

Project Proposal

“Exploring the Impact of MS Media on Shoot Proliferation in In-Vitro-Grown Citrus Hystrix Explants”

Or

Optimizing In-Vitro Growth of Citrus Hystrix: A Comparative Analysis of Various Murashige and Skoog Medium Concentrations

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Abstract:

This research investigates the in-vitro growth of Citrus hystrix in a controlled environment, emphasizing a comprehensive comparative analysis under varying amounts of Murashige and Skoog (MS) media. The concentrations analyzed include an absence of MS media, 2.17 g/L of MS media, 4.33 g/L of MS media, and 8.66 g/L of MS media. The growth was systematically assessed by parameters, including changes in mass after each replication, shoot length, root-to-shoot ratio, and changes in plant color. The innovative approach of time-lapse imaging is utilized for a more in-depth analysis and to further differentiate between results. The results show that [add couple of sentences after the research is done about what we found + recommendations]

Introduction:

Murashige and Skoog (MS) media has had a profound impact on tissue culture research. First developed in 1962 by Toshio Murashige and Folke Skoog, MS media is a staple of tissue culture research; being the most widely used variant of media for plant tissue culture. MS media mainly consists of a myriad of vitamins, amino acids, and growth hormones, allowing a wide application in shoot proliferation, organogenesis, and callus induction. In this study, its applications in shoot proliferation will be examined.

Originating in southeast Asia, the C. Hystrix has a wide application with medicinal purposes, herbal supplements, cleaning, and cuisine.

MS Media is economical when compared to other forms of media, however, its price becomes concerning in large-scale research projects. Without information regarding the most efficient, cost-effective, and prolific solution for in-vitro growth, researchers are unable to perform studies with these optimal conditions.

This research aims to address gaps in current knowledge of MS Media usage for in-vitro growth by providing insights for improving explant regeneration in the fruit species Citrus Hystrix. This paper argues that H_0 is the optimal amount of MS Media for in-vitro growth of C. Hystrix. It was hypothesized that H_A Amount of MS media powder would have no effect on Citrus Hystrix growth and H_A amount of MS media powder would affect Citrus hystrix growth. (clarify amount) This paper first discusses the methodology behind the design, approach, and data collection, then goes on to present and analyze the findings of the research before summarizing the main findings and listing the sources cited in the paper (which citation style?) and ending with acknowledgements.

Review of Literature:

Also idk the background research can we do that later?

Hypothesis:

H_0 : Amount of MS media powder will have no effect on Citrus hystrix growth.

H_A : Amount of MS media powder will affect Citrus hystrix growth.

At alpha level =? 0.05?

Objective:

Determine the optimal amount of MS media for in-vitro growth of Citrus hystrix to determine the most cost-effective and prolific solution for in-vitro growth.

Importance/Significance:

Despite not being widely cultivated for commercialization (despite its limited commercial cultivation?), this plant possesses therapeutic properties and is valued for its medicinal

applications, as well as its role in Southeast Asian cuisine. In order to alleviate the severity of certain illnesses, the leaves, fruits, and rinds of the C. Hystrix are utilized for the various beneficial phytochemical compounds present. Numerous bioactive compounds that have potential pharmaceutical effects need to be further studied, however, C. Hystrix has traditional usage in native tribes for treating a myriad of physical ailments, such as stomach aches, fevers, and diabetes. In nature, abiotic and biotic stressors reduce crop species yield due to effects on the growth and development of the plants. Rearing these plant species in-vitro using plant tissue culture would allow agricultural methods to become simultaneously more efficient and cost-effective, allowing it to be deemed the future of the agriculture industry. Given the impending need for this essential technology, it is imperative to explore the optimal procedures and determine the most efficient methods as soon as possible. Reducing and optimizing the concentration of MS Medium utilized in the creation of these media solutions would exponentially decrease the costs of current in-vitro plant studies on the C. Hystrix

- Explanation of how Citrus hystrix are used in Southeast Asian cuisine & aromatherapy
- Citrus hystrix bioactivity linked to antioxidant properties
 - Contains vitamin A, carotenes, xanthin, and folates (aid in red blood cell formation)
- Plant tissue culture is the future of agriculture
- If plant tissue culture is to become widely used (environmental preservation or extraterrestrial human colonization) a wide variety of plants must be cultured and the most efficient methods must be determined now
- Lower levels of MS Media (if exhibiting successful growth) will reduce costs of in-vitro studies

