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CALLUS INDUCTION ANTHER CULTURE OF HONEYDEW (*CUCUMIS MELO*) USING NAA

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A Thesis Submitted in Fulfilment of the Requirements for the
BACHELOR OF APPLIED SCIENCE (AGROTECHNOLOGY)
WITH HONORS

FACULTY OF AGRO-BASED INDUSTRY
UNIVERSITY MALAYSIA KELANTAN

2022

DECLARATION

I hereby declare that the work embodies in this report is the result of my own research except individual citations and summaries that I have explained their sources.

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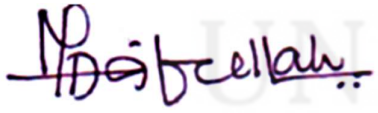

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ACKNOWLEDGEMENTS

I'd want to recognise and express my heartfelt gratitude to my supervisor Dr. Mohammed Aurifullah, Co-supervisor Dr. Suhana, Lab assistant Nurabila Nabila Ismail, my team master student Fadzlin Qistina binti Fauzan who made this work possible. his or her guidance and advice carried me through all the stages of writing my project.

I'd also want to express my admiration to my parents Ibrahim bin Taib and Zohra Mat Nor and my friends as a whole for their continuous support and understanding when undertaking my research and writing my project. you're praying for me was what sustained me this far.

ABSTRACT

Anther culture is a quick and easy way to make haploids from the microspores found in the intact anther. The effect of cultivated anther is more prevalent at various maturation phases in young anther, particularly in the field-growed than in average the grown plants. Callus is created to produce embryos where haploid plants are made and transferred to another media. Callus induction is more common. The significance of this study is to observe the development and growth of callus from anther culture of *Cucumis melo* treated with 1 naphthaleneacetic acid (NAA). The objective of the project is to find the suitable amount of NAA to be used in media for inducing callus develop post-induction.

Keywords: honeydew, callus induction, 1 naphthaleneacetic acid (NAA), anther culture.

ABSTRAK

Kultur anter ialah cara cepat dan mudah untuk membuat haploid daripada mikrospora yang terdapat dalam anter yang muda. Kesan anter yang ditanam lebih ketara pada pelbagai fasa pematangan dalam anter muda, terutamanya yang ditanam di rumah atau di ladang berbanding rata-rata tumbuhan yang ditanam. Kalus dicipta untuk menghasilkan embrio di mana tumbuhan haploid dibuat dan dipindahkan ke media lain. Induksi kalus adalah lebih biasa. Kepentingan kajian ini adalah untuk melihat perkembangan dan pertumbuhan kalus daripada kultur anter Cucumis melo yang dirawat dengan 1 asid naftalenasettik (NAA). Objektif cadangan adalah untuk mencari jumlah NAA yang sesuai untuk digunakan dalam media untuk mendorong perkembangan kalus selepas induksi.

Kata kunci: embun madu, aruhan kalus, 1 asid naftalenasettik (NAA), kultur anter

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Chapter 1

Introduction

1.1 Research Background

Another culture may be used in the production of haploids. The process involves selecting cell lines and producing homozygous lines for further breeding programmes. Anther culture means the regeneration of plants from haploid microspore cells in order to make haploid and dihaploid plants. In other cultures, homozygous inbred lines develop instantaneously to prevent the length of time needed using traditional self-pollination methods. Haploid plants, easily recognized by their smaller sterile flowers, result in another culture. Chromosomes are treated with colchicine to double the haploid numbers. There may also be spontaneous diploids. Double haploids can be applied as hybrid parents or as "cultivars" for themselves according to incompatibility status. The procedure in calabrese is successful (Dal, et al., 2016).

Early attempts to grow haploid melon and other cucurbit crops using ovule culture failed in plant breeding programmes. Double Haploids (DH), liberation of parthenogenetic rudiments caused by cross-fertilization with radiated pollen was the first success reported in obtaining haploid or DH melons (Asadi, A., et al, 2018). Low percentages of haploid embryos induced, difficulties in the detection and excision of these embryos, and a small number of haploid plantlets recovered limiting the use of the technology in application for breeding programmes. The soft X-ray procedure can be used in order to detect seed parthenogenetic embryos, but not many labs have the necessary devices. As an alternative approach to DH recovery, tested unpollinated ovary gynogenesis but did not compare it with the results of irradiated pollens use. The Cornell melon-breeding project focuses on multiple virus resistances, and hybrids have been obtained from lines of various resistances (Dal, B., et al, 2016).

