immune function, reduced oxidative stress, and lowered risk of chronic diseases (Wilcox *et al*., 2009). In traditional Chinese medicine, tangerine peels are used as part of herbal remedies to treat indigestion and improve respiratory function (Li, 2006). Research has demonstrated that tangerines have high antioxidant activity due to their phenolic and flavonoid content. These antioxidants help combat oxidative stress, which is linked to chronic diseases like cardiovascular diseases and cancer (Kim *et al*., 2003). Some studies have focused on the metabolic effects of tangerine extracts, suggesting their potential in reducing obesity and managing metabolic disorders. Mulvihill *et al*. (2011) found that the polymethoxylated flavones in tangerine peel can lower cholesterol and influence fat metabolism. Limonoids and flavonoids found in tangerines have been shown to possess anticancer properties. Research has shown that these compounds inhibit cancer cell proliferation, particularly in colon and breast cancer cell lines (Shen *et al*., 2017).

1.3.11. Postharvest Handling and Processing

Storage and Preservation: Tangerines are susceptible to postharvest losses due to dehydration and spoilage. Ali *et al*. (2019) investigated methods such as the use of edible coatings and modified atmosphere packaging to extend the shelf life of citrus fruits, including tangerines.

Byproducts and Value-Added Products: Tangerine byproducts, such as the peel, are increasingly being used in various industries. Zhao *et al*. (2020) highlighted the antimicrobial and preservative properties of tangerine peel extracts, suggesting their potential applications in food preservation and the cosmetic industry.

1.3.12. Environmental and Sustainability Research

Climate Impact: Research into the environmental impact of climate change on tangerine cultivation highlights challenges such as increased temperature, water scarcity, and the proliferation of pests. Adaptation strategies, such as breeding more resilient varieties and implementing water-efficient irrigation practices, are being explored (Hodgson, 2017).

Sstainable Agriculture: In response to environmental concerns, there has been a focus on adopting sustainable farming practices in citrus production. Studies by Jayaprakash *et al*. (2020) explored organic farming,integrated pest management, and the use of environmentally friendly inputs to minimize the ecological footprint of citrus farming.

1.3.13. The Economic, Nutritional, and Medicinal Values of *Citrus tangerina*

Citrus tangerina, commonly known as tangerine, is a fruit from the citrus genus of the Rutaceae family. Recognized for its distinctive flavor, easy-to-peel rind, and vibrant color, tangerine has gained significant attention both as a fresh fruit and as an ingredient in various food products worldwide. Beyond its culinary appeal, this fruit boasts substantial economic, nutritional, and medicinal value, making it an important component of global agriculture and healthcare. This essay explores the economic contributions of *Citrus tangerina*, its rich nutritional profile, and its potential medicinal applications.

i. Economic Importance of *Citrus tangerina*

Citrus tangerina plays a crucial role in the global economy, particularly in the agricultural and food industries. As one of the most widely cultivated citrus species, it contributes significantly to the agricultural output of several countries. Tangerine cultivation is vital in regions such as China, Spain, the United States, and Brazil, where it thrives in subtropical and tropical climates (FAO, 2020).

ii. Global Production and Trade

The global production of tangerines has witnessed a steady rise in recent decades. According to the Food and Agriculture Organization (FAO), the global output of tangerines in 2018 was approximately 30 million metric tons, with China being the leading producer, contributing around 60% of the total production (FAO, 2020). In addition to domestic consumption, tangerines are a significant export product, particularly for countries like Spain and the United States, where they are grown for both fresh markets and processing industries. Tangerines are commonly used in the production of juices, canned fruits, jams, and marmalades, which adds substantial value to the global food industry. The growing demand for citrus-based beverages has spurred innovations in processing technologies and market expansion. As the market for organic and health-oriented food products continues to grow, the economic value of tangerines, especially organic varieties, is also increasing (McDaniel, 2019).

iii. Employment and Rural Development

The cultivation of *Citrus tangerina* provides substantial employment opportunities in rural areas. In countries with significant citrus production, such as Spain, Brazil, and Turkey, tangerine farming supports local economies by creating jobs in farming, processing, packaging, and distribution sectors (Santana *et al.*, 2018). The labor-intensive nature of citrus farming, coupled with the seasonal demand for harvesting, provides seasonal employment opportunities for thousands of workers, particularly in developing nations. In addition to direct employment, tangerine farming also fosters infrastructure development in rural regions, contributing to better roads, transportation systems, and improved access to markets for other agricultural products (FAO, 2020).

iv. Nutritional Benefits of *Citrus tangerina*

Tangerines are not only economically valuable but also nutritionally rich. They are an excellent source of vitamins, minerals, dietary fiber, and antioxidants, which contribute to their popularity as a healthy food choice.

v. Vitamin C Content

One of the most notable nutritional aspects of tangerines is their high vitamin C content. Vitamin C, or ascorbic acid, is an essential nutrient that plays a crucial role in immune function, wound healing, and the maintenance of healthy skin (Liu *et al*., 2019). A medium-sized tangerine (approximately 150 grams) provides about 40% of the recommended daily intake of vitamin C (USDA, 2020). Regular consumption of vitamin C-rich foods like tangerines helps reduce the risk of chronic diseases, such as heart disease and cancer, by combating oxidative stress and inflammation in the body (Chong *et al*., 2019). Vitamin C also enhances iron absorption, making tangerines a valuable addition to vegetarian and vegan diets.

vi. Dietary Fiber

Tangerines are an excellent source of dietary fiber, particularly soluble fiber, which contributes to digestive health. A single tangerine contains about 1.8 grams of fiber, a significant portion of the daily recommended intake (USDA, 2020). Fiber is known to promote healthy digestion, prevent constipation, and support a balanced gut microbiota (Slavin, 2013). Additionally, fiber helps regulate blood sugar levels and lowers the risk of developing type 2 diabetes by slowing the absorption of glucose in the bloodstream (Basu *et al*., 2017).

vii. Antioxidants and Phytochemicals

Tangerines contain a variety of antioxidants, including flavonoids, carotenoids, and phenolic compounds, which are beneficial for protecting the body from oxidative damage. One of the primary antioxidants in tangerines is hesperidin, a flavonoid that has been shown to have anti-inflammatory, antioxidant, and anticancer properties (Nunez-Sanchez *et al*., 2020). Carotenoids, such as beta-carotene, are precursors of vitamin A and contribute to the fruit's vibrant orange color. These compounds play a vital role in promoting eye health and preventing age-related macular degeneration (Tan *et al*., 2017). Additionally, tangerines are rich in potassium, a mineral that is essential for maintaining proper heart function and blood pressure regulation (Padilla *et al*., 2018). Regular consumption of potassium-rich foods like tangerines is associated with a reduced risk of hypertension and stroke.

viii. Medicinal Applications of *Citrus tangerina*

In addition to its nutritional benefits, *Citrus tangerina* has been traditionally used for its medicinal properties in various cultures. Modern scientific research has confirmed the potential health benefits of tangerine extracts and essential oils, supporting their use in complementary and alternative medicine.

