Arabian Journal for Science and Engineering (2026) 51:1241–1259
https://doi.org/10.1007/s13369-025-10282-y

RESEARCH ARTICLE-BIOLOGICAL SCIENCES

Effect of Medium Compositions and Different Culture Conditions on the Physiological Properties and Clonal Micropropagation of *Ficus carica* L. cv. Sarılop Plant

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Received: 19 August 2024 / Accepted: 27 April 2025 / Published online: 23 May 2025
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Abstract
The fig (*Ficus carica* L.), a crop of significant economic value, necessitates the development of innovative production techniques. This study seeks to establish an optimized protocol for the clonal micropropagation of the 'Sarılop' cultivar under in vitro conditions, focusing on determining the ideal medium compositions and environmental conditions (LED-lighting and culture vessel type) to maximize shoot and root regeneration. Various experiments on micropropagation were conducted using different nutrient media, plant growth regulators, and their combinations, as well as varying culture vessels and conditions. The highest mean number of shoots per explant (1.23) was observed in DKW medium. An even higher shoot count per explant (3.2) was recorded using DKW medium supplemented with 2 mg/L BAP and 0.5 mg/L IBA. The greatest mean shoot number occurred under white LED lighting in 265 mL glass jars and Magenta (2.33 and 2.30 respectively). The highest mean leaf lenght was observed under white lighting in Magenta vessel as 0.89 cm. In subculture experiment, node explants were subcultured four times at four-week intervals in 55 mL glass tubes, achieving the highest multiplication rate (3.27) during the third subculture. Subsequently, rooted plantlets (53.33%) were acclimatized with a 70% success rate. Ultimately, DKW medium was identified as the most suitable basal nutrient medium, with 2 mg/L BAP and 0.5 mg/L IBA being the optimal hormone combination for shoot propagation, and Magenta as the preferred culture vessel according to physiological parametres. These findings facilitated the development of a protocol that supports commercial production.

Keywords *Ficus carica* L. · LED · Culture vessel · Clonal micropropagation · Multiplication rate · Subculture

1 Introduction

Ficus is a largest angiosperm genus belonging to the family Moraceae, consisting of approximately 800 species and 2000 varieties, occurring mostly in tropical and subtropical forests worldwide. More than 845 species of the genus *Ficus*, known as figs, are plants with edible fruit and valuable wood, and were frequently used in ancient Egypt during ancient times. [1–3]. *Ficus* spp. has many different biological activities such as antibacterial, antimicrobial, anticancer, antioxidant, anti-inflammatory and antidiabetic properties. In addition, several pharmacological studies have been supported by the ethnomedical uses of *Ficus* species due to their beneficial activities [4–6]. Some of the most important species of *Ficus* spp. are *F. carica*, *F. bengalensis* and *F. religiosa* are used as medicines in traditional medicine to treat various diseases [7, 8].

Ficus carica Linn. is an irregularly branched tree or large scattered shrub, growing up to 7–10 m high, deciduous every year [9–12]. Common fig or *Ficus carica* L. is the most popular *Ficus* species known for its outstanding commercial importance [13]. Fresh and dried fruits of fig used for cancer, ulcers, liver and spleen enlargement, its latex is used in the treatment of ulcers and gout, and its leaves are used in the treatment of cancer, tumors and dermatitis. The fruit of *F. carica* has antiplatelet effect as well as shows spasmolytic activity through activation of K^+-ATP channels. Therefore, it

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is used in intestinal motility and inflammatory disorders [14]. As a result of archaeological evidence, fig which has been claimed to have been cultivated for more than 11,000 years, has been reported to be one of the oldest tree crops and medicinal plants used by humans [5, 15]. In addition, according to the International Nut and Dried Fruit Council (INC), it is stated that world dried fig consumption is over 161 thousand tons and global dried fig export is 148,400 thousand tons [16]. Besides, among the world countries, Turkey ranks at the top in dried fig exports and meets 58% of world exports [17].