Melon (*Cucumis melo*) is a Cucurbitaceae eudicot diploid plant genus. Pitrat, (2016) identified 19 groups of melon, including agrestis, ameri, ibericus cantalupensis, makuwa, chandalak, chate, chinensis, chito, conomon, dudaim, acidulus, flexuosus, inodorus, cassaba, indicus, kachri, momordica, and tibish are some examples. Melon was among the most commercially significant fruits. Cucurbitaceae plants, alongside cucumber (*Cucumis sativus*) and watermelon (*Citrullus lanatus*).

1.2 Problem Statement

Melon production was estimated to be about 1 million tonnes in 2021 (FAO, 2021), with China, Turkey, Iran, Egypt, India, Kazakhstan, the United States of America, and Spain being the top producers by yield. The melon fruit is genetically diverse, and

each nation has its own preferences for cultural reasons (Monforte et al., 2014). In Spain, the most popular melon varieties are Inodorus and Cantalupensis. Pathogens pose a significant danger to melon output.it is estimated that over 200 pathogens, including fungi, bacteria, viruses, and mycoplasma species, affect cucurbit productivity. Diseases are thought to cause yield losses of more than 30–50 percent in melon production (El-Naggar et al., 2012). In melon and cucurbit plants, powdery mildew, fusarium wilt, and melon necrotic spot virus (MNSV) are the most severe diseases. Many modern breeding programs have been introduced to obtain resistant cultivars as a result of the high impact of pathogens in cucurbits (Kuzuya et al., 2003; Lotfi et al., 2003). The problem statement is to establish honeydew cultivars (*Cucumis melo)* in Malaysia using anther cultivation supplied with NAA hormone.

1.3 Hypothesis

The hypothesized that the application of these NAA in culture medium could influence androgenesis induction and plant regeneration from honeydew anther cultures.

1.4 Significant of Study

The significance of study is to observe the treatment of the plant through the anther culture *Cucumis melo* using one type of treatment which is using NAA hormones to enhance the growth. Anther culture is a quick and easy way to make haploids from the microspores found in the intact anther. There is always the risk that diploid anther somatic cells may react to the culture condition and develop unwanted diploid calluses or plantlets during this phase. As growth inhibiting substances leak out of the anther wall and come into contact with the nutrient medium, the production of microspore within the anther can be disrupted. The culture of free pollen has been studied in an effort to prevent these issues. The following are some of the additional benefits of pollen culture (Rajcan, Boersma, & Shaw, 2011).

Pollen grains are not overcrowded in the anther, and isolated pollen grains are similarly exposed to the nutrient medium. Unwanted anther wall and other associated tissue growth is removed. Starting with a single cell, the steps of androgenization can be observed. Various androgen-regulating factors can be better regulated. Pollens can be uniformly exposed to chemicals and physical mutagens, making them suitable for absorption, transformation, and mutagenic studies. Pollen can be converted directly into an embryoid. As a result, it's ideal for learning about androgens' biochemistry and physiology. Pollen culture is expected to produce more haploid plants per anther than anther culture (Shaw,2011)

1.5 Limitation of study

The limitation of this study was the stringent timeline which was only 4-weeks. Meanwhile, the callus had to appear for at least 55 days. Due to lack of skill I failed to contamination. Furthermore, the limitation of flower buds due to the heavy raining season also contributed to shortage of flower bud collection. Ideally three different stages of flower buds needed for this study.

1.6 Research Objective

The objective of the proposal is to find the suitable media for the anther to develop, which will induce callus from the anther. To find the concentration of NAA eligible for callus induction from melon anther cultures.

Chapter 2

Literature Review

Initial culturing at high temperatures (35 °C), which stimulates embryogenesis, for up to 48 hours is also optimal for anther cultivation. Pretreatment of floral buds with high temperature also stimulates embryogenesis. Anther culture is a potential technique for haploid production and is simpler than a culture of pollen and androgen. In sorghum, haploids developed through anther cultures are not successful. Sorghum germplasms (*Sorghum almum* and *S. versicolor*) are used by various studies and include inbred grain and drill type lines from the United States and China (Parasorghum).

2.2 2.1 Induction of callus

Haploid and double haploid growth was reported in sorghum-Hybrid CSH5, but cytological evidence was not provided to prove the haploid nature of progeny plants. The induction frequency of the CSH9 sorghum hybrid was fairly at 60%.