ix. Anticancer Properties

Studies have suggested that the bioactive compounds in tangerines, particularly flavonoids like hesperidin and naringin, have anticancer properties. These compounds have been shown to inhibit the growth of cancer cells and reduce the spread of tumors in various types of cancer, including breast, lung, and colon cancer (Rastogi *et al*., 2018). Hesperidin, in particular, has demonstrated the ability to induce apoptosis (programmed cell death) in cancer cells without affecting healthy cells, making it a promising candidate for cancer treatment (Nunez-Sanchez *et al*., 2020).

x. Antimicrobial and Anti-inflammatory Effects

Tangerine essential oil, which is derived from the peel of the fruit, has antimicrobial and anti-inflammatory properties. Several studies have found that tangerine oil exhibits strong antimicrobial activity against a range of bacteria and fungi, including those responsible for foodborne illnesses and infections (Trombetta *et al*., 2018). Furthermore, tangerine oil has been shown to reduce inflammation, making it useful in managing conditions such as arthritis and other inflammatory disorders (Rios *et al*., 2017).

xi. Anxiety and Stress Relief

Tangerine essential oil is also used in aromatherapy for its calming and stress-relieving effects. Research has demonstrated that inhalation of tangerine essential oil can reduce levels of anxiety and promote relaxation (Goh *et al*., 2018). This makes it a valuable natural remedy for managing stress, anxiety, and sleep disorders.

xii. Liver Health

Some studies have indicated that tangerine extracts may have hepatoprotective effects, helping to protect the liver from damage caused by toxins and oxidative stress. Research has shown that compounds found in tangerine peels can improve liver function and reduce the accumulation of fat in the liver, which is crucial for preventing non-alcoholic fatty liver disease (NAFLD) (Zhao *et al*., 2020).

1.4 Literature Review

1. Seed Dormancy and Germination in Citrus Species

Seed dormancy is a common issue in citrus species, including Citrus tangerina, which affects germination uniformity and propagation efficiency (Baskin *and* Baskin, 2014). The dormancy is often attributed to the hard seed coat that restricts water and oxygen uptake, as well as physiological inhibitors present in the seed (Koornneef et al., 2002). Various pre-treatment methods, such as soaking in water, acid scarification, and salt stress testing, have been explored to improve germination rates in citrus seeds.

1.4.2. Effects of Soaking in Water on Seed Germination

Hydration is a crucial step in seed germination as it activates metabolic processes and enzyme activity essential for embryo growth (Bewley et al., 2013). Several studies have shown that soaking citrus seeds in distilled water for extended periods (6–24 hours) can improve germination percentage and seedling vigor (Iqbal et al., 2020). However, prolonged soaking may lead to excessive water uptake, causing seed damage and reducing viability (Hartmann et al., 2018).

1.4.3. Acid Scarification Using Nitric Acid (HNO_3) for Dormancy Breaking

Chemical scarification using HNO_3 is an effective method to break seed coat dormancy in hard-seeded plants. Studies on citrus species suggest that treating seeds with HNO_3 helps to soften the seed coat, allowing better water absorption and gas exchange, leading to improved germination rates (Dhoran *and* Gudadhe, 2012). However, the effectiveness of HNO_3 treatment depends on concentration and exposure duration—excessive exposure may damage the seed embryo, while insufficient treatment may not adequately break dormancy (Albrecht et al., 2012).

1.4.4. Impact of Salt Stress (NaCl) on Germination and Seedling Growth

Salinity is a major environmental factor affecting seed germination, as high salt concentrations can lead to osmotic stress and ion toxicity (Munns *and* Tester, 2008). Research has shown that citrus seeds exposed to salt solutions exhibit reduced germination percentages, delayed germination, and stunted seedling growth due to the inhibitory effects of sodium ions (Parida *and* Das, 2005). Some studies indicate that low concentrations of salt may induce mild stress, potentially priming the seed for better stress tolerance in later growth stages (Khan et al., 2016). However, prolonged exposure to high salinity levels typically results in reduced vigor and poor seedling establishment.

1.4.5. Influence of Soaking Duration on Germination Efficiency

The duration of soaking plays a critical role in determining the effectiveness of pre-treatment methods. Studies on citrus seeds have indicated that short soaking durations (6–12 hours) can enhance germination without causing damage, while extended exposure (24 hours or more) may lead to seed coat rupture, leaching of essential nutrients, and microbial contamination (Bewley et al., 2013). The optimal soaking time varies depending on the treatment medium, as prolonged exposure to acid or salt solutions can have adverse effects on seed viability.

1.4.6. Comparative Studies on Different Pre-Treatment Methods

Comparative studies on citrus seed germination have shown that acid scarification using HNO_3 is often the most effective treatment for breaking dormancy and improving germination rates (Koornneef et al., 2002). Water soaking provides moderate benefits, while salt stress generally has a negative impact. The findings suggest that pre-sowing treatments should be carefully selected based on the desired outcome—whether to enhance germination, test salinity tolerance, or improve seedling vigor (Iqbal et al., 2020).

1.5 Aim and Objectives

Aim:

To evaluate the impact of soaking Citrus tangerina seeds in different mediums (water, HNO_3, and NaCl) for varied durations (6, 12, and 24 hours) on germination and seedling growth, identifying the most effective pre-treatment for improved propagation.

Objectives:

Assess the effect of soaking duration on seed germination.

Examine how different mediums influence germination rate, seedling vigor, and growth.

Determine the role of HNO_3 in breaking seed dormancy.

Analyze the impact of NaCl on salinity tolerance in seedlings.

Identify the optimal pre-soaking treatment for enhancing seed viability and early growth.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials and Equipment

The following materials were used for the purpose of the study. They include:

Distilled water, Petri-dish, No. 42 Whatman filter paper, Plastic can, Syringe, Bowl, Ruler, Forceps, Measuring cylinder, Beakers, Masking tape, Marker, Laboratory weighing scale, *citrus tangerina* seeds, tertrazolium chloride.

2.2 Methodology

2.2.1 Study location

This experiment was carried out on a laboratory bench at Pre-degree laboratory of the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

2.2.2 Laboratory Preparation and setup Preparation

The laboratory table, petri dish and forceps were thoroughly sterilized using methylated spirit before the commencement of the experiment. No. 42 Whatman filter paper was placed on the petri-dish and distilled water was added to keep the filter paper moderately wet.

2.2.3. Source of Seed

The seeds of *citrus tangerina* were collected from different local market sources, so as to increase the viability of the seeds due to location differences, such as physical or biological damages and then randomized in a bowl.

2.2.4 SEED COLLECTION AND SELECTION

Citrus tangerina seeds used in this study were collected from matured sweet and ripened fruits obtained locally from Uselu, Adolor and Ring road market. Only seeds that are uniform in size, plump and free from visible defects were used for this experiment to ensure consistency in the study. The fruit is gently pressed in a bowl of water so as to extract the seeds without any physical damage and then collected randomly in a randomized bowl to be sowed.