Materials and Methods:

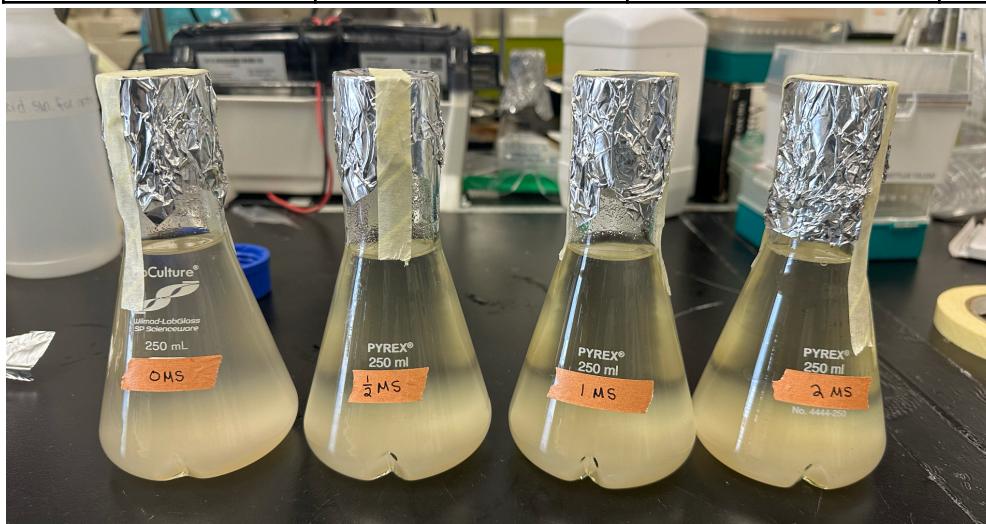
MS media preparation:

The preparation of experimental MS media was created using the same processes as traditional MS media preparation with the only difference being the amount of MS media added to each replication. Traditionally, MS media for plant tissue culture is made using 4.33 grams of MS media powder. In this experiment, 4 types of media were prepared with differing amounts of MS media powder: 0% MS media, 50% MS Media, 100% MS Media, and 200% MS Media.

In addition to the MS media powder, other phytohormones and vitamins are added. [add list here]

To ensure our experiment was conducted in a controlled manner, all media solutions were prepared at the same time. 1 Liter of media solution was prepared with all reagents, minus MS media. Then, the 1L solution was distributed into 4 250mL glass containers. Since MS media is not homogeneous before it is heated, each container was filled 50mL at a time, alternating between the containers with each round. Between each round of pouring, the mother solution was stirred using an electric stir rod for 30 seconds. After filling each container, the correct amount of MS media powder was added to each replication.

T-1	T-2	T-3	T-4
0%	50%	100%	200%
0.0g	.542g	1.083g	2.167g



All 4 media containers were then sterilized in the autoclave with 64 50mL centrifuge tubes at 121 °C for 20 minutes to reduce the chance of infection in the explants. Sterilized media was then distributed to the centrifuge tubes (16 tubes per media type.) All tubes were then covered with their lids and parafilm and placed in the greenhouse to solidify for 1 week.

Explant preparation:

All explants were harvested from the same mother *C. hystrix* plant. Explants were collected from a variety of new growth sites on the tree. Newer grown plants were chosen to increase the possibility of successful tissue culture. Explants were cut to about 6 inches in length and 90% of each leaf was cut off to prevent contamination. For the very young and delicate shoots, they were cut off from the rest of the branch and kept in a separate sterilizing group.

To sterilize explants, each sample collected was washed under running water for 2 hours with liquid dish soap. After, each plant was washed and shaken with distilled water for 10 minutes, then washed and shaken with a 20% bleach solution for 10 minutes. The young and delicate new shoots were only in the bleach solution for 5 minutes to prevent damage.

Tissue culture process:

All tissue culture procedures need to be conducted in a sterile environment to prevent contamination of samples. To achieve a sterile environment tissue culture is done in a laminar air flow hood and all equipment is sterilized using 70% ethanol and a bunsen burner.

Each treatment had 6 young explants cultured in media and 10 older explants, for a total of 16. To culture, an explant is carefully placed inside the semi-solid MS media after being soaked in 70% ethanol solution for around 5 minutes. The container is then covered with parafilm.