The high rate of recurrence of callus stimulation and plant rejuvenation was also reported by Nakamura et al. in 1997 which is by (14.3 percent). Callus induction is more common nowadays. The regeneration rate for green seedlings in honeydew was 27 percent on average and for most regenerate seedlings, the number of chromosomes was unstable and 20. In the 1990, Guha and Maheshwari first reported successful anther culture by in vitro methods. It is mainly used in rice (*Oryza sativa*) and tobacco (*N. tabacum*). The benefits are that haploid plants are high frequency, easily divided into cells in most species and no high degree of expertise is required. In a breeding program, it involves selecting suitable parents, depending on the goals. The appropriate parents can easily have excellent agronomic characteristics, interesting characteristics and culture. F1 is the supplier of young pollen grains in the anthers. The growing environment of contributor parents have to be monitored in all areas including light, nutrients, moisture and temperature. The plant's developmental stage is also significant when selecting, it is also important to take note of. At the right phase, flowers must be selected and this varies between the species. Depending on the variety and media obligations, the flowers or else buds may also have to be sterilized. After elimination of the bud or bloom, it has maintained on ice, and the anthers are microscopically examined and disinfected. The anther is then relocated to solid media that are plentiful in nutrients and a callus is developed. Cultivators should make an effort to grow before scaling up of tissue culture is done, since several genetic constitutions are the most important factor in haploid establishment compared to others within a species. Subsequently, a callus is created to produce embryos where haploid plants are made

and transferred to another media. Subsequently, callus are created to generate embryos where haploid plants are made and transferred to another media. The haploid plant is located on the soil or rooting media after sufficient growth afterward doubled artificially.

There are weaknesses to this technique, including the fact that not everything constructed in plants is haploid, that in some species there are difficulties in removing anthers, that unexceptional proficiency is required. However, in the case of rice anther culture, species and genotype were found to be responsible more than growing conditions and media for the effectiveness of the development of calluses and haploid making.

The first DH techniques discovered in many plant breeding programmes and played an important role was one of anther culture. As the technology improves, numerous plant growers and scientists hope that this practice will become extra competent and can remain transferred to various species.

2.3 History of *Cucumis melo*

Cucurbitaceae is a family of more than 30 species belonging to the genus Cucumis. Cucumber (*C. sativus* L.), melon (*C. melo* L.), and are among the recognised organisms and impaled cucumber or kiwano (*C. metuliferus* E. mey ex Schrad) are all annual plants. Melon, muskmelon, and cantaloupe melon are all common names for *Cucumis melo.* It is an affiliate of the Cucurbitaceae family, also known as the cucurbits or gourd family. Thirty o*f Cucumis'* 32 species, including *C.melo*, have n = 12 basic chromosome number. *Cucumis sativus* L. and

Cucumis hystrix Chakrav. are the only exceptions, with chromosome number n = 7 as reported by Kerje Grum (2000).

Melon must have been widely used as a wild plant in the past, and selection has resulted in sweeter fruits. At least 3000 years ago, early trade spread selected (wild) species from Africa to Asia, where today's diversity is high. Natural selection and hybridization with cultigens separated the two subspecies, agrestis and melo, over time. Today's sweet wild agrestis and melon fruits may be the products of hybridization with cultigens and should not be considered genuinely wild. Part of the distribution should be considered feral as a result of agriculture. Melon domestication took place primarily in Asia, where early trade and migration spread the fruit rapidly. Asia has a greater diversity of landraces and cultivars than Africa due to heavy trade, higher population, and domestication. The bitter, inedible fruits are thought to be mainly found in Africa (Grum, 2000).

2.4 Taxonomy

Elongated petioles, wide gaunt leaves, modest tendrils, hirsute roots, long branches, and ascending or sprawling growth habits are all features of this species. *Cucumis melo* is the most polymorphic species, which is an important trait in taxonomic research. More than a few taxonomic explorations have made an effort to classify melons into botanical varieties, groups, or subspecies. According to taxonomic research, there are seven intra-specific classes of melons: cantalupensis (sweet melon), agrestis (wild melon), flexuosus (snake melon or snake cucumber), inodorus (winter melon), momordica (pomegranate melon), dudaim (mango melon or pomegranate melon), mom (snap melon), and conomon (pickling melon). On account of the

regular manifestation of uninhabited Cucumis species with the same basic chromosome number n=12.5, Africa is thought to be the melon's origin (Bezirganoglu, 2018).

Turkey, Syria, Iran, Afghanistan, India, Turkmenistan, Tadjikistan, and Uzbekistan are the secondary sources of melon. Subordinate centres of variety for the particular species were correspondingly identified as China, Korea, Portugal, and in eastern humid Africa south of the Sahara Spain is only present *C. melo*, according to Whitaker & Bemis (1976). Wild types are popular in the Sudano-Sahelian region also stated by Pitrat et al.(1999), in addition to the findings. In the inferior centre of diversity encompasses from Asia to the Japan and Mediterranean, melon comes in many forms and sizes and is used in a variety of ways. Cucumis melo is one of the most significant horticultural yields in the world and confronts an imperative role in intercontinental trafficking (Bezirganoglu,2018).