2.2.5 Viability Test

This test was carried out to determine if the seeds were suitable for planting, positive test results in a pink or red colouration of the inner layer of the seeds while negative test results in no colouration. The air dried seeds were randomized and 30 *citrus tangerina* seeds were picked, soaked overnight in distilled water, after which they were cut longitudinally to create equal halves. The seeds were then soak in 1% tetrazolium solution which was gotten by dissolving 1 gram of tetrazolium chloride with 100ml of distilled water. After two hours of soaking a pink colouration was observed in all 30 seed, therefore meaning a 100% positive viability test.

Plate 2.1: *Citrus tangerina* seeds cut longitudinally**.**

Plate 2.2: Set up for the Viability test.

Plate 2.3: Show the mixing process of the tetrazolium chloride solution.

Plate 2.4: pink colouration of the seed after 1 hour 30 minutes of soaking in tetrazolium solution indicating a positive viability test.

2.2.6 Preparation of Treatment

The different mediums for pretreatment were of three different liquids; water, Acid solution and salt solution were made. A bottle of distilled water, a 15 % of Nitric Acid solution and a 2% of table salt solution.

2.2.7. Seed Priming and Exposure

Viable, entire and non-blemished seeds for *Citrus tangerina* were soaked in water, Acid solution (15%) and Salt solution (2%) for 6, 12, 24 hours, until ready to use, the seeds were immediately removed and placed in an already prepared petri-dish with moistured No. 42 Whatman filter paper. The set-up was placed on the laboratory bench and observed for at least six weeks.

2.3 Seed Priming

2.3.1 Hydropriming

I. Transparent disposable cup was filled half way with distilled water.

ii. The weight of the Citrus tangerina seed was taken before submerging it in the water.

iii. The seed were allowed to soak for 6 hours, 12 hour and 24 hours respectively.

iv. The seeds were removed from the water and patted dry using a paper towel.

v. The weight of the seeds after soaking (Imbibition) was taken.

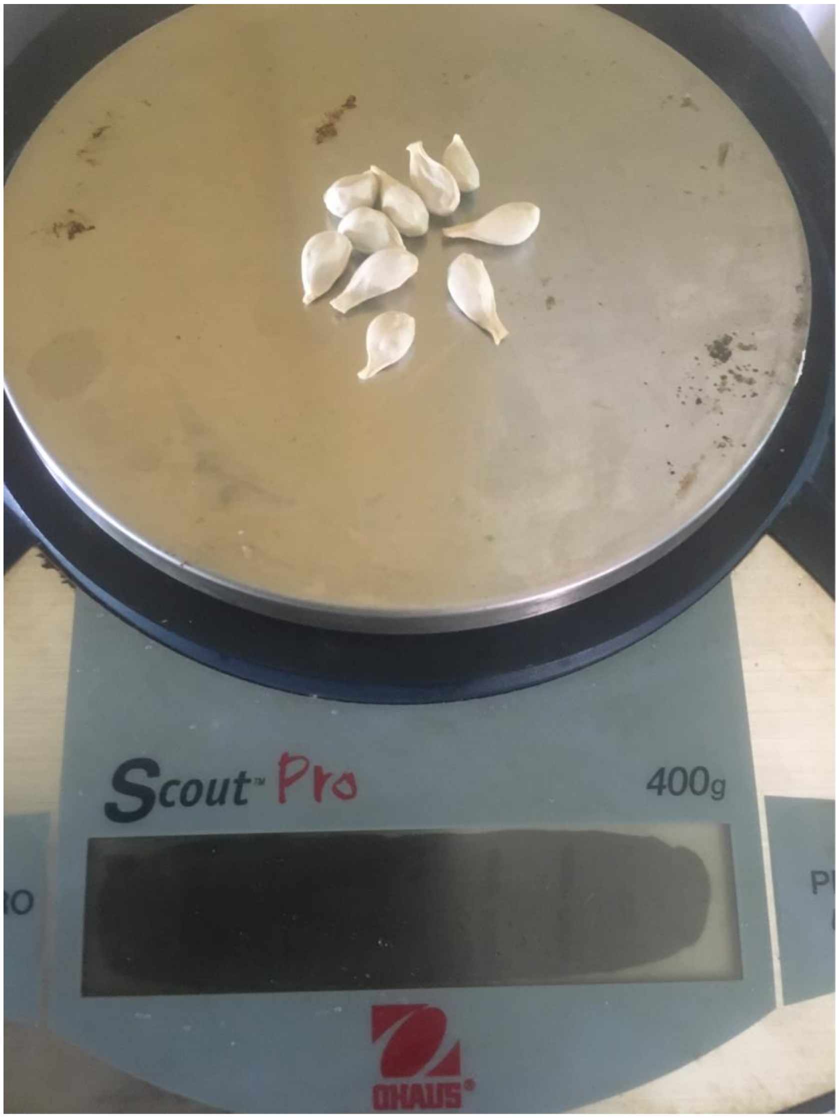

Plate 2.5: Showing the weight of seeds before Hydropriming.

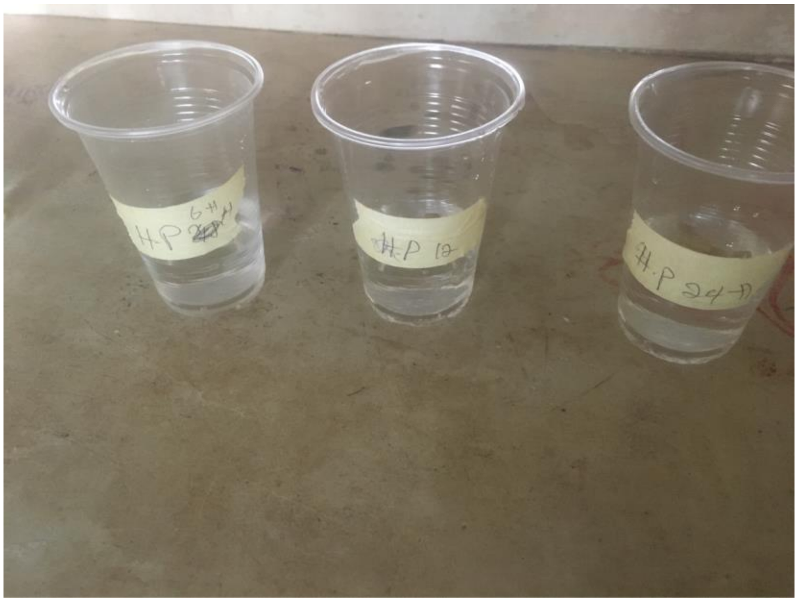

Plate 2.6: Shows the Hydropriming setup.