Different production techniques can be used to develop healthy and productive fig orchards. Traditional propagation methods used in fig propagation are seed propagation, cutting propagation, layering and grafting. However, figs grown from seed are not preferred due to their heterozygosity and low vitality [18–23]. Trees propagated by methods such as layering or root shoots are not preferred because they tend to produce too many root shoots and the problem of cleaning these root shoots requires additional labor [20]. In the propagation by cutting method, environmental conditions significantly affect the success rate [22, 24]. In addition to these, problems such as high frequency of insects and diseases that damage the plant in orchards, rotting of fruits during the rainy season, poor management of planting areas and frost damage occur [25, 26]. Various abiotic and biotic stress factors prevent the growth of the fruit, resulting in a decrease in income and gradual cessation of planting [27]. When all these conditions are evaluated, alternative methods are needed instead of traditional production methods [28–31]. Plant tissue culture, which covers a significant part of biotechnology, is an important factor in agricultural systems to support many pharmaceutical and industrial products [32]. Plant tissue cultures are in vitro aseptic cultures of plant cells, tissues, organs and components used in basic and applied studies and commercial applications and are carried out in solid or liquid media under defined physically and chemically controlled conditions [33, 34].

The success of a plant tissue culture study mostly depends on the selection of the right medium compositions [35]. Optimum growth and development of plant tissues varies between plants and depends on the nutritional requirements of the plants. Therefore, the medium is selected according to the type of plant to be cultured [36]. The most commonly used medium composition for plant tissue culture is Murashige and Skoog (MS) (1962) [37] or modifications of this formulation [38]. MS is a "high salt" medium due to its content of potassium and nitrogen salts. Gamborg (B5) (1968) [39] is another medium composition containing macro and micro elements, vitamins, potassium iodide and calcium chloride commonly used in plant tissue culture [40]. Gamborg's B5 medium, developed for callus culture of soybeans, contains less nitrate and ammonium salts than MS medium. Although B5 was originally developed for the purpose of obtaining callus or for use in suspension culture, it also functions as a basal medium for whole plant regeneration [41, 42]. Lloyd and McCown (1980) [43] developed WPM media for use in woody plants to counteract the salt sensitivity of some woody species [42, 44]. However, the DKW (Driver and Kuniyuki Walnut) medium developed by Driver and Kuniyuki (1984) [45] has an ammonium:nitrate ratio similar to MS, but contains less nitrogen and different salt sources than MS, this causes an increase in sulfate dose. Thus, some woody plants can benefit more from sulfate in this medium [46]. DKW, which was originally used as a medium for shoot multiplication and callus development in walnut plants, has a higher total salt content. It also has much higher doses of sulfur (~ 7x), calcium (~ 3x), and copper (~ 10x) than MS [40, 47].

Plant growth regulators (PGRs), is a term that encompasses naturally occurring hormonal compounds (phytohormones) and their synthetic analogues and can be applied to induce any physiological response in plants [48, 49]. PGRs which can be active at very low doses in plant cells as biostimulants and bioinhibitors, have the ability to regulate growth and development [50]. PGRs are grouped under different groups [51]. The most effective PGRs are, in particular, auxins, cytokinins, and auxin-cytokinin interactions. However, abscisic acid, ethylene, gibberellins and other hormone-like synthetic compounds also have regulatory roles in culture systems that should not be ignored. The most commonly used auxins in plant tissue culture media are listed as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxy-acetic acid (2,4-D) and naphthalene acetic acid (NAA). IAA is the only auxin found naturally in plant tissues. Auxins differ according to their physiological activities and the degree to which they are metabolized in tissue. In plant tissue cultures, auxins are generally used in callus production and stimulation of cell growth, induction of shoot and root formation, as well as stimulation of somatic embryogenesis [52]. It is also known that auxins increase rooting homogeneity and rooting percentage and capacity [53]. In a study conducted on "Salti Kodari" fig variety, the number of shoots per explant was 4.2 in MS medium containing 0.4 mg/L BAP and 0.2 mg/L IBA [54]. In a study conducted on Celeste variety, the number of shoots per explant was 5.83 in MS medium containing 2 mg/L BAP and 0.2 mg/L IBA [55]. The root formation-inducing ability of IBA is due to its relatively high stability. It was also found that IBA present in the medium was more stable than the cytokinin IAA after the medium was exposed to high pressure and temperature during autoclave sterilization. IBA also acts as a slow-release hormone and its effects persist longer in the medium, thus ensuring continued growth of roots [56]. In a study conducted on "Sarılop clone 37" fig variety, the multiplication coefficient was determined as 2.69 in MS medium containing 1 mg/L IBA and 1 GA3 and 5 mg/L BAP. In the study, it was