2.5 Male Flower

Fruits from hermaphroditic flowers were longer than fruits from female blooms (Risser 1984). Male melon flowers have stamens, which are pollen-covered stalks that protrude from the centre of the flower. Female flowers will contain a sticky knob within the flower called a stigma (to which pollen will adhere), and the female flower will also sit on top of an immature, small melon. In muskmelon, four male sterility genes have been identified (1,2,3,4) Rutkowska-Krause (2014). However, Clause Seed Company has long recognised and employed the fifth gene for the development of F1 hybrid seeds, but its description and inheritance have never been published. This mutation was discovered in 1966 during a breeding experiment to introduce PMR 45 powdery mildew resistance into the Charentais muskmelon type (M. Pitrat and G. Risser, 1990). Because of abortion at the bud stage, the number of male flowers

blossoming on male-sterile plants is smaller than on male-fertile plants. The anthers of male or hermaphroditic flowers are smaller and empty. At the meiotic stage, pollen begins to degrade (Lecouviour,1990).

Male flowers are distinguished by tiny, short anthers containing pollen in the centre of the bloom and a slender, bulging-free stem reaching up to the flower. Female flowers, on the other hand, have a roundish folded stigma in the centre of the bloom and a thicker immature form that holds the unpollinated fruit (Jay, 2013). Cucumis melo can develop exclusively male flowers in response to high temperatures or fertility, resulting in poor fruit set.

2.6 Anther Culture

Anther culture is a method for causing undeveloped pollen to rift and develop into tissue (either embryonic tissue or callus) in order to create haploids (plants with a N chromosome number). Androgenesis is the process of producing haploids from anther culture. As an alternative, the greatest common procedures designed for producing haploids on a false culture matrix is through anthers or microspores. There now are countless procedures for producing haploids, nevertheless, they are extremely infrequent. In this process, pollen-containing anthers are isolated from a flower and placed in a suitable culture medium. During this process, some microspheres survive and grow into tissue. Some of the fruit develops into embryonic tissue, which is then used for shoot and root growth when combined with a suitable medium. However, if a callus develops, it can separate into shoot and root tissue following hormonal therapy.

In the 1970s, Guha and Maheshwari published the first effective anther culture using in vitro methods. It's been used in a variety of plants, mostly tobacco (*N. tabacum*) and rice (*Oryza sativa*). The advantages include a high prevalence of haploid plants, the ease with which most

organisms can cause cell division, and does not require a high degree of expertise. It entails deciding on appropriate parents for an engendering system based in working order objectives not to mention then performing a interbreed. Commencement who have good agronomic characteristics, a trait of interest, and can easily culture their offspring are good choices. The F1 is used to obtain immature pollen grains from the anthers. Light, nutrients, humidity, and temperature must all be monitored in the donor parents' growing climate.

It's also worth noting that the plant's developmental stage plays a big role in the assortment. Flowers must be selected at the right time, which varies depending on the species. Depending on the species, the buds or flowers will as well prerequisite designate sterilized, and the media specifications may vary. The anthers are dissected using sterilized forceps under the microscope as soon as the bud or flower has been separated and kept going with ice. The anthers were then moved to nutrient-rich solid media, where they form a callus. Breeders should explore culturing before pursuing large-scale production since those genotypes within such a population are more suited for haploid manufacturing than others. The callus is subsequently moulded and transported to a different medium to form embryos, by which the haploid plant is created. After the haploid plant has developed sufficiently, it is placed in the soil as well as rooting media and treated to artificial chromosomal doubling. (Bhatia,2015).

Several drawbacks consist of the fact that not every plant generated is haploid, the difficulty of removing anthers in the field of a variety of species, the need for adequate capability, and the prevalence of albinism in cereals and other monocots. There are a lot of breeding programs and experts out there hope the aim of as technology advances, this this practice will become more effective and convenient to a variety of other plant species (Asadi,2018).

2.7 Media Composition

According to Arditi (2008), the medium composition is critical in ensuring that the explants have adequate nutrients to grow. MS medium, which is abundant in vitamins and microelements, had the best results for tropical plants compared to other media, with the greatest mean shoot length and number of multiplied shoots (Zahara et al.,2017). The table shows the chemical composition of commonly cultivated tissue culture media, which was presented in the formulation of culture material MS (Murashige and Skoog) medium (Esfandiari, 2015).