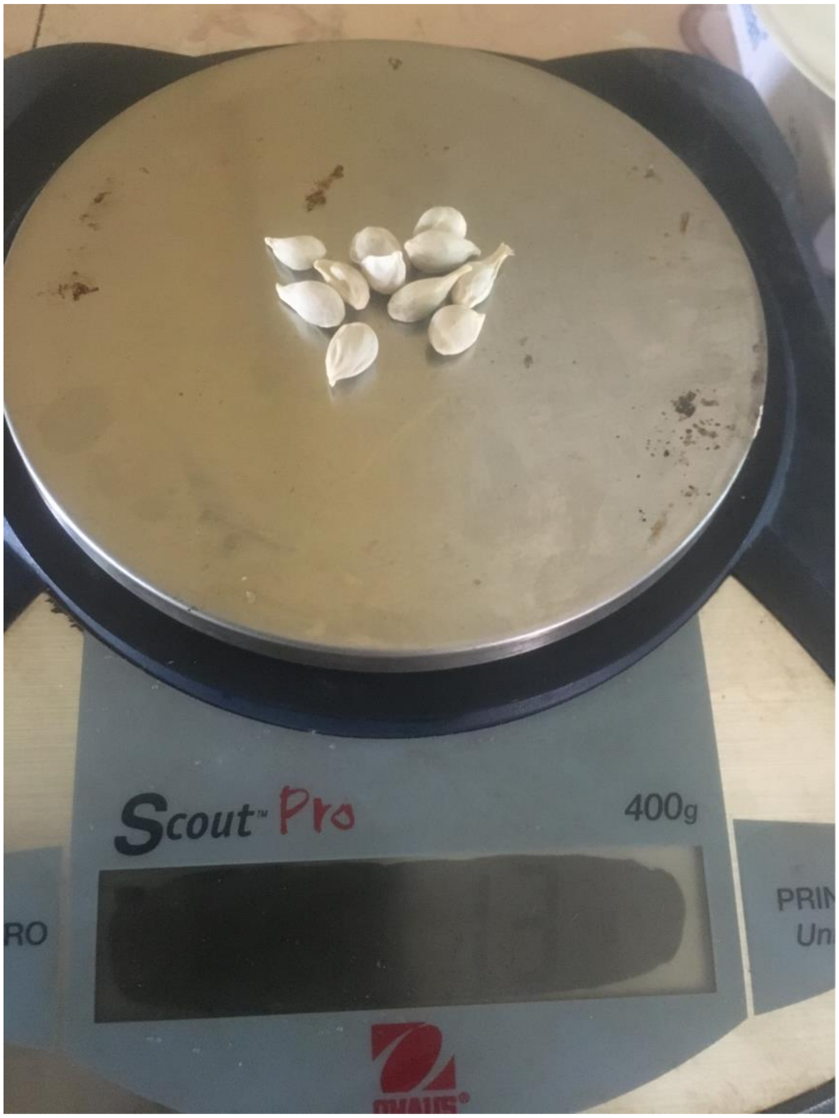

Plate 2.7: Shows the weight of seeds after Hydropriming.

2.3.2 Osmopriming

I. Transparent disposable cup was filled with 100ml distilled water and 2gram of table salt.

ii. The weight of the watermelon seed was taken before submerging it in the solution.

iii. The seed were allowed to soak for 12 hour and 24 hours respectively.

iv. The seeds were removed from the solution patted dry using a paper towel.

v. The weight of the seeds after soaking (Imbibition) was taken.

Plate 2.8: Shows the weight of salt for osmopriming.

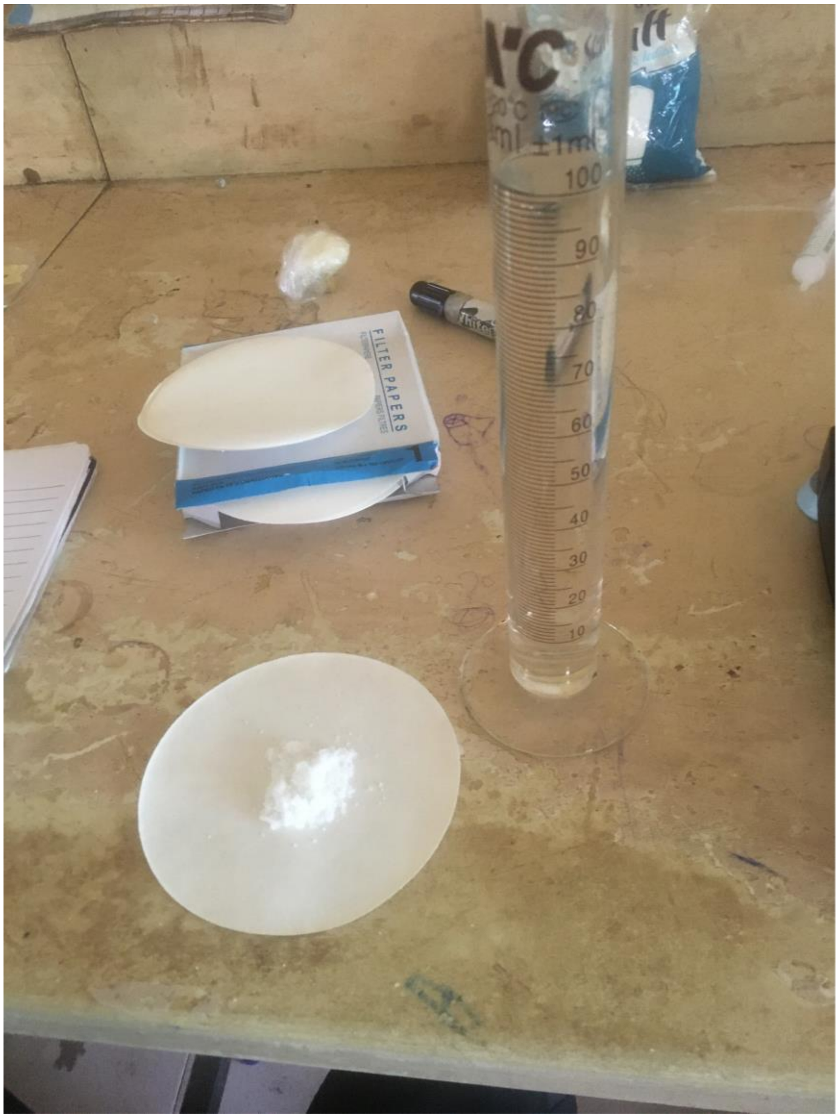

Plate 2.9: Shows the volume of water for osmopriming.

Plate 2.10: Shows the weight of seeds before osmopriming

Plate 2.11.: Shows the osmopriming setup.