determined that PGRs doses were effective on micropropagation parameters. It was determined that when PGRs doses were reduced, the multiplication coefficient decreased and explant growth was negatively affected [57].

Cytokinins have a shoot proliferation promoting effect, especially because they are a signal molecule that plays a role in the division of meristematic cells. BAP, one of the important cytokinins, triggers multiple shoot regenerations by reducing apical dominance and promoting lethal shoot proliferation. In addition, BAP has a more stable structure than other cytokinins due to its slow metabolic rate [56]. In two different studies carried out on fig plants with different explant sources (root and axillary shoot tips) in MS medium, the number of shoots per explant was determined to be 2.57 [58] when 1 mg/L BAP was used, while it was determined to be 1.67 when 2 mg/L BAP was used [56]. A study was conducted on the effects of the medium on fig plants in "Sultani", "Aboudi" and "Conadria" varieties. In the study, the longest shoots were 3.8 cm for "Aboudi" variety and 3.5 cm for "Sultani" variety in MS medium containing 1 mg/L KIN and 1 mg/L BAP. In all experiments, successful results were obtained in KIN containing medium for all 3 varieties [59]. It was thought that this was due to the difference between KIN and BAP metabolisms. In addition, differences in endogenous PGRs levels of plant cells may also cause different responses to cytokines [60]. In another study conducted to determine the effects of BAP and KIN cytokinins on multiple shoot production in fig plant, explants were cultured in MS medium containing 3.0 mg/L BAP, the highest number of leaves per explant was recorded as 11.573, while in the presence of 2 mg/L KIN, this value was 4.91. KIN showed less stimulatory effect on shoot compared to BAP cytokinin. The reason for this situation can be explained by the fact that there are two double bonds in the BAP structure compared to the single bond in the KIN molecule [61]. In the study on "Brown Turkey" fig variety, the highest number of leaves along with longer shoots was observed on BAP alone or the combination of BAP and a lower dose of KIN (0.3 μM). However, suboptimal KIN doses showed toxic effects and the resulting shoots were short and did not form enlarged leaves [62].

Light is a vital environmental factor that affects plant photosynthesis and photomorphogenesis. Various factors such as light signals, spectral quality and intensity of light, and photoperiod affect the regulation of plant physiology, growth and other metabolic processes [63, 64]. In commercial micropropagation laboratories, the light source is one of the most important factors controlling plant morphogenesis and the metabolism of plant cell, tissue and organ cultures [65]. The lighting systems initially used for in vitro plant growth were fluorescent tubes, high-pressure sodium, metal halide and incandescent lamps with wavelengths ranging from 400 to 700 nm. Among these, fluorescent light is the most popular in plant tissue culture laboratories, consuming approximately 65% of total electricity [66]. However, fluorescent light, which is of poor quality to support plant growth, consumes high levels of energy and produces heat, so it should be placed away from plants to avoid the effects of photo-stress. In this respect, it is necessary to have an energy-efficient light that can reduce costs and increase the production and quality of tissue culture plants [67]. Light quality; affects plant germination, flowering, stomatal regulation and other developmental and biochemical processes, but plant species respond differently to light quality. One way to improve light quality for plant tissue culture is to use light emitting diodes (LED) as light sources. LEDs are considered the most economical and powerful light sources available for this purpose due to their ease of installation and maintenance [68]. LED lamps with different wavelengths (< 400 ultraviolet (UV); 400–450 violet; 450–500 blue; 500–570 green; 570–590 yellow; 590–610 orange/amber; 610–760 red; and > 760 nm infrared color) can be used alone or in combination to optimize photosynthesis. In the meantime, the selection of appropriately colored LEDs containing phytochromes that absorb red and far-red light and cryptochromes and phototropins that absorb blue light from different photoreceptors found in plants to enhance photosynthesis in in vitro conditions should be taken into account [69]. The absorption of the light spectrum varies depending on the plants. While red light plays an important role in controlling stem, petiole growth and reproductive system function, blue light regulates plant growth, leaf expansion, photomorphogenesis, stomatal opening, photosynthesis and pigment accumulation [70]. The lighting condition of the culture environment should be determined before the plant to be studied is cultured. Because light has different effects especially on plant regeneration [71].