MS medium was first created for tobacco and was based on mineral analysis of tobacco tissue. The MS medium is notable for its high concentrations of nitrate, potassium, and ammonia. MS medium is less expensive than other media such as White's medium. It stimulates organogenesis and plant regeneration. MS medium is used for micropropagation, organ culture, callus culture, and cell suspension culture (Murashige T, Skoog F, 1962.).

Chemical	Formula	Concentration
Macronutrients (10 X)		100 mL/L
Ammonium nitrate	NH_4NO_3	16.5
Potassium nitrate	KNO_3	19.0
Calcium chloride	$CaCl_2.2H_2O$	4.4
Magnesium sulfate	$MgSO_4.7H_2O$	3.7
Potassium dihydrogen orthophosphate	KH_2PO_4	1.7
Micronutrients (100 X)		10 mL/L
Manganese sulphate	$MnSO_4.4H_2O$	2.23
Zinc sulphate	$ZnSO_4.7H_20$	0.86
Potassium iodide	KI	0.086
Cupric sulphate	$CuSO_4.5H_2O$	0.0026
Sodium molybdate	$Na_2MoO_4.2H_2O$	0.025
Cobalt (ous) chloride	$CoCl_2.6H_2O$	0.0026
Boric acid	H_3BO_3	0.62
Vitamin source (100 X)		10 mL/L
Nicotinic acid	$C_6H_5NO_2$	0.05
Thiamine hydrochloride	$C_{12}H_{17}ClN_4OS.HCl$	0.01
Pyridoxine hydrochloride	$C_8H_{12}N_2O_2.2HCl$	0.05
Glycine	$C_6H_{12}O_6$	0.2
Iron source (100 X)		10 mL/L
Sodium EDTA	$C_{10}H_{14}N_2O_8Na_2H_2O$	2.78
Ferrous sulphate	$FeSO_4.7H_2O$	3.72
Myo-inositol		0.1 g (freshly add)
Sucrose	$C_2H_5NO_3$	30 g
Phytagel		2 g

Table 1 : Murashige and Skoog (MS) basal medium with vitamin

Source : Esfandiari, T. & Ghavidel, R. & Foroghian, Sh. (2015). The Effect of Phytohormones on Lavender (*Lavandula angustiflia* Mill.) Organogenesis. Journal of Pharmacy and Pharmacology. 3. 10.17265/2328-2150/2015.07.004.

Due to the change in medium and other regeneration circumstances, assessing the influence of basal induction media on plant regeneration was challenging (Sirkka Immonen and Hanna Anttila, 1999).

2.8 Plant Growth Regulator

Plant growth regulators (usually referred to simply as plant hormones) are substances that regulate plant development. It is a class of chemical molecules that have a significant impact on the development and disparity of tissues, organs, and plant cells. Plant development mechanisms act as chemical emissaries, allowing cells to communicate with one another. Sun

et al(1980). described the effect of four growth regulators on flax anther culture. When eight auxins were introduced to callus-induction media, NAA, Picloram, and 2,4-D, were much more conducive to anther callus initiation, whereas IAA, IBA, and TIBA produced callus at the lowest frequencies (Tejklova, 1996). Zeatin was shown to be more effective than BAP for anther callogenesis and also bud renewal in anther calli (Tejklova, 1998). When MT was added to callus-induction media, it resulted in a greater rate of recurrence of callogenesis in anthers than BAP at the equivalent dose. Because once calli receiving MT media had been transferred to rejuvenation medium carrying the same amounts from either meta-Topolin (MT) rather than benzylaminopurine (BAP), bud regeneration was much greater in the BAP medium.

According to Soroka (2004), callus expanded and developed more effectively. with a BAP concentration of 2 mg/L in culture media compared to 6 and 4mg. L1 in growth medium. Root and shoot redevelopment occurred modest in addition to being unaffected by BAP levels. Explants were transferred to fresh media, which facilitated regenerated structure de-differentiation. Breton and Gonzales (2005) investigated the effect of kinetin concentrations on callus initiation in anthers. Kin at 2 mg.L1 was the most effective. Higher amounts of zeatin replaced by Kinetin had no effect on embryogenesis in the anther calli. (Gonzales et al., 2005). There are presently no progressive methods available for flax that accord acquiesce for the enhancement and increase in efficiency of double-haploid production.

Because anthers have a poor ability to stimulate haploid shoots, the impact of novel spin-offs on a qualified model of multiple shoot culture, conduct experiment, was initially evaluated (Smkalova et al., 2010). The latest research on anther culture explains the superior carbon source concentration inside the medium and, BAP and NAA (2: 1 and 1: 2) with the volume of genotype callogenesis being genotype-dependent relative (Blinstrubiene, and Burbulis (2006); Obert et al., (2004)). Many attractive lineage, however, have been obstinate (a

circumstance in which poor regeneration occurs in vitro or revitalisation does not develop at all).