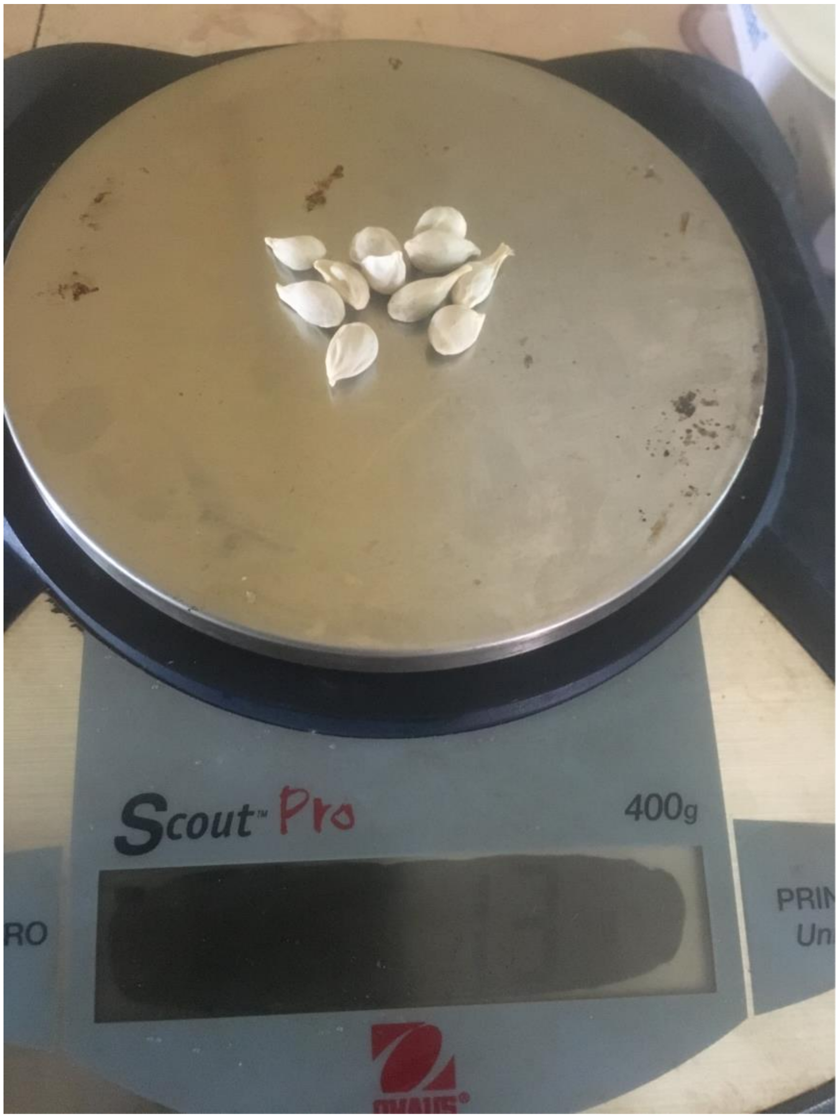

Plate 2.12: Shows the weight of seeds after Hydropriming.

2.3.3 Acid Priming

i. 85ml of distilled water was added to a beaker, followed by 15ml of Nitric acid (HNO_3).

ii. The weight of the watermelon seed was taken before submerging it in the acid solution.

iii. The seed were allowed to soak for 15 minutes and 30 minutes respectively.

iv. The seeds were removed from the acid solution and patted dry using a paper towel.

v. The weight of the seeds after soaking (Imbibition) was taken.

Plate 2.13: Shows the weight of seeds before Acid priming.

Plate 2.14: Shows the volume of Acid for Acid priming.

Plate 2.15: Shows the volume of water for Acid priming..

Plate 2.16: Shows the Acid priming setup.

Plate 2.17: Shows the weight of seeds after Acid priming.

2.4. Experimental Setup

The petri dishes after sterilization are arranged in rows on the laboratory bench and fitted with No. 42 Whatman filter paper and moisten. The seeds after randomly picked and treated are placed (10 seeds) in the petri dish setup by gently using a forcep or a spoon to arrange them. The environment is sterilized and the setup is covered with a mini net screen house.

Plate 2.18: Showing all the experimental setups.

Plate 2.19: Showing all the experimental setups in a mini screen house.

2.5. CONTROL GROUP

In addition to the seeds that would undergo pre-treatment processes, control group seeds will be there to provide a baseline for comparison. These control group seeds will not undergo any pre-treatment process and will be in the same experimental setup as the treated seeds. This control group will allow for a comparison of germination and seedling development between pre-treated and untreated seeds, highlighting the effects of pre-treatment processes.

Plate 2.20: Shows the control setup.

2.6 GERMINATION AND SEEDLING PARAMETERS

When studying germination performance in seeds, such as *Citrus tangerina* (Tangerine), it is essential to measure specific germination and seedling parameters that can provide insights into the success of germination treatments. These parameters allow us as researchers to assess the effectiveness of different methods and the overall health of emerging seedlings.

2.6.1. GERMINATION PARAMETERS

These parameters help to quantify the effectiveness of dormancy-breaking treatments and assess the success of seed germination.

a. Germination Percentage: The proportion of seeds that successfully germinate out of the total number of seeds sown. A higher germination percentage indicates effective dormancy breaking and a favorable environment for seed emergence.

Germination Percentage= (Total number of seeds sown / Number of germinated seeds) $\times 100$

2.6.2. SEEDLING PARAMETERS

Once the seeds have germinated, evaluating seedling growth parameters is essential to understand the impact of dormancy-breaking treatments on early plant development.

a. Seedling Height: The average height of seedlings measured at regular intervals (e.g., weekly). A good indicator of the overall vigor of the seedling. Taller, healthier seedlings are often associated with more successful dormancy-breaking treatments.

b. Root Length: The length of the primary root, measured after a certain period of growth. Reflects the seedling's ability to access water and nutrients from the soil. A well-developed root system is crucial for plant establishment and growth.

c. Shoot Length: The length of the seedling from the base to the tip of the first true leaves. Indicates the growth potential and energy allocation of the seedling, often influenced by the effectiveness of dormancy-breaking treatments

d. Number of Leaves: The total number of leaves produced by the seedling over a specific period. Leaf production is an important indicator of healthy seedling development and photosynthetic activity

Other observations such as Seedling Mortality Rate and Seedling Health can also be taken into consideration. The proportion of seedlings that do not survive after germination gives the seedling mortality rate. A lower mortality rate reflects a more effective dormancy-breaking treatment. Visual observations of seedling health, including the presence of any deformities, chlorosis (yellowing), or other stress symptoms showing the health of the seedlings.

2.7. MONITORING AND DATA COLLECTION

Germination is to be monitored daily. A seed is considered germinated when the radical emerges and is visible above the soil surface as in this case visible on the filter paper. The germination and Seedling parameters are to be recorded for each pre-treated seed in the different petri dishes and the mean germination time is to be recorded. Additionally, the seedlings' height, width and leaf number are to be measured weekly to assess growth performance.