Growth Environment:

The ideal amount of MS Media was investigated using a prospective study, where #? Citrus *Hystrix* explants were randomly assigned to each experimental group. Newly budding shoots were severed from the mother plant before undergoing rigorous sterilization. To mitigate the risk

of infection, the shoots were treated with a solution of dishwashing soap and water for two hours. Following this sterilization, they were subjected to an additional five-minute rinse of liquid soap and water in the laboratory. After being held under tap water and deionized (DI) water for five cycles each, they were shaken with a 10% bleach solution for twenty to thirty minutes. The final stage of plant preparation involved a second wash under DI water five times. Additional safety procedures included thoroughly spraying the laminar flow hood and any equipment with 70% ethanol. To eliminate microorganisms, the UV light was turned on thirty minutes beforehand, while the fan setting regulated airflow.

[insert part abt making media in diff. concentrations]

Necessary materials were sterilized and transported into the laminar flow hood, such as the previously collected explants, scalpels, forceps, Petri dishes, spirit lamp, ethanol, parafilm, and a beaker of DI water. Before beginning work, the tools were carefully heated in an open flame and soaked in the concentrated ethyl alcohol to cool. With the forceps, the Citrus Hystrix shoots were placed in a Petri dish for excision of the remaining leaf. Lateral cuts were made below two of the explant's nodes and at the exposed sites to induce proliferation. One to two cuttings were vertically inserted into the media-containing tube by holding the forceps at a straight angle. After the lid was tightly secured, parafilm was tightly wrapped around the rim and the tube was labeled with the appropriate date, specimen, and media type.

The tubes were stored in the controlled setting of a greenhouse, which maintained consistent temperatures of 25 °C and a 16:8 Photoperiod Regime.

[insert part abt data collection + designing color chart]

In-Vitro Growth

The Citrus Hystrix tissue culture commences with the preliminary step of its explant procurement from the field. The explants, measuring in 6-8 inches, were transported to the research laboratory for detailed examination. Employing precision instruments such as seeateurs, scissors and blades, roughly 75% of the leaves were terminated from the obtained explants.

The dissected shoots were arranged in a container for an extensive two hour cleansing protocol by a running flow of tap water (H_2O) infused with dishwasher soap (**add the chemical formula for the soap**). Subsequent to this initial cleaning, a supplementary disinfection process was also implemented where the shoots were cleansed under 10% sodium hypochlorite ($NaClO$)

~~and were also run under deionized water (dH₂O) in five constant one minute repetitions. Concluding the preparatory juncture, the shoots are carefully stored in a controlled environment at 4°C. The antisepsis of these explants formulates the imminent phase of Citrus Hystrix growth inquiry.~~

In-vitro growth (pre-experiment)

- Explant Collection Procedure
- Normal media preparation procedure
- Tissue culture of explant procedure
- Explanation of controlled growth environment
- Reculture procedure of explants procedure

Media Solution Preparation (experimental groups)

- Differences from normal media preparation procedure (concentrations of 0x, 0.5x, and 2x normal)
- T-3 (normal media) used for control

In-vitro growth (experimental groups)

- Reculture procedure of explants to experimental group media
- Continue for _?_ weeks
- Data gathering procedure (use color sheets to observe colors, weigh bottles, use measuring tape)

Limitation:

- Sample size of explant
- # of mother plants used for experiment
- Time
- Space in greenhouse

- High risk of bacterial, fungal infections
- Possible confounding variables (ex: exposure to pathogens, unsterile bottles, human error)
- MS media
- Using only C. Hystrix

Data Analysis:

Independent Variable: Amount of MS Media added to solution relative to 1.0 value of 4.33 g

T-1	T-2	T-3	T-4
0.0	0.5	1.0	2.0

Dependent Variables: We will be measuring a variety of variables to determine the effect of MS media powder amounts on a holistic scale.

% change in plant mass (Quantitative)	% change in shoot length from root to tip (Quantitative)	Color Observations (Qualitative)
From 0 to 5 weeks	From 0 to 5 weeks	Recorded Weekly

% change in plant mass		Treatment			
		T-1	T-2	T-3	T-4
Replication	R-1				
	R-2				
	R-3				
	R-4				
	R-5				

% change in shoot length from root to tip		Treatment			
		T-1	T-2	T-3	T-4
Replication	R-1				
	R-2				
	R-3				
	R-4				
	R-5				

Presentation/Publications:

Works Cited: