ESTABLISHMENT OF TISSUE CULTURE PLANTING MATERIALS OF *Ficus carica* L. Red Libyan CULTIVAR

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ESTABLISHMENT OF TISSUE CULTURE PLANTING MATERIALS OF *Ficus carica* L. Red Libyan CULTIVAR

by

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LIST OF ACRONYMS AND ABBREVIATIONS

%	Percentage
µ mol/s	Micromole per second
µg/mL	Microgram per microlitre
µL	Microlitre
µM	Micromolar
ºC	Degree Celsius
µ mol/s	Micromole per second
2,4-D	2,4-Dichlorophenoxyacetic acid
2-iP	6-(γ,γ-Dimethylallylamino)purine
A	Absorbance
AC	Activated charcoal
AFLP	Amplified fragment length polymorphism
AgNPs	Silver nanoparticles
ANOVA	Analysis of variance
BAC	biologically active compounds
BAP	6-Benzylaminopurine
bp	Basepair
BR	Blue and red
BSA	Bovine serum albumin
bp	Basepair
CO_2	Carbon dioxide
CI	Cultural importance index
cm	Centimetre
DAMD	Directed amplification of minisatellite DNA

DMHDA	Dimethylhexadecylamine
DNA	Deoxyribonucleic acid
ddH_2O	Double distilled water
EM	Electromagnetic
EDTA	Ethylenediaminetetraacetic acid
FAA	Formalin: Acetic acid: 95% alcohol
FR	Far-red
g	Gram
g/L	Gram per litre
GaN	Gallium nitride
h	hour
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
InGaN	Indium gallium nitride
Kn	Kinetin
ISSR	Inter simple sequence repeats
LEDs	Light-emitting diodes
mg/L	Milligram per litre
mL	Millilitre
mm	Millimeter
MS medium	Murashige and Skoog's (1962) medium
NAA	1-Naphthaleneacetic acid
NaOH	Sodium hydroxide
nm	Nanometer

PCIB	p-chlorophenoxyisobutyric acid
PAR	Photosynthetically active region
PCR	Polymerase chain reaction
PGR	Plant growth regulator
PPFD	Photosynthetic Photon Flux Density
pH	Potential hydrogen
PSI	Photosystem I
PSII	Photosystem II
R^2	R-squared, coefficient of determination in regression
RAPD	Random amplified polymorphic DNA
RGB	Red: green: blue
rpm	Revolutions per minute
SEM	Scanning electron microscope
SPAR	Single primer amplification reaction
SSR	Simple sequence repeats
T_a	Annealing temperature
TBA	Tert-butyl alcohol
TBE	Tris-Borate-EDTA
TDZ	Thidizuron
TEM	Transmission electron microscopy
TIBA	2,3,5-triiodobenzoic acid
Zn	Zeatin
UV	Ultraviolet
v/v	Volume per volume
w/v	Weight per volume

UV	Ultraviolet

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PENUBUHAN BAHAN PENANAMAN KULTUR TISU *Ficus carica* L. KULTIVAR LIBYA MERAH

ABSTRAK

Ficus carica L. var. Red Libyan, di bawah keluarga Moraceae adalah sejenis kultivar pokok tin yang terkenal dengan buahnya yang berwarna merah ungu. Kultivar ini dikenali dengan buahnya yang manis dan berperisa, yang menjadikannya popular dengan peminat dan pekebun buah tin. Mikropropagasi *Ficus carica* L. var. Red Libyan telah berjaya ditubuhkan melalui beberapa siri eksperimen dan penyelidikan secara sistematik. Penanaman dan propagasi *Ficus carica* L. var. Red Libyan adalah mencabar melalui kaedah tradisional. Ini disebabkan oleh beberapa faktor seperti pertumbuhan yang perlahan, mudah terdedah dengan penyakit, dan kekurangan sumber bahan penanaman. Tambahan pula, kekurangan protokol kultur tisu yang optima khusus untuk kultivar ini menghalang penggandaan secara besaran dan penambahbaikan genetik. Oleh itu, penyelidikan ini bertujuan membangunkan protokol mikropropagasi yang efisien untuk kultivar buah tin ini dan menilai kestabilan genetik dan fisiologinya. Kajian ini melibatkan pensterilan permukaan tisu meristem tunas, penjanaan semula pucuk, dan pertumbuhan secara *in vitro* menggunakan pelbagai pengawalaturan pertumbuhan dan pencahayaan LED. Kombinasi media MS dengan 20 µM TDZ + 1 µM NAA bersama 1.0 gL^{-1} karbon aktif menunjukkan parameter pertumbuhan yang paling baik, termasuk untuk ketinggian pucuk, bilangan daun, bilangan akar, dan panjang akar. Pancaran cahaya LED merah:biru didapati membantu pertumbuhan pucuk yang lebih tinggi. Protokol aklimatasi telah berjaya dilaksanakan, dan menghasilkan kadar pertumbuhan anak

pokok pada 100 %. Penambahan nanopartikel perak (AgNPs) telah mempengaruhi pertumbuhan tumbuhan secara positif dan negatif, menangani masalah seperti perubahan warna dan nekrosis hujung pucuk. Analisis klorofil dan karotenoid menunjukkan bahawa pucuk *in vitro* yang terdedah kepada pancaran cahaya merah:biru mengandungi pigmen yang lebih tinggi dan signifikan yang sama dengan pokok induk. Kestabilan genetik telah disahkan melalui analisa histologi dan penanda molekul, dan tiada variasi somaklonal yang diperhatikan. Penyelidikan ini telah memberikan maklumat penting mengenai mikropropagasi *Ficus carica* L. cv. Red Libyan dan juga berpeluang untuk diaplikasikan dalam bidang hortikultur dan pertanian.

ESTABLISHMENT OF TISSUE CULTURE PLANTING MATERIALS OF *Ficus carica* L. Red Libyan CULTIVAR

ABSTRACT

Ficus carica L. cv. Red Libyan, a member of the Moraceae family, is a cultivar of the common fig tree known for its purplish red fruit. This variety is known for its sweet and flavoursome figs, making it popular with fig lovers and gardeners. The micropropagation of *Ficus carica* L. cv. Red Libyan has been successfully established through a series of systematic experiments and studies. The cultivation and propagation of *Ficus carica* L. cv. Red Libyan can be challenging using traditional methods. This is due to factors such as slow growth, susceptibility to diseases and limited availability of planting material. In addition, a lack of optimised tissue culture protocols specifically for this variety hinders its efficient mass multiplication and genetic improvement. Therefore, the aim of this study was to develop an efficient and reproducible micropropagation protocol for this fig cultivar as well as to evaluate its morphological and genetic stability. The study included sterilisation of apical bud surfaces, regeneration of mature plantlets and *in vitro* propagation with different growth regulators and culture conditions. In particular, the combination of 20 µM TDZ + 1 µM NAA + 1.0 g L^{-1} activated charcoal medium showed the most favourable growth parameters, including shoot length, leaf number, root number and root length. Red:blue LED light was found to promote higher shoots during plantlet development. An acclimatisation protocol was successfully carried out and resulted in a 100 % survival rate of the plantlets. In addition, the addition of silver nanoparticles (AgNPs) had both positive and negative effects on plant growth and treated problems such as discolouration and necrosis of the shoot tips. Chlorophyll and carotenoid tests showed

that the plantlets irradiated with red-blue light had significantly higher pigment contents similar to those of the mother plant. Genetic stability was confirmed by histological examinations and molecular markers, and no somaclonal variations were observed. This research provides valuable insights into the micropropagation of *Ficus carica* L. cv. Red Libyan and paves the way for potential applications in horticulture and agriculture.

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Ficus carica L. belongs to the Moraceae family and is a flowering plant known for its significance within the Ficus genus. Typically found as deciduous shrubs or small trees, it is commonly called as "fig." This tree is native to southwest Asia and the eastern Mediterranean and holds historical importance as one of the earliest cultivated plants by humans. The fig tree's syconium, known as the common fig, is the primary edible part, characterized by its fleshy, hollow receptacle. The dried fruits of *F. carica* are notably rich in vitamins, minerals, carbohydrates, sugars, organic acids, and phenolic compounds, making them an essential dietary source (Jeong & Lachance, 2001; Veberic *et al*., 2008; Slatnar *et al*., 2011). Both fresh and dried figs are abundant in dietary fibre and polyphenols, contributing to their nutritional value (Vinson, 1999; Vinson *et al*., 2005). Recent researches has shown that figs are rich in bioactive compounds that give them various beneficial properties, including anti-hyperlipidaemic, antioxidant, antibacterial, anti-proliferative, anti-diabetic, anti-obesogenic and hepatoprotective effects (Palmeira *et al*., 2019).

The fig fruit is formed from multiple drupelets that develop from the ovaries in a closed receptacle - the syconium, which is part of the inflorescence (Weiblen, 2002; Machado *et al*., 2005; Borges *et al*., 2011). Inside the fig is a small entrance called an ostiole. This ostiole has evolved in line with certain fig wasps from the Agaonidae family and allows them to enter and pollinate (Machado *et al*., 2005; Borges *et al*., 2011; Kjellberg & Lesne, 2020). The pollination process in *F. carica*, commonly referred to as caprification, is host specific. Susceptible figs from female plants release volatile attractants to lure *Blastophaga psenes*, the specific wasp species

involved in pollination (Weiblen, 2002; Machado *et al*., 2005; Kjellberg & Lesne, 2020). This unique pollination mechanism makes manual pollination ineffective for figs. As a result, the availability of figs on the market has declined due to problems with seed viability and the absence of these host-specific pollinators in nature.

The cultivation of traditional figs involves a number of important steps, ranging from soil preparation to soil improvement and planting. An essential aspect of fig cultivation is pruning, which regulates the height of the plants and the positioning of fruit development. In regions with warm and temperate climates, figs are usually propagated by methods such as cuttings, air layering, or grafting (Gholami *et al*., 2012; Gaaliche *et al*., 2016; Silva *et al*., 2019). Air layering and cuttings are the most important methods for propagating figs via stems, as fig seeds are not viable (Flaishman *et al*., 2008; Gholami *et al*., 2012; Bussmann *et al*., 2019). In addition, traditional agricultural techniques such as stem cuttings and seed germination, although effective, are time-consuming and economically challenging. It can also be uncertain whether substantial and economically viable yields can be achieved using conventional farming methods (Gopi *et al*., 2006; Gana, 2011).

Due to the lack of effective horticultural practises for fig propagation, there is a gap in research regarding survival rates and optimal soil media for cultivation. Traditional cultivation methods require controlled growing conditions, a significant amount of labour and extensive knowledge of figs, given their susceptibility to pathogens and the vulnerability of fig quality (Flaishman *et al*., 2008; Faccoli *et al*., 2016). The fig plant is particularly susceptible to diseases such as rust, which visibly attacks the leaves, and beetles, which serve as disease vectors for the plants (Rahmani & Aldebasi, 2017). Therefore, exploring plant tissue culture as an alternative for the propagation of fig plants in Malaysia is promising.

Plant tissue culture represents a reliable alternative for the *in vitro* mass propagation of plants, ensuring a uniform and accelerated growth rate. The challenges associated with the production of large quantities of planting material can be effectively overcome through the application of plant tissue culture techniques. The term "micropropagation" refers to the use of *in vitro* methods to achieve rapid propagation of a selected plant species (Singh, 2015). The resulting plantlets are totipotent, i.e., they are genetically identical to their mother plant (Caponetti *et al*., 2018). Consequently, the nutrients and medicinal ingredients obtained from *F. carica* through tissue culture match those of the mother plants (Caponetti *et al*., 2018). Since the entire process takes place in a carefully controlled and sterile environment, variables such as seasons and weather are no longer problematic factors. This facilitates the production of new plantlets at any given time, regardless of external conditions (Ahloowalia *et al*., 2002).

Light-emitting diodes (LEDs) have established themselves as highly efficient devices for converting electrical energy into electromagnetic radiation. Their application in plant tissue culture has gained significant importance due to their energy efficiency, extended lifetime and minimal heat emission (Anjali, 2022). LEDs have been used in plant tissue culture studies since the 1980s, with a focus on evaluating the effects of light quality and intensity on the growth of tissue-cultured seedlings (Anjali, 2022; HortiPower, 2022). In contrast to conventional lighting systems such as fluorescent lamps and incandescent bulbs, LEDs have been shown to be particularly effective in regulating plant growth in tissue culture environments (Xu *et al*., 2020; Anjali, 2022).

The use of LED lamps in tissue culture laboratories is crucial to promote the growth and development of plant tissue while maintaining a near-sterile environment

(HortiPower, 2022). LED growth lights, in particular, are increasingly being used in tissue culture due to their superior efficiency, minimal energy consumption, increased safety, reliability and remarkable longevity (Mickens *et al*., 2019). These LED growth lamps are even suitable for tissue culture in tubes, and the choice of the optimal photoperiod is crucial for the success of tissue culture. The effects of LED lamps on plant growth depend on the light spectrum used. For example, red LEDs contribute to greater plant height, greater internode length and faster rooting, while blue light is closely linked to chlorophyll synthesis and stomata opening (Higrowsir, 2021). To summarise, the use of LED lamps in plant tissue culture brings numerous benefits and is a promising technology to advance the field of plant propagation.

Silver nanoparticles (AgNPs) comprise tiny silver particles with a size of 1 to 100 nm (Zivic *et al*., 2018). These nanoparticles possess special properties, including remarkable thermal and electrical conductivity, chemical stability and antimicrobial capabilities (Vera-Reyes *et al*., 2022; Mahajan *et al*., 2022). Their application spans a variety of industries, with a significant presence in the field of plant tissue culture. In this context, AgNPs are used as growth regulators and elicitors that increase the production of secondary metabolites (Arshad *et al*., 2022; Zaeem *et al*., 2022). In the field of plant tissue culture, silver nanoparticles have been shown to promote biomass accumulation of *Stevia rebaudiana* calli (Arshad *et al*., 2022). These nanoparticles were found to cause a significant increase in various parameters including total phenolic content, total flavonoid content, SOD activity, GSH content, antioxidant activity, FRAP and DPPH in *S. rebaudiana* calli compared to control groups (Arshad *et al*., 2022). The use of AgNPs in plant tissue culture requires adaptation of the culture media to facilitate their effective penetration into the plant tissue (Pérez-Caselles *et al*., 2022). Furthermore, the cultivation of plants in chloride-free liquid media is essential

for the successful integration of AgNPs into plant tissue (Pérez-Caselles *et al*., 2022). Through a careful review of the relevant research, AgNPs have been identified as prime candidates for experiments to evaluate their effects on *in vitro* cultures of *F. carica*.

The quality of the regenerated plantlets was assessed using anatomical, genetic and biochemical methods. The main objective of *in vitro* propagation is to ensure consistent genetic stability in all plantlets. This preservation of genetic integrity allows the plants to retain their original histochemistry and genetic make-up. In the evaluation of the clonal traits, both the genetic stability of the plants and the morphological similarity were analysed using biochemical techniques and microscopy. These analyses provide conclusive evidence on the effectiveness of the clonal propagation methods used (Martins *et al*., 2004; Chandrika *et al*., 2008; Zafar *et al*., 2019). Histological analysis is a valuable tool for evaluating plant consistency and understanding *in vitro* culture systems. Numerous histological protocols provide a valuable understanding of cellular processes. In addition, histological analysis has confirmed the successful regeneration of vascular and meristematic tissue (Vilela *et al*., 2019).

The study focusses on the cultivar *Ficus carica* L. cv. Red Libyan. The aim of this project was to investigate whether a refined micropropagation protocol for *F. carica* L. cv. Red Libyan combined with the application of LED lighting could produce high quality fig plantlets with robust root systems on a significant scale and within a short period of time. By establishing an *in vitro* tissue culture method for fig plants, the yield and quality of the fig fruit should be improved. At the same time, the project aimed to investigate the potential of using plant tissue culture methods for fig cultivation. This initiative not only promises to increase market supply for this

relatively little-known fruit in the country, but also offers research prospects and benefits for local growers and communities.

1.2 Objectives

The objectives of the present study are:

1. To establish contamination-free *in vitro Ficus carica* L. cv. Red Libyan explants via surface sterilisation of *ex vitro* planting materials,
2. To determine an efficient regeneration system for *in vitro Ficus carica* L. cv. Red Libyan plantlets supplemented by plant growth regulators and LEDs,
3. To investigate the effectiveness of silver nanoparticles and LEDs for micropropagation of *Ficus carica* L. cv. Red Libyan,
4. To investigate anatomical arrangement of the plantlets via histological analysis,
5. To identify polymorphisms in tissue culture plants using DAMD and ISSR molecular markers.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Moraceae family

The Moraceae family is an extremely diverse group of flowering plants, comprising about 1100 species and 40 genera, as documented by García-Cox *et al*. (2023). This family is known for its remarkable richness of individuals and species and is one of the ten families with the highest number of tree species worldwide (García-Cox *et al*., 2023). Its distribution is extensive and covers different regions around the world, including the evergreen foothill forests of the Ecuadorian Amazon (García-Cox *et al*., 2023).

One of the peculiarities of the Moraceae family is its mutual relationship with the insect family Agaonidae, which serve as important pollinators for the genus *Ficus* (Marchiori, 2022). Within the Moraceae family, Ficus is the most widespread genus, with *Ficus sp*. being the most abundant species (García-Cox *et al*., 2023). In addition, the Moraceae family has been found to make a significant contribution to carbon storage in biomass, as its trees have a remarkable carbon uptake capacity of 35.09 mg per hectare (García-Cox *et al*., 2023). This emphasises the ecological importance of the family and its potential impact on carbon sequestration efforts.

Apart from its ecological importance, the Moraceae family is also of great value to humans due to its various practical uses. Certain species within the family serve important medicinal purposes. *Broussonetia papyrifera*, for example, is known for its antimicrobial, antifungal, antioxidant, anti-inflammatory and antinociceptive properties, as highlighted in a study by Verma *et al*. 2022. The family also includes plants that are used as a food source. For example, *Ficus carica*, *F. semicordata* and

F. auriculata are commonly consumed as food, used in herbal medicine and serve as fodder plants in northeast India, as documented by Das *et al*. 2022. This emphasises their importance in nutritional and medicinal practises in the region.

Overall, the Moraceae family is characterised as a diverse and valuable group of plants that play a crucial role in ecological balance, have important medicinal properties and contribute to cultural practises through their use as a source of food and herbal medicine.

2.2 *Ficus carica* L.

Ficus carica L., commonly known as Injeer in Pakistan, shows remarkable adaptability to dry climates with high temperatures. As detailed in a review by Stover and Aradhya (2007), the six largest fig-growing countries, namely Turkey, Algeria, Egypt, Greece, Iran and Morocco, together account for about 70 % of the world's annual fig production.

Ficus carica L. is a deciduous woody plant with characteristic features. Fig trees typically grow sprawling, resulting in a greater width than their height. Their height can vary between 3 and 10 metres, depending on the genotype. They are characterised by single or multiple trunks with smooth, grey-brown bark. The leaves of *Ficus carica* L. are large, palmately lobed and deeply divided into 3 to 5 main lobes that resemble the shape of a hand. These leaves are arranged alternately and measure between 10 and 30 centimetres in length. They have a rough texture on the upper side and a lighter, somewhat velvety texture on the underside.

Fig trees are dioecious, i.e. they have separate male and female trees. The small and inconspicuous flowers of the fig tree are enclosed in a hollow, pear-shaped structure called a syconium, a modified receptacle. The fig fruit ripens from the

syconium and can vary in colour from green to purple or black depending on the degree of ripeness. The fruit contains numerous tiny seeds, although some cultivars are seedless.

The *Ficus carica* is known for its unique growth habit with a spreading canopy of leaves and roots that can become invasive if not cared for properly. This distinctive tree has cultural significance in various regions of the world.

Four different fig varieties are characterized by their pollination characteristics: common figs, Smyrna, San Pedro and Caprifigs. Common figs are persistent and do not require pollination to bear fruit. Turkey, Morocco, Iran, Egypt, and Spain are the five largest producers of figs. The annual global production of figs reaches 1.265 million tonnes (FAOSTAT, 2020).

2.2.1 Botanical classification, taxonomy, and distribution of *Ficus carica* L.

Ficus carica L., commonly known as fig, is one of the oldest cultivated fruit trees in the world with a significant presence in Morocco, Turkey and Algeria (Hmimsa *et al*., 2012; Mezhenskyj *et al*., 2022; Abdelkader *et al*., 2023). The tree, which belongs to the Moraceae family, is native to the Middle East and Western Asia, but has been introduced and cultivated in various regions (Hmimsa *et al*., 2012).

The phenology of fruiting in *Ficus carica* L. was shaped by short-term selection pressure on male function, indicating a possible evolutionary transition from monoecy to dioecy in response to seasonal climate (Hmimsa *et al*., 2012). The Western Anatolian region of Turkey plays a crucial role in the global production of dried figs, contributing almost a quarter of the total production (Ergül *et al*., 2021). In the Khorol Botanical Garden in Ukraine, a diverse collection of subtropical plants includes *Ficus carica* L. and 24 other species from different genera, families and orders of flowering

plants (Mezhenskyj *et al*., 2022). The leaves of the fig tree show considerable diversity in shape and size. Their morphological parameters prove useful for distinguishing different cultivars, regardless of their growing environment (Abdelkader *et al*., 2023).

Studies carried out in Morocco and Turkey have provided important insights into *Ficus carica* L.. In Morocco, significant differences were found between five local fig cultivars in terms of various parameters such as average fruit weight, peelability and titratable acidity (Tikent *et al*., 2022). Meanwhile, the first occurrence of *Neoscytalidium dimidiatum*, a pathogenic fungus associated with dieback and canker of common fig, was reported in a study in Turkey (Güney *et al*., 2022a). On a positive note, a recent study has developed an efficient protocol for the mass propagation of fig plantlets, particularly for the 'Violette de Solliès' variety, highlighting the potential of plant tissue culture for large-scale production (Ling *et al*., 2022). This achievement could make an important contribution to the cultivation and availability of fig plants.

2.2.2 Monoecious and dioecious *Ficus carica* L. varieties

The varieties of *Ficus carica* L. can be categorised as either monoecious or dioecious based on their flower structure and reproductive characteristics. In monoecious varieties, each individual tree has both male and female flowers. Consequently, figs produced by monoecious trees contain both male and female flowers, with the female flowers arranged in multiple ovaries inside the fig (Galil & Eisikowitch, 1971). Notable examples of monoecious *Ficus carica* L. cultivars include *Ficus carica* cv. sativa, commonly known as the edible fig.

In contrast, the dioecious varieties of *Ficus carica* L. consist of separate male and female trees. The female trees, such as *Ficus carica* cv. caprificus (caprifig), require pollination by wasps to enable fruit development. The male trees of dioecious

varieties, on the other hand, only produce pollen and wasp offspring that carry pollen. An important difference is that dioecious *Ficus carica* L. cultivars generally have less synchronised reproductive periods compared to their monoecious counterparts (Marcotuli *et al*., 2023).

The transition from monoeciousness to dioeciousness in *Ficus carica* L. is considered an evolutionary process (Marcotuli *et al*., 2020). Although morphological data indicate that *Ficus carica* L. is gynodioecious, it is functionally considered dioecious with two distinct tree morphs (Mawa *et al*., 2013). Understanding the difference between these two types of cultivars is crucial for the cultivation and propagation of fig trees, as their reproductive characteristics play an important role in fruit production and yield.

2.2.3 Types of fig plants (*Ficus carica* L.)

The genus Ficus includes various fig plants, with *Ficus carica* being the best known, originating from the Mediterranean region and western Asia. Among the 700 named varieties, there are only four species: Caprifigs, Smyrna, San Pedro and common figs (Bayoudh *et al*., 2018; Bussmann *et al*., 2019b; Cui *et al*., 2019).

Caprifigs are used exclusively to pollinate female fig trees, as they only produce male flowers and do not bear fruit. Smyrna figs, on the other hand, have only female flowers but require pollination by caprifigs in order to bear fruit. San Pedro figs lie between Smyrna and common figs in terms of their characteristics (Flaishman *et al*., 2008; Kjellberg & Lesne, 2020). In contrast, common figs, such as Brown Turkey, Celeste, Chicago Hardy, Kadota and Violette de Bordeaux, are often grown in home gardens. They do not require pollination by other trees and have no opening for pollinating wasps, which makes them less susceptible to rotting by insects and

rainwater entering the fruit (Flaishman *et al*., 2008; Taleb *et al*., 2019; Kjellberg & Lesne, 2020).

2.2.4 Reproduction system of *Ficus carica* L.

Ficus carica L. can reproduce both sexually and asexually. The fruit of *Ficus carica* L., commonly known as syconium or fig, has a unique characteristic: it can only be pollinated by certain agaonid wasps associated with the plant (Mawa *et al*., 2013). The reproductive biology of figs is complicated, with some varieties requiring pollination while others do not (Marcotuli *et al*., 2020). The relationship between fig plants and their tiny pollinating wasps is highly specialised and specific (Valdeyron & Lloyd, 1979).

In addition to sexual reproduction, fig plants can also reproduce asexually through so-called vegetative propagation. In this method, cuttings are taken from the plant and their roots are stimulated to develop, which ultimately leads to the formation of new plants (Mars, 2001). This asexual reproduction allows figs to reproduce without pollination and helps them to spread and grow successfully in different environments.

2.2.5 Economic and medicinal values of *Ficus carica* L.

Ficus carica L., commonly known as the fig tree, has traditionally been of great ethnobotanical importance to the inhabitants of the Himalayan Pakistan region. The leaves and milky latex of this plant are traditionally used to treat diseases such as boils, warts and tumours. In addition, the fruits of *Ficus carica* L. are known for their laxative properties (Abbasi *et al*., 2013). Some members of the community cultivate and commercialise this plant for its fruits and as a source of income through the sale of firewood.

In a study conducted in the Lesser Himalaya, *Ficus carica* L. received the highest average cultural importance index (CI) among edible wild vegetables in five locations. This indicates that the plant is popular and well-known in the culture studied (Abbasi *et al*., 2013). In addition, *Ficus carica* L. is highly regarded as a medicinal plant in south-west Morocco, whose leaves and fruits are consumed orally for various health problems such as diabetes, hypertension and bone fractures (El-Ghazouani *et al*., 2021). In a study by Serraclara *et al*. (1998), the blood sugar-lowering effect of a decoction made from the leaves of *Ficus carica* L. was documented for a short duration in patients with type I diabetes.

In addition, Canal and colleagues (2000) conducted experiments on streptozotocin-induced diabetic rats and confirmed the hypocholesterolemic properties of the chloroform extract from the leaf decoction of *Ficus carica* L.. This plant also has commercial importance as a drug in various herbal markets (Zahoor *et al*., 2021). An ethnopharmacological study by Akhtar *et al*. (2015) found that inhabitants in remote areas where medicinal plants are abundant consume *Ficus carica* L. orally to relieve bone pain.

In a preliminary study, Belattar and co-workers (2021) reported a remarkable anticoagulant effect of polyphenol extracts from *Ficus carica* L. in exogenous and *in vitro* coagulation assays, suggesting potential applications in the treatment of blood clotting disorders. In addition, *Ficus carica* L. has antibacterial properties, as shown in a study by Doro and colleagues in 2018. Methanolic extracts from the stem, leaves and latex of *Ficus carica* L. were found to be effective against various bacteria, including Gram-positive (e.g. *Staphylococcus aureus*) and Gram-negative (e.g. *Escherichia coli* and *Klebsiella pneumoniae*) strains.

2.2.6 Uses of *Ficus carica* L. in traditional medicine

Ficus carica L., commonly known as fig, has a long history of use in traditional medicine, where it is used for a variety of health problems related to the digestive, endocrine, reproductive and respiratory systems (Badgujar *et al*., 2014). This versatile plant is recognised as a valuable source of traditional remedies for diseases such as anaemia, cancer, diabetes, inflammation, stomach problems and liver and spleen disorders (Badgujar *et al*., 2014; Hajam & Saleem, 2016). In Unani medicine, fig is used as a mild laxative, expectorant, diuretic and deobstruent (Badgujar *et al*., 2014).

Various parts of the fig tree, including its fruits, roots and leaves, are used in traditional medicine to treat a variety of ailments such as gastrointestinal problems (colic, indigestion, loss of appetite and diarrhoea), respiratory diseases (cough, bronchitis and asthma) and skin diseases (Mawa *et al*., 2013). Through phytochemical research, primary and secondary metabolites, plant pigments and enzymes such as protease, oxidase and amylase have been isolated from the fig plant. These components, whether in fresh plant material, crude extracts or isolated forms, have shown a variety of biological and pharmacological activities (Badgujar *et al*., 2014).

2.2.7 Economic value of figs

Figs have considerable economic value, especially as a profitable and profitable alternative crop for farmers. A study carried out in Spain has shown the potential profitability of sustainable fig production in greenhouses as a promising complement to intensive horticulture in the south-east region. By growing figs, the Almeria region could strengthen its economy without relying on the import of foreign products, thus reducing direct dependence on other countries (Batlles-delaFuente *et al*., 2022).

In some regions, fig consumption is hampered by the lack of supply on the market during the season and the rapid spoilage during transport from the north. For example, figs play a crucial role in the export market in Afghanistan with significant trade with India, Pakistan, the USA, Canada and the United Arab Emirates (Mushair & Mohammadi *et al*., 2022). However, the perishability of fresh figs and limited understanding of postharvest physiology pose a challenge for prolonged storage (Flaishman *et al*., 2008; Levaj *et al*., 2010; Khapre *et al*., 2015). To solve this problem, a study in Algeria investigated how figs can be preserved by solar drying to enable year-round consumption. The study evaluated the microbiological and physicochemical properties of the dried figs and confirmed that they meet the quality standards required by Algerian commercial legislation (Loumani *et al*., 2022).

2.2.8 Diseases and pests

Fig trees are susceptible to numerous diseases and pests that can cause considerable damage to both the tree and its fruit. Some of the diseases that affect figs are vascular wilt, canker and twig blight caused by *Ceratocystis ficicola*, *Neofusicoccum parvum* and *Neoscytalidium dimidiatum*, respectively (Güney *et al*., 2022b). In addition, fig trees planted in sandy soils can be affected by root-knot nematodes (Andersen & Crocker, 2009). Studies on diseases and pests on *Ficus carica* L. plants are still lacking, especially in Malaysia. First report on the fungal diseases were leaf blights and stem canker associated with *Lasiodiplodia theobromae* and *L. brasiliensis* (Nur-Shakirah *et al.*, 2022). Collection of fig plants and fruit samples from a few states in Malaysia later has also revealed that fig plants infected with leaf blight, stem canker and fruit rot, mainly associated with fungal strains from the

Colletotrichum species, namely *Colletotrichum fructicola*, *C. siamense*, and *C. truncatum* (Nur-Shakirah *et al.*, 2023).

Regarding pests, fig trees can be attacked by various insects like psyllids, whiteflies, and the black weevil (*Aclees sp*. cf. foveatus) (El Nasr *et al.*, 2012; Lodolini *et al.*, 2020). Among these, the Saycamora fig psyllid (*Pauropsylla trichaeta* Petty) is particularly common in Egypt (El Nasr *et al.*, 2012). Leafy mistletoes from the genus *Phoradendron* can also parasitize fig trees and reduce their vigour (Lichter *et al.*, 1991).

To combat these pests, diverse methods can be employed, including the use of organic products like Laser™ to control the black weevil (Lodolini *et al.*, 2020). Moreover, dried leaves of *Artemisia annua*, *Azadirachta indica*, and *Ocimum gratissimum* have been employed to control bruchid pests during cowpea storage (Brisibe *et al.*, 2011).

2.2.9 *Ficus carica* L. cv. Red Libyan

Ficus carica L. cv. Red Libyan is a fig tree cultivar originated from Libya (Lance, 2022). Its medium-sized fruits can turn dark red when fully ripe (Figaholics, 2020). FigVarieties.com describes the main fruit figs of *Ficus carica* L. as having a berry flavour, a chewy texture and a moderate cracking of the seeds. They are known to be very sweet, with a noticeable hint of tartness (Lance, 2022). The morphology and anatomy of the *Ficus carica* L. cv. Red Libyan plant and its fruits are shown in Plates 2.1 and 2.2.

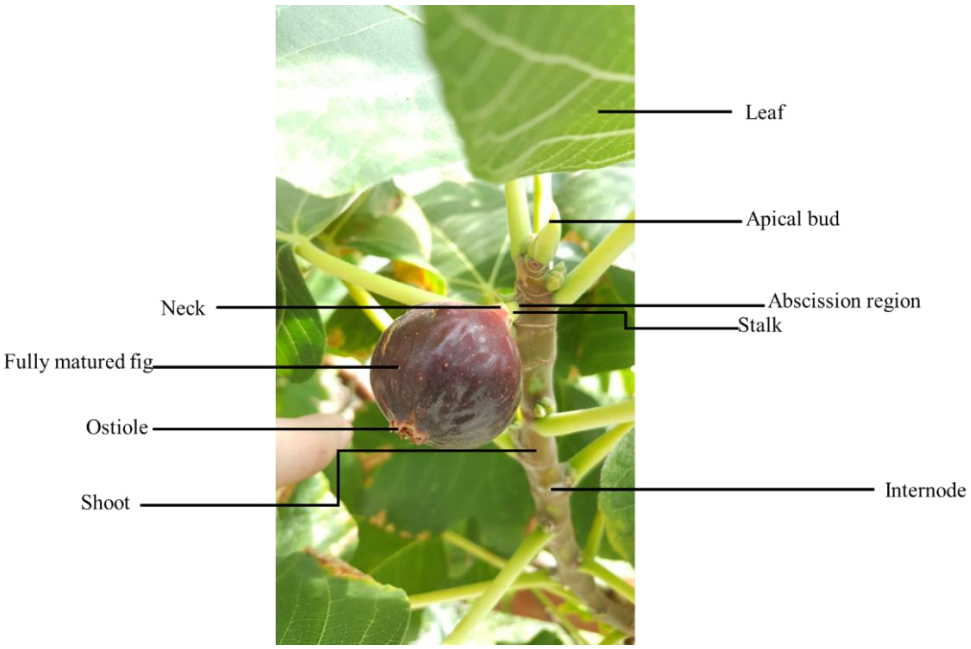

Plate 2.1: Branch of *Ficus carica* L. cv. Red Libyan with fruits. (Scale bar = 1cm)

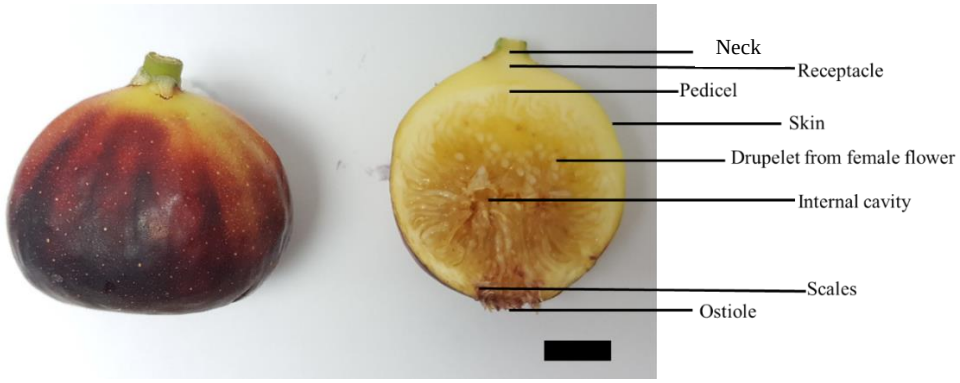

Plate 2.2: Cross-section of a matured infructescence of *Ficus carica* L. cv. Red Libyan. (Scale bar = 1 cm)

2.3 Plant tissue culture technology

There are various methods for propagating plants, but the traditional methods often require a large amount of plant material and can damage the donor plants (Sahraroo *et al*., 2019). These conventional methods are time-consuming, lead to poor root formation and result in a low survival rate.

Plant tissue culture is an established technique for culturing aseptic plant cells, tissues, organs or whole plants under sterile, controlled conditions, including nutrition and environment. This method utilises the totipotency of plant cells and tissues to generate a large number of clones in a relatively short time (Akin-Idowu *et al*., 2009; Hussain *et al*., 2012). Plant tissue culture has diverse applications in plant biotechnology and enables the mass production of high-quality seedlings for ornamental plants, medicinal plants, fruit trees, plantation crops and forest trees (Shahzad *et al*., 2017; Amare & Dugassa *et al*., 2022). It also plays a crucial role in the production of disease-free planting material for horticultural crops that are vegetatively propagated (Devanakonda & Revati, 2021). In tissue culture systems, adventitious root formation is highly dependent on the interaction between various endogenous and exogenous factors (Sahraroo *et al*., 2019).

Plant tissue culture techniques were originally used in the 1980s by commercial laboratories for the mass propagation of plants in the horticultural sector. They have evolved over time and are now used for various purposes, including somatic embryogenesis, disease and virus elimination and the production of secondary metabolites (Akin-Idowu *et al*., 2009; Hussain *et al*., 2012).

Plant tissue culture technology enables efficient, rapid and extensive plant propagation. Its widespread application can potentially preserve important medicinal

plant species while meeting the needs of a growing world population (Akin-Idowu *et al*., 2009; Faisal *et al*., 2018). The aseptic clones produced using plant tissue culture technology are genetically uniform and disease-free, resulting in higher yields, especially when pathogen-free germplasm is used (Hussain *et al*., 2012). This technology also enables the production of single-variety clones for continuous utilisation and germplasm conservation (Faisal *et al*., 2018).

Meristem size is crucial for meristem culture (Sahraroo *et al*., 2019). In a 2018 review by Wang and colleagues, they pointed out the significant influence of shoot tip size on virus elimination, with the size of shoot tip explants used positively influencing explant survival and regeneration. However, they found that the rate of virus elimination is inversely proportional to the size of the shoot tips. Therefore, the ideal or appropriate shoot tip size depends on the condition of the donor plants and the severity of the infection.

Molecular studies are often carried out to ensure the genetic uniformity of plant tissue cultures. This is crucial as somaclonal and gametoclonal variations can occur during the process, potentially affecting elite germplasm. Therefore, it is essential to perform genetic stability testing of the regenerated clones (Faisal *et al*., 2018). However, the generation of genetic variation in plants during the micropropagation process can lead to the development of disease- and insect-resistant varieties, improved growth, higher yields and drought-tolerant species in different plant species (Bridgen *et al*., 2018).

Plant tissue culture involves the aseptic cultivation of plant parts in a nutrient-rich medium in a controlled environment that is not affected by seasonal changes (Shahzad *et al*., 2017; Phillips *et al*., 2019; Amare & Dugassa *et al*., 2022). The growth

medium contains essential macro- and micronutrients, gelling agents, carbon sources and other additives required for proper plant development (Phillips *et al*., 2019).

The media used in plant tissue culture contain macro- and micronutrients, gelling agents, carbon sources and other additives that are important for plant growth and development (Phillips *et al*., 2019). The media and practises used in plant tissue culture and developmental biology have been extensively studied and reviewed (Phillips *et al*., 2019; Neumann *et al*., 2020). To reduce the cost of plant tissue culture, alternative options such as optimising the use of equipment, using bioreactors and replacing gelling agents, macro- and micronutrients and carbon sources have been proposed (Amare & Dugassa *et al*., 2022).

2.4 Plant tissue culture medium

2.4.1 Murashige and Skoog (MS) medium (1962)

Plant tissue culture media consist of several key components, including macro- and micronutrients, vitamins, gelling agents and sucrose. Numerous media formulations have been developed over the years. However, Murashige and Skoog medium (MS medium), which was introduced in 1962, is the most widely and commonly used medium for various applications in plant tissue culture. MS medium has been extensively modified and supplemented with components such as auxin, kinetin, sucrose and organic additives to tailor its effectiveness to different plant species and tissue culture methods. These adaptations aim to optimise certain growth and development parameters, such as shoot multiplication, seed germination and *in vitro* rooting. The MS medium is used and customised in a wide range of plant tissue culture applications, demonstrating its versatility and suitability for different plant species and processes.

2.4.2 Plant growth regulators

Plant growth and development depend on both nutrients and plant growth regulators (PGRs). These regulators, also known as phytohormones or plant hormones, play a crucial role in influencing different phases of a plant's life, from germination to reproduction (Gaba, 2005). PGRs are organic compounds synthesised in the plant that facilitate intercellular communication and exert their effect on physiological growth and development even at low natural concentrations (Davies, 2010; Adak *et al*., 2020).