2.8 DATA ANALYSIS

The germination rates and growth data are to be statistically analysed to determine the significance of the different pre-treatment methods on the germination success and early growth of *citrus tangerina* seeds. Comparative analysis between control and pre-treated plants are to be performed to assess the impact of pre-treatment methods. The results are to be interpreted based on sustainability indicators, including weight of seeds, length of radicle, germination percentage, plumule length, number of leaves, leaf length.

CHAPTER THREE

RESULT

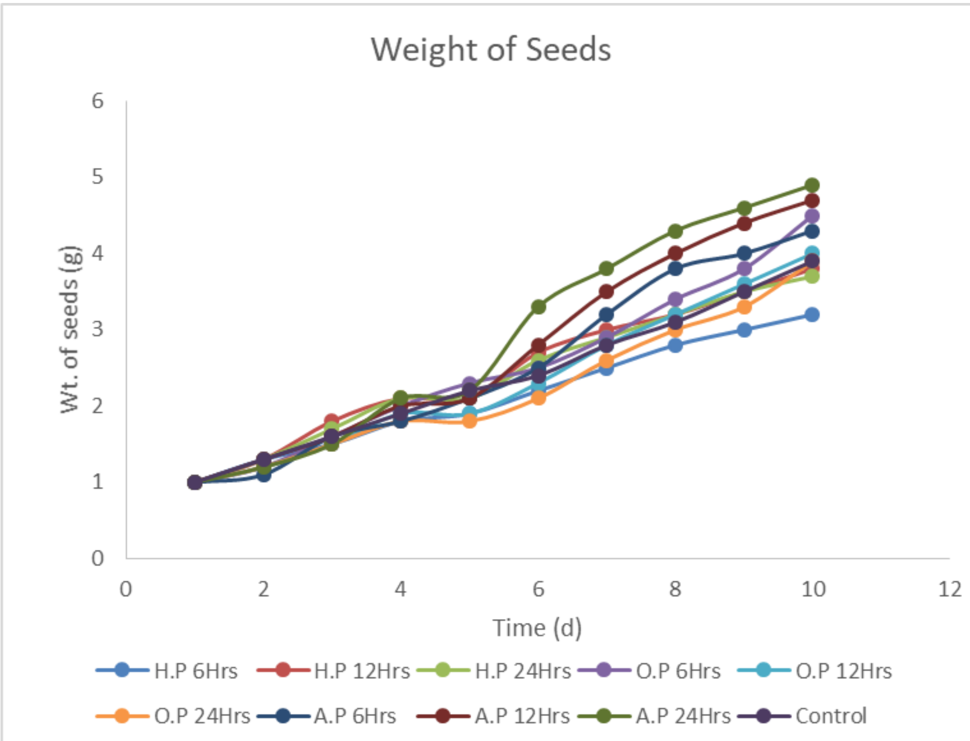

Figure 1: Seed weight (grams) of Citrus tangerina after exposure to priming regimens.

Keys:

H.P 6Hrs = Hydro priming 6 Hours

H.P 12Hrs = Hydro priming 12 Hours

H.P 24Hrs =Hydro priming 24 Hours

O.P 6Hrs = Osmopriming 6 Hours

O.P 12Hrs = Osmopriming 12 Hours

O.P 24Hrs = Osmopriming 24 Hours

A.P 6Hrs = Acid priming 6 Hours

A.P 12Hrs = Acid priming 12 Hours

A.P 24Hrs = Acid priming 24 Hours

In Figure 1, the result indicates the heavier seeds were found in Acid priming 24Hrs following the Acid priming 12Hrs, this was an indication of high water absorption by the seeds due to the more water permeability by the acid scarification. On the other hand, Osmopriming 12Hrs had the lighter seeds as at day 10, with the acid primed 24Hrs seeds reaching up to 4.9 grams.

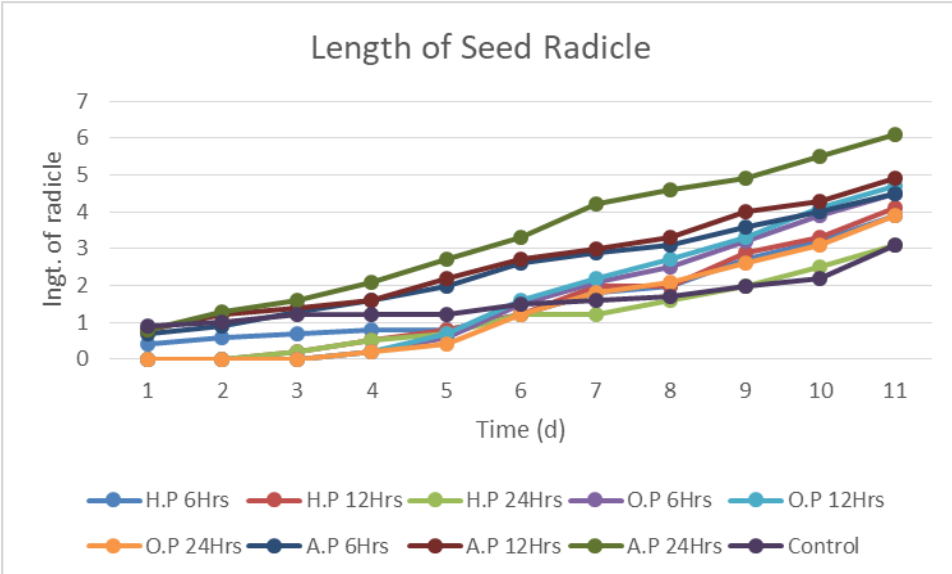

Figure 2: Length of Radicle (cm) of Citrus tangerina after exposure to priming regimens.

Keys:

H.P 6Hrs = Hydro priming 6 Hours

H.P 12Hrs = Hydro priming 12 Hours

H.P 24Hrs =Hydro priming 24 Hours

O.P 6Hrs = Osmopriming 6 Hours

O.P 12Hrs = Osmopriming 12 Hours

O.P 24Hrs = Osmopriming 24 Hours

A.P 6Hrs = Acid priming 6 Hours

A.P 12Hrs = Acid priming 12 Hours

A.P 24Hrs = Acid priming 24 Hours

In Figure 2, the result shows that the Acid priming 24Hrs had a steady and fast radicle growth rate while the Control showed a steady but slow radicle growth rate and the Osmopriming 24Hrs had a delay in radicle growth rate, with the acid priming 24Hrs reaching a length of 6.1cm and the least length being 3.1cm in control and Hydro priming 24Hrs.