The spectral energy distribution of RB LED light increases the net photosynthesis rates of plants cultured under this light, as the chlorophylls in the plant absorb the incoming light, and it is generally accepted that it positively affects plant growth, development and extension [72]. Plants growing in vitro and *ex vitro* exhibit different photosynthetic photon flux density or absorption capacities of light photons depending on the availability of light and the photoreceptors present in plants [73]. However, the morphological characteristics of plantlets cultured in vitro under various light spectrums vary depending on both light quality and plant species [74]. Plants are sensitive to wavelengths of light from UV (280–400 nm) to far-red (700–800 nm). Plant responses to light begin with the activation of pigments and photoreceptors (phytochromes) that are sensitive to certain wavelengths of light. The wavelengths of blue (420–450 nm) and red (600–700 nm) light are the most effective light spectrum regions for plant growth. Lights at these wavelengths are absorbed by chlorophyll a and b pigments, which initiate the photosynthetic process in plants [75]. Thus, multiple molecular forms of these

two light receptors; defined by different spectrophotometric, biochemical and physiological properties, therefore, the corresponding genes in the plant are believed to respond differently in response to environmental and physiological signals [55]. Light quality also plays an important role in photosynthesis and affects the absorption of light by chlorophyll [65]. In the study carried out on the "Black Jack" fig variety, the highest shoot number (1.47) and leaf number (3) were observed in white LED light, while the highest shoot length value (1.20) was obtained in RB LED light [65, 72, 76]. It has also been reported that the use of RB LED at a 1:1 ratio promotes multiple shoot regeneration and 1.8 multiple shoot numbers are obtained. In the study, it was determined that morphologically healthier plants developed under RB LED lights instead of blue and white LED lights. It has been observed that the leaf color of cultures grown under RB LED light is darker than the leaves of cultures grown under white LED light [72].

LEDs deliver a targeted flow of photons towards cultures in vitro. Thus, photochemical activities controlled by chlorophyll in plants are positively affected by the use of LEDs. In addition, it has been observed that the type of culture vessel and especially the type of closure used can have significant effects on the physiology and development of plantlets [77]. The type of culture vessel and lid affects both the gas composition in the vessel and the quality of light penetrating into the vessel [78]. Adequate ventilation of culture containers used for micropropagation depends on the way the culture container is closed. However, lids that allow gas exchange prevent ethylene accumulation in the culture vessel and provide the CO_2 required for assimilation [79].