Growth regulator (abbreviation/name)	*Chemical name*
Auxins	
IAA	Indole 3-acetic acid
IBA	Indole 3-butyric acid
NAA	1-Naphthyl acetic acid
2, 4-D	2, 4-Dichlorophenoxy acetic acid
2, 4, 5-T	2, 4, 5-Trichlorophenoxy acetic acid
4—CPA	4-Chlorophenoxy acetic acid
NOA	2-Naphthyloxy acetic acid
MCPA	2-Methyl 4-chlorophenoxy acetic acid
Dicamba	2-Methoxy 3, 6-dichlorobenzoic acid
Picloram	4-Amino 2, 5, 6-trichloropicolinic acid
Cytokinins	
BAP	6-Benzyl aminopurine
BA	Benzyl adenine
2 iP (IPA)	N^6-(2-isopentyl) adenine
DPU	Diphenyl urea
Kinetin	6-Furfuryl aminopurine
Zeatin	4-Hydroxy 3-methyltrans 2-butenyl aminopurine
Thidiazuron	1-Phenyl 3-(1, 2, 3-thiadiazol-5 yl) urea

Table 2: List plant regulator used in media culture

Source : Biology Discussion, 2015

2.9 Auxins

Plants employ a wide range of hormones, including steroids and peptides, as well as the five conventional classes of phytohormones (auxins, abscisic acid, cytokinins, ethylene, and gibberellins), all of which are tiny molecules. The amount and relevance of phytohormone transport for all of these classes is unknown, but it is particularly important to the action of auxin and the narrative of its discovery. When Charles and Francis Darwin published The Power of Movement in Plants, they recorded the impact of auxin for the first time. They discovered that when light is seen in one part of a grass coleoptile, an "influence is transferred" that promotes bending towards the light in another.

This messenger was isolated from plant tissues 45 years later, in 1926, simply by allowing it to disseminate into agar blocks, which again preserved a growth-stimulating action. Initially, three types of auxin were discovered in plants, one of which was also discovered in human urine. Following that, the first published findings on the crystallisation and structural properties of auxin began to appear. Only one of the structures, indole-3-acetic acid (IAA), was successfully recognised in those early days (Teale, et al, 2006).

Using 2,4-D and NAA, the effects of different kinds of auxins on anther-derived callus production were studied after a 3-week incubation period (Arisandi, et al, (2020)). Furthermore, strong green plantlets were regenerated from *Hordeum vulgare* microspore culture in the presence of auxins such as indoleacetic acid (IAA) or naphthaleneacetic acid (NAA) (Castillo et al., 2000). The inclusion of plant growth regulators in culture media, namely IAA, NAA or 2,4-D and BAP as well as kinetin, was critical in boosting callus development on another soybean culture, whether it's on Merubetiri or Wilis cultivars. Merubetiri, on the other hand, was shown to produce a better in vitro reaction than Wilis (Arisandi, et al., 2020). Among the

auxins studied, 2,4-D was more efficacious than IAA or NAA, as evidenced by a larger number of explants producing callus. Previous studies have also documented the use of 2,4-D as an auxin source for callus induction in legumes, with 1-2 mg/L, 2,4-D being the most efficient (Kiran et al., 2005; Kaviraj et al., 2006; Kumari et al., 2006; Ahlawat et al., 2006) . This is understandable given that 2,4-D is classified as a strong auxin that plays an essential function in encouraging callus development in an in vitro culture system.

2.10 Temperature

The use of a low-temperature (4℃) pretreatment to flax inflorescences and isolated anthers did not appear to improve the fraction of callusing and renewing plant anthers (Rogalska and Rutkowska-Krause, 1994; Poliakov et al., 1995). Later, Rutkowska-Krause et al. (1995) observed that chilling flowerings ahead of anther mining inhibited callogenesis induction appearing in the anthers.

Cold treatment of excised anthers cultivated on medium increased anther reactions. Tejklova (1996) found that isolated anthers were subjected to a cold pretreatment. (4℃, 15 days) decreased anther callogenesis while storage of flower buds (4℃, 28 days) did not promote anther callogenesis. The quantity of callus producing anthers was dramatically reduced after three days of pretreatment of flower buds in the dark at 4℃. (Kurt and Evans, 1998).

A multivariate culture trial was carried out, in which the hypocotyls of something like the Jitka, Tabor, and Venica kinds were grown alongside the AGT genetic line. The

performance of these types in vitro has already been documented in previous investigations (Smkalova, 2010), and the gathered data on growth performance may be compared.