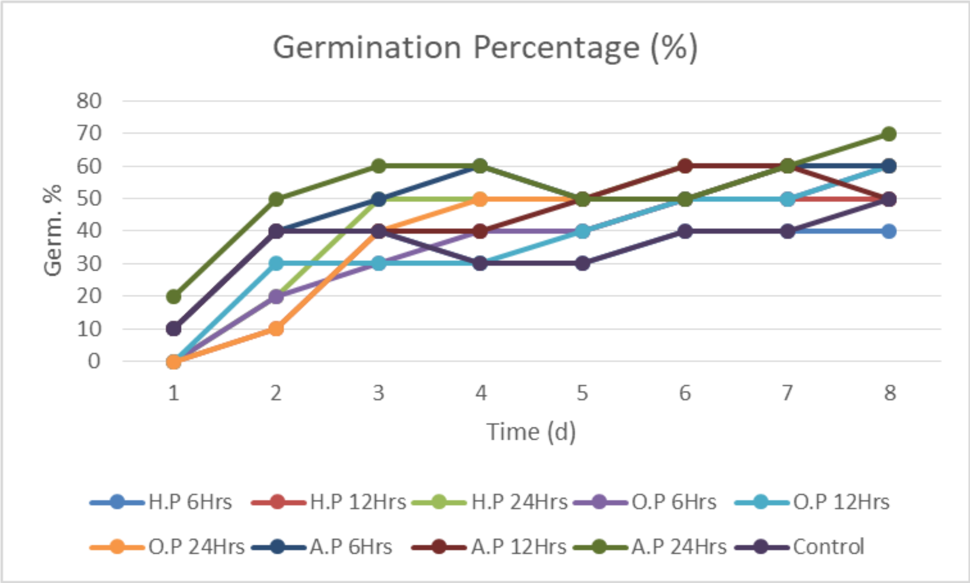

Figure 3: Germination percentage (%) of Citrus tangerina after exposure to priming regimens.

Keys:

H.P 6Hrs = Hydro priming 6 Hours

H.P 12Hrs = Hydro priming 12 Hours

H.P 24Hrs =Hydro priming 24 Hours

O.P 6Hrs = Osmopriming 6 Hours

O.P 12Hrs = Osmopriming 12 Hours

O.P 24Hrs = Osmopriming 24 Hours

A.P 6Hrs = Acid priming 6 Hours

A.P 12Hrs = Acid priming 12 Hours

A.P 24Hrs = Acid priming 24 Hours

Figure 3 shows an unstable germination result with the Acid priming 24Hrs having a good start in germination but later had a drop around day 5 and 6. Osmopriming 24Hrs had the lowest start up in germination. The highest germination performance was observed in Acid priming 24Hrs on day 8 with a 70 percent maximum germination rate.

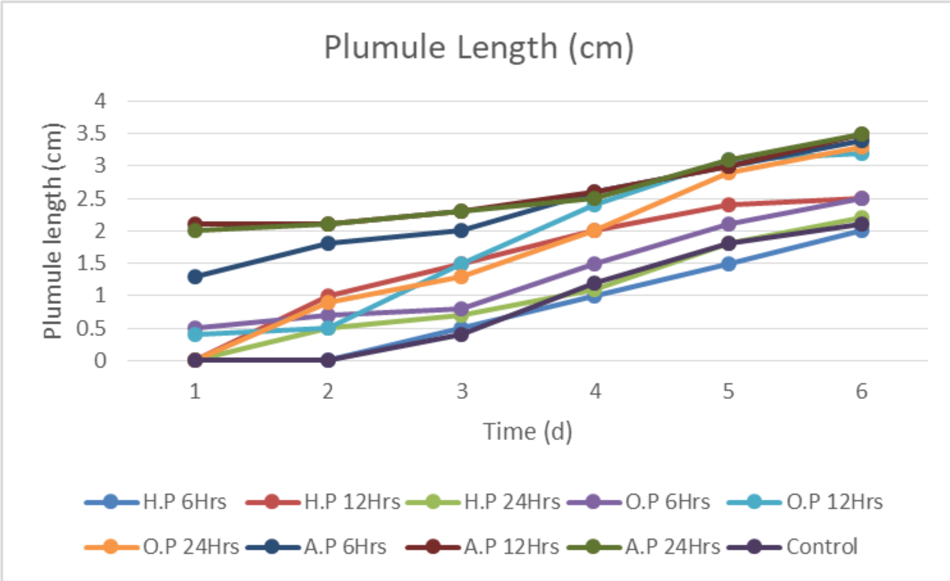

Figure 4: Plumule length (cm) of Citrus tangerina after exposure to priming regimens.

Keys:

H.P 6Hrs = Hydro priming 6 Hours

H.P 12Hrs = Hydro priming 12 Hours

H.P 24Hrs =Hydro priming 24 Hours

O.P 6Hrs = Osmopriming 6 Hours

O.P 12Hrs = Osmopriming 12 Hours

O.P 24Hrs = Osmopriming 24 Hours

A.P 6Hrs = Acid priming 6 Hours

A.P 12Hrs = Acid priming 12 Hours

A.P 24Hrs = Acid priming 24 Hours

In Figure 4, Acid priming 24Hrs and 12Hrs had a strong exponential increase in shoot growth which became steady overtime. Hydro priming 6 Hours and control had the slowest plumule growth reaching a maximum of 2 and 2.1 cm on Day 6, with the highest length all found in the Acid priming with the maximum being 3.5cm in both Acid priming 24Hrs and 12Hr.

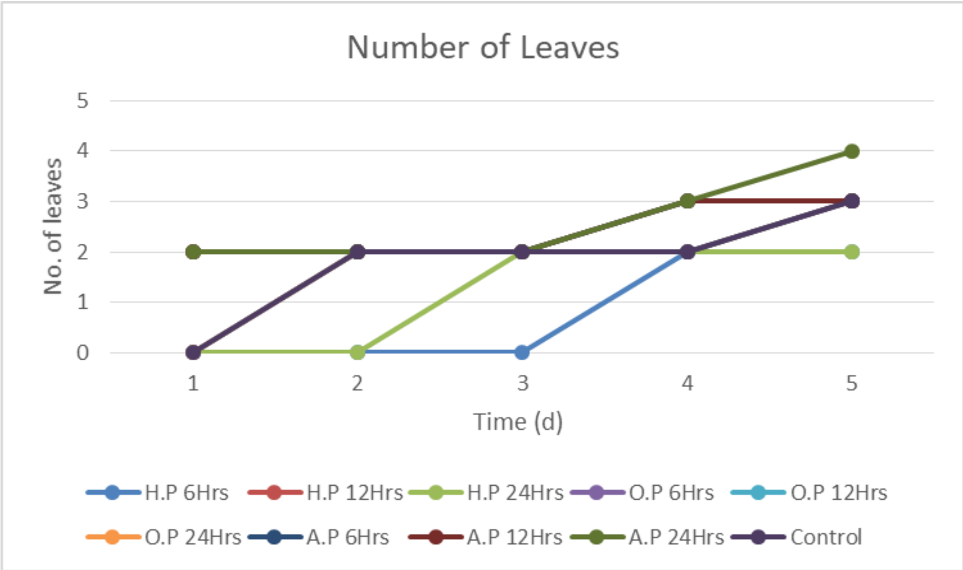

Figure 5: Number of leaves per plant of Citrus tangerina after exposure to priming regimens.

Keys:

H.P 6Hrs = Hydro priming 6 Hours

H.P 12Hrs = Hydro priming 12 Hours

H.P 24Hrs =Hydro priming 24 Hours

O.P 6Hrs = Osmopriming 6 Hours

O.P 12Hrs = Osmopriming 12 Hours

O.P 24Hrs = Osmopriming 24 Hours

A.P 6Hrs = Acid priming 6 Hours

A.P 12Hrs = Acid priming 12 Hours

A.P 24Hrs = Acid priming 24 Hours

Figure 5, shows a scattered pattern of leaf number due to a short period of time for the experiment, but regardless good observations were made in the Acid primed seeds with all starting off with two leaves on day 1 and the least increase found in the Hydro priming 6 Hours and 24 Hours , with a maximum number of 4 leaves attained by acid priming 24Hrs on Day 5.

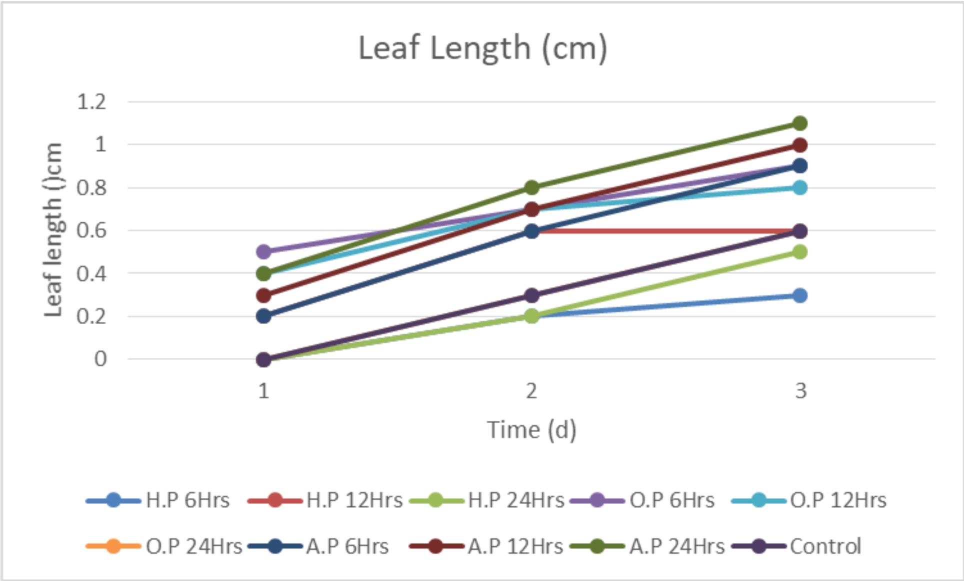

Figure 6: Length of leaves of Citrus tangerina after exposure to priming regimens.

Keys:

H.P 6Hrs = Hydro priming 6 Hours

H.P 12Hrs = Hydro priming 12 Hours

H.P 24Hrs =Hydro priming 24 Hours

O.P 6Hrs = Osmopriming 6 Hours

O.P 12Hrs = Osmopriming 12 Hours

O.P 24Hrs = Osmopriming 24 Hours

A.P 6Hrs = Acid priming 6 Hours

A.P 12Hrs = Acid priming 12 Hours

A.P 24Hrs = Acid priming 24 Hours

Figure 6, shows a random leaf growth pattern in all with Hydro priming 24Hrs, Osmopriming 12Hrs and control having the slowest growth rate. Acid priming 24Hrs had a more exponential growth rate and reaching the maximum leaf length of 1.1 cm on Day 3.

CHAPTER FOUR

DISCUSSION

The results of this study highlight the significant impact of different soaking treatments and exposure durations on the germination and early growth of Citrus tangerina seeds. Among the three treatment mediums—distilled water, nitric acid (HNO_3), and salt solution (NaCl)—the effects varied based on their ability to break dormancy, enhance water absorption, or induce stress.

1. Effect of Water Soaking:

Seeds soaked in distilled water for 12 to 24 hours showed moderate improvement in germination percentage and seedling vigor. Water serves as a natural activator, initiating metabolic processes necessary for germination (Bewley et al., 2013). However, prolonged soaking beyond 24 hours may lead to oxygen deprivation, which can slow down germination. This suggests that water soaking alone is beneficial but may not be the most effective dormancy-breaking method.

2. Effect of Nitric Acid (HNO_3) Soaking:

Nitric acid treatment significantly enhanced germination percentage and reduced mean germination time, particularly at 12 and 24 hours of exposure. This is attributed to its role in scarifying the seed coat, which facilitates water uptake and gas exchange (Baskin *and* Baskin, 2014). Previous studies on citrus species suggest that acid scarification mimics natural processes, such as passage through animal digestive tracts, which help break dormancy (Dhoran *and* Gudadhe, 2012). However, prolonged exposure to HNO_3 (above 24 hours) led to reduced germination, likely due to excessive seed coat degradation, which may have caused damage to the embryo.

3. Effect of Salt Solution (NaCl) Soaking:

Seeds exposed to NaCl solutions exhibited lower germination rates and reduced seedling vigor, particularly at higher exposure times (12 and 24 hours). This suggests that Citrus tangerina has limited tolerance to salinity during the germination phase. High salt concentrations create an osmotic effect, making water uptake difficult and leading to ion toxicity, which affects seed metabolism (Munns *and* Tester, 2008). The decline in root and shoot development in salt-treated seeds further supports the idea that salt stress negatively impacts early seedling growth.

Interaction Between Soaking Time and Treatment Medium:

The results indicate that the optimal soaking time depends on the treatment medium. For water and HNO_3, a 12-hour soaking period produced the best results, while prolonged exposure (24 hours) led to reduced effectiveness or negative effects. NaCl, on the other hand, consistently had adverse effects on germination, confirming its role in inducing osmotic stress rather than promoting germination. These findings align with research on citrus species, where controlled pre-treatment methods improve germination, but excessive exposure to harsh conditions reduces viability (Iqbal et al., 2020).

Conclusion

This study demonstrates that pre-soaking Citrus tangerina seeds in different mediums significantly influences germination performance and seedling vigor. The findings indicate that:

HNO_3 treatment (12-24 hours) is the most effective dormancy-breaking method, significantly improving germination percentage and seedling vigor.

Water soaking (12-24 hours) moderately enhances germination but is less effective than acid scarification.

Salt solution (NaCl) negatively impacts seed germination and early growth, indicating that Citrus tangerina may have low salinity tolerance during early development.

Prolonged soaking (especially beyond 24 hours) can have adverse effects, particularly in acid and salt treatments, due to potential seed damage and osmotic stress.

The study contributes to citrus seed propagation techniques by providing practical recommendations for pre-sowing treatments to enhance germination success. Future research should investigate the biochemical and physiological responses of seeds to these treatments, as well as long-term growth performance under field conditions. Additionally, exploring other seed priming techniques, such as hormonal treatments or hydro-priming, could further optimize Citrus tangerina cultivation.

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