Tissue culture plantlets are traditionally grown in culture vessels containing sterile medium containing essential nutrients, carbohydrates, and growth regulators [80]. The vessels are placed on horizontal shelves arranged in the culture room. The growth of plantlets is affected by conditions such as the amount, quality, distribution and air temperature of light in the inner microclimate of the vessels. External conditions and physical properties of culture vessels affect the internal microclimate of culture vessels. The most important features required for vessels are to provide homogeneous and sufficient light quality, prevent microorganism contamination, and allow gas exchange [81]. Glass materials normally used in plant tissue culture (test tubes, glass vials, glass and plastic petri dishes) are expensive and various containers are used at different stages of micropropagation. However, pre-sterilized disposable plastic petri dishes, glass bottles, and baby food jars with polypropylene lids are quite affordable and have been found to be a low-cost option [82]. Clear plastic vessels, such as "Magenta", resistant to autoclaving and washing, are often used for micropropagation but are very expensive. Additionally, repeated autoclaving of plastic vessels makes them blurred and thus reduces the transmission of light [83]. In the micropropagation study conducted on *Musa spp.* cv. Grande naine banana variety, the effects of two types of illumination (LED and fluorescent light) systems and two types of temporary immersion systems (5-L glass culture vessel, 40-L polycarbonate plastic culture vessel) were examined. The uniform distribution of white LED light from the top of the glass culture vessel resulted in a higher propagation rate for banana shoots than white fluorescent light. In addition, the wavelength range passing through the polycarbonate culture vessel shows a 10% lower transmittance compared to the wavelength passing through the glass bottle. Therefore, choosing a polycarbonate plastic vessel as the culture condition was thought to have a negative effect on banana shoot formation [76]. In another study conducted on *Vanilla planifolia* to determine the effects of different LED light qualities on in vitro shoot reproduction, the highest number of leaves was observed in plantlets grown under fluorescent light (3.6) and white LED light (3.5) culture conditions. This is because the chlorophyll content of in vitro plantlets grown under different light qualities can be correlated with the plant species or variety. Blue LED light plays an important role in chlorophyll synthesis, but in the study, it was determined that chlorophyll doses were higher in plantlets grown under white and fluorescent LED light applications compared to blue LED light [65].

Under in vitro studies carried out on different varieties of the fig species *Ficus carica* L., in addition to shoot tips [30, 57, 84–93], shoots [94], meristems [95], apical buds [29], axillary buds [96–98], leaves [98, 99], nodal segments [100–105], stem, roots and petioles [58] were also used as explant sources. For this purpose, the use of MS [29, 90–92], LS [94], WPM [96, 100] media containing different PGRs types and doses was preferred. In vitro regeneration and clonal micropropagation values had varied depending on the various fig genotypes, media and composition, and culture conditions used in the experiments. In the literature, there is only one study on the effect of DKW medium on shoot regeneration in fig plant. In this study, it was determined that the number of axillary shoots increased up to 4–5 axillary shoots as a result of subculture every three weeks by culturing the shoot tip and meristem tip explants in DKW medium [106]. In a study carried out to determine the most suitable hormone-free medium composition at different strengths for in vitro propagation of "Purple Valinhos" variety, shoot tip explants were cultured and the highest shoot percentages obtained were at WPM media, 72% and 68%, respectively [90]. DKW medium is generally known for its use in in vitro propagation of elm, walnut, ash, hazelnut, olive and hornbeam trees. In a study carried out to determine the effect of medium on shoot regeneration in pear (*Pyrus communis* L.), axillary shoot explants were cultured in four different mediums and the highest number of leaves per node was 1.70 for the "Beurre Bosc" variety and 1.53 for the 'Bartlett'

variety when they were cultured on DKW media. The differences between the media tested in the study cannot be explained solely by the high level of total ionic strength, which can inhibit the in vitro growth of woody plant species. Because DKW medium gave superior results than MS and WPM mediums for shoot regeneration and production of quality leaf explants, even though it was 8% higher than the total ion dose of MS. Although DKW contains extremely low doses of Ni^{+}, it is the only medium that contains almost twice the Zn^{++} dose of the other three media. In addition, the organics and vitamins contains are different from the MS medium, which doesn't contain pyridoxine–HCl, but contains two times nicotinic acid and 20 times thiamine-HCl. This may have contributed to the positive preconditioning effect of the DKW medium. Moreover, it was thought that the yellowing observed in explants cultured in WPM medium could be due to low nitrogen content and in this regard, it was stated that both the number of leaves and shoot regeneration were negatively affected [107]. In the study carried out on "Sultany", "Aboodi" and "White Adcy" fig varieties, explants were subcultured 3 times and it was determined that the number of subcultures was an effective parameter. As the number of subcultures increased, the number of shoot multiplications increased significantly. In addition, it was determined that as shoot elongation increased in parallel with the number of subcultures, the number of leaves decreased. It was thought that this may be due to the accumulation of the tested PGRs in higher doses than the optimum doses required by the plant for leaf, callus formation and shoot elongation. It has been stated that the accumulation of more tested PGRs is needed to increase the shoot multiplication coefficient [97].

Within the scope of this study, it was aimed to create