2.11 Naphthaleneacetic acid

Naphthalene acetic acid has a variety of physiological functions, including promoting cell division and cell development. Naphthalene acetic acid can reach plants through a variety of routes, including leaves, roots, and tender skin, and can have an effect on plant and fruit properties. Naphthalene acetic acid increases the rate of fruit development, resulting in a larger size at harvest without a decrease in yield, and ultimately improving the quality of the fruit. At high concentrations, naphthalene acetic acid is harmful to plants. Naphthalene acetic acid in excess has unfavourable effects and can cause growth rate. Many metabolic processes can be slowed by high concentrations. The importance of NAA in extending produce's post-harvest existence cannot be overstated. NAA can stimulate plant development, prevent flower drop, and so on at low concentrations, but it can also inhibit plant growth at high concentrations. It also causes wilting, and since the effect lasts longer, the damage to the fruit plant is greater, and flower drop is more likely. As a result, the concentration (to be used) is critical. The NAA treatment causes the fruit to grow in size, which leads to an increase in yield and a noticeable improvement in fruit quality (Singh, et al., 2017).

Chapter 3

Methodology

2.12 3.1 Materials and Apparatus

1) Flower buds of *Cucumis melo*

Flower Buds collection: Remove any nearby leaves and cut the buds with a length of 5-6 mm and a breadth of about 1.8 mm in linseed and a length of 4-5 mm and a width of about 1.5 mm in flax (that is, cut the buds of maximum size when it does not have coloured petals). The most effective regeneration occurs in the anthers of the plant's initial few buds during the bud development stage.

Material for anther culture selection: Because anthers of flower buds from other sections of the plant are normally not acceptable for culture, the closed flower buds were removed

from the top of the plant for the study. The sample was taken at 3 different stages of flower buds.

3.2 Methods

Male flower buds containing microspores at the mid to late uninucleate (vacuolate) stages (10–15 mm in length) collected, inserted into double-layered, moistened cheesecloth, deposited in a glass plate, and cold pre-treatment at 4 °C for 2 days, unless otherwise specified in each experiment. Buds will be surface sterilised for 30 minutes in fungicide, rinse in running tap water in 30 minutes, surface sterilisation involves treating all explants with an appropriate chemical in order to destroy any contaminating bacteria that may be present on their surface. The surface sterilisation technique will be mostly determined by the source and kind of explants, which will define the contamination load and sterilising agent tolerance (Saurabh Bhatia,2015), followed by washing the anthers with soap, followed by sodium hypochloride . Using tweezers, grasp the flower bud, cut a 1 mm segment of the calyx from the stem side of the bud with a scalpel (without damaging the anthers), push aside the sepals, and petals, and remove the anthers without filaments. Anthers removed and plated in petri dishes with basal MS salts and vitamins (Murashige and Skoog 1962), transfer into laminar flow rinse it again with 70% ethanol followed by DH_2O and subcultured every 21 days with the freshly prepared medium until calli and/or embryos appear.

The preparation of culture medium is best done in a compartment specifically designed for this purpose. This compartment should be built in such a way that it is easy

to clean and has a low risk of contamination. Tap and distilled water should be available. Appropriate methods for water sterilisation or deionization are also required. A refrigerator, freezer, hot plate, stirrer, pH metre, electric scales with different weighing ranges, heater, Bunsen burner, as well as glassware and chemicals, are necessary for optimal performance. It is now generally understood that errors in the tissue culture process are most usually caused by incorrect media preparation, which is why clean glassware, good quality water, pure chemicals, and precise measurement of media components should be encouraged (Abobkar et al., 2012).

In both versions, anthers buried in agar culture media produced less callus than anthers put on the medium surface (Tejklova, 1996). The orientation of the anthers on the culture medium surface had no influence on callogenesis or regeneration. (Chen et al., 1998).

3.3 Data Analysis

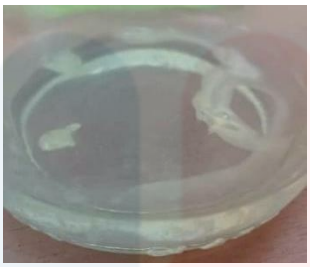
The data was collected by observing the media of anther culture. Unfortunately, the explant was initially contaminated due to inadequate sterilising. Endogenous bacteria in the tissue explant frequently cause latent contamination because they multiply and reproduce long after culture begins. 35- 55 days after commencing or subculturing, the culture should be checked for contamination. Fungi are among the most common microorganisms and are particularly dangerous because they grow directly in the nutritional medium, competing with plants for nutrients and producing phytotoxic metabolites.

Chapter 4

Results And Discussion

Although had 2 anthers had survive, due to limited time, the observation still not get enough time to callus to forms up. There are different types of containmination on my media in Tabel 2

Date of culture	Num. of cultured anther	Containminated	Survive
28/10/21	6	6 	0

15/11/21	15	15 	0
16/11/21	11	11 	0
29/11/21	7	7 	0
15/12/21	12	12	0

			
27/12/21	8	6 	2

Table 2: Results for cultured anthers

Contamination may enter the environment in a variety of ways. Initial contamination as a result of insufficient sterilisation of the explant. Latent contamination is often caused by endogenous bacteria existing in the tissue explant that proliferate and reproduce long after culture beginning. The culture should be examined for contamination 35 days after starting or subculturing. Bacteria are the most common pollutants. Fungi can enter culture via tissue explants or airborne spores.

In many circumstances, the contaminating sources are difficult to identify; nonetheless, the most prevalent ones are related to microbes from the environment and the manipulating individual. A common way for spores and microbe cells to enter the workplace is by air currents brought by air conditioning equipment and persisting in the environment owing to insufficient aseptic conditions. Fungi are among the most prevalent microorganisms and are extremely harmful because they grow directly in the nutritional medium, vying for the

medium's nutrients with the plants and creating phytotoxic metabolites. Endophytic infection, or germs that invade vegetable tissues inside, is extremely detrimental to in vitro plant cultures. The elimination of culturable fungal and bacterial contaminants is required for the development of an in vitro culture. Antibiotics and fungisides, alcohols, mercuric chloride, and oxidising biocides such as halogen compounds (e.g., chlorine, bromine, and iodine) and hydrogen peroxides are among the chemical approaches utilised. The techniques employed are determined by the plant species, explant type, phytoxicity, kind of pollutants, and cost (Sumarmi, 2014).

The main elements that impact flower buds include temperature, solar radiation, altitude, rain, wind, and air pressure (Fischer and Melgarejo, (2020); Restrepo-Daz and Sánchez-Reinoso, (2020)). The tropics, on the other hand, lack the distinct temperature seasons observed in temperate zones; hence, the wet and dry seasons determine the seasons to which plants respond physiologically. Fruits and vegetables are among the species most affected by climate change, with Shukla et al. (2019) warning that production and quality would decline as warming continues, particularly in tropical and subtropical countries. Carr (2013), concluded that climate change and fluctuation had altered growing conditions in honeydew production locations, potentially affecting the yield and economic viability of these crops.

Chapter 5

Conclusion And Recommendation

5.1 Conclusion

As conclusion, callus do not formed and all the cultured were contaminated. due to lack of skill, buds and not get enough time. Anther culture success is dependent on a variety of parameters, including cold-pretreatment, genotype, medium additions, and growth regulators.

5.2 Recommendation

In terms of enhancements for this study, the next researcher should learn the skill of anthers culture. Surface sterilisation involves treating all explants with an appropriate chemical in order to destroy any contaminating germs that could be on their surface. The surface sterilisation technique will be determined mainly by the source and kind of explants, which will define the contamination load and sterilising agent tolerance.

Based on the result, researchers need to continue in order to get the callus forms. This also can improve the skill on anther culture. According to our tests, the flowering time for anther culture in 'Halep Karas' is one day before anthesis and two days before anthesis in 'Crimson Tide'. Tulukolu (2014) investigated the influence of buds obtained at various blooming times on anther culture in four distinct watermelon types. According to the data, callus development was more remarkable in the one-day-before-anthesis blossoms stage in the cultivars 'Halep Karas,' 'Zeugma F1,' and 'Starburst F1,' although it was more productive in the two-days-before-anthesis stage in 'Crimson Tide.' According to Cihad and Solmaz (2019) findings, 'Halep Karas' and 'Kar 375' were successful in callus production one day before anthesis, whereas 'Kar 234' and 'Crimson Tide' were more successful two days before anthesis.

Following the breach of the anther wall, all regenerated callus propagated from within the anthers. This suggests that the callus might have started from microspores inside the anthers. As a result, the ploidy level of regenerated callus is likely the same as that of the microspores, i.e. haploid. This notion, however, must be supported by studying and counting the number of chromosomes under a microscope. Inducing embryogenesis from within the

callus mass was another technique to verify it was haploid. It was thought that embryoids generated from callus were haploid as well as matured into complete haploid plants. This study discovered that the presence of plant growth regulators, particularly auxin, in culture media had a substantial effect on callus development.

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