Auxins and cytokinins are generally used as primary PGRs in plant tissue culture. Auxins promote both cell division and cell elongation, while cytokinins stimulate cell division and differentiation. In addition, other regulators such as gibberellins and abscisic acid are also used in plant tissue culture (Chandler *et al*., 2000; Rakesh *et al*., 2021; Aftab, 2022). These PGRs play a crucial role in modifying cell division, expansion and differentiation, leading to significant changes in plant growth and development (Coggins & Lovatt, 2014).

Remarkably, even minute concentrations of these hormones can have significant effects, making them highly potent in most cases (Ferguson & Grafton-Cardwell, 2014). Consequently, the addition of PGRs to culture media allows precise control of *in vitro* plant development. By strategically manipulating the types and concentrations of these regulators, it is possible to produce a large number of plants or secondary metabolites with medicinal value, enabling commercial applications.

Research on fig propagation has shown that the optimal PGRs for this purpose are cytokinins, in particular BAP, TDZ and kinetin. These cytokinins have been shown to be extremely effective in producing multiple shoots from a single explant. Fig cultivars such as Sultany, Aboudi and White Adcy responded favourably to treatment

with BAP (Mustafa & Taha, 2012). Similarly, significant regeneration and multiplication of several shoots was observed in the fig cultivar Conadria when treated with TDZ. In addition, auxins such as IAA and IBA have been shown to efficiently form roots in the process of *in vitro* shoot formation. These auxins are crucial for stimulating root formation on shoots produced in tissue culture (Zayed *et al*., 2018).

2.4.3 Cytokinin

Cytokinins, N6-substituted adenine derivatives, are important plant growth regulators that are mainly synthesised in the roots. However, they are also produced in various parts of the plant, including the cambium (Chen *et al*., 1985). *In vivo*, cytokinins are mainly found in mitotically active regions of the plant, such as shoot and root meristems, where they can effectively stimulate cell proliferation. Conversely, cytokinins occur in lower concentrations in regions where cell growth is halted (Kieber & Schaller, 2014).

Cytokinins play a central role in regulating various processes, including growth, response to nutrients, response to biotic and abiotic stress, root nodule formation, stress physiology, and various metabolic networks and pathways (Hwang *et al*., 2012; Kieber & Schaller, 2018). Recent studies have revealed the complex physiological functions of cytokinins, particularly their interactions with auxin and other signalling pathways (Hwang *et al*., 2012). In addition, cytokinins co-operate with other plant growth regulators such as auxin in various physiological and developmental processes (Haberer & Kieber, 2002; Kieber & Schaller, 2014). These processes include seed germination, dehorning, chloroplast differentiation, apical dominance, plant-pathogen interactions, flower and fruit development and leaf senescence.

As reviewed by Kieber and Schaller (2014), exogenous cytokinins have been shown to stimulate cell division in plants lacking endogenous cytokinins. Kinetin, originally isolated from the DNA of herring sperm by Miller and colleagues (1955), was identified as a cell division stimulant. It promotes the continuous growth of tobacco stem segments when accompanied by indole-3-acetic acid (IAA) and is physiologically active only in the presence of IAA. As explained by Kieber and Schaller (2014), cytokinins act as positive regulators in the proliferation of cells in the shoot apical meristem of the plant. Cytokinin response regulators modulate cytokinin signalling in plants in the shoot apical meristem.

2.4.4 Auxin

Auxin plays a crucial role in the cytokinin signalling mechanism in plants and influences the sensitivity of specific subdomains within the shoot apical meristem to cytokinin (Kieber & Schaller, 2014). These studies and reviews have highlighted the intricate interplay between auxins and cytokinins in plants.

A 2002 review by Haberer and Kieber, based on the 1957 work of Skoog and Miller, found that the relative ratio of these two plant growth regulators in the culture medium can significantly influence the initiation of rooting or shoot development in undifferentiated callus cultures. They emphasised that it is the ratio between these regulators that determines the outcome and not the absolute amount added to the medium.

Undifferentiated callus cultures tend to remain undifferentiated when exposed to a balanced cytokinin-auxin ratio. Conversely, a high cytokinin-auxin ratio promotes the induction of shoot growth, while a low cytokinin-auxin ratio promotes rooting in the culture medium (Haberer & Kieber, 2002).

2.5 Surface sterilisation

Surface sterilisation is a fundamental step in plant tissue culture technology, especially when introducing explant material from external environments into the laboratory for aseptic cultivation. Its importance lies in the removal of external contaminants from plant explants, preventing microbial contamination and ensuring the success of *in vitro* culture (Ayele & Tefera *et al*., 2018). The main goal of surface sterilisation is to eliminate all microorganisms that could grow on the culture media and plant tissue *in vitro*, while maintaining the viability and regenerative capacity of the explants (Telci *et al*., 2011). Failure to effectively sterilise the explants can pose a significant risk to the success of the *in vitro* culture procedure and result in a waste of time, effort and resources.

During surface sterilisation, the explants are usually washed under running tap water for 30 to 90 minutes immediately after being cut from the donor plants. This step is important to remove dust particles, impurities and phenolic compounds that adhere to or are secreted by the explants, which could lead to oxidation (Pasqual & Ferreira, 2007). The duration of this washing step may vary depending on the plant species, explant type and origin of the plant samples.

Sodium hypochlorite (NaOCl) is a highly reactive sterilising agent known for its strong oxidising properties. It effectively targets amino acids, nucleic acids, amines and amides and is therefore very effective in the sterilisation of plant explants against various fungi, bacteria and viruses. During the sterilisation process, NaOCl reacts with amino acids, producing corresponding aldehydes, ammonium chloride (NH_4Cl) and carbon dioxide (CO_2) (Yildiz *et al*., 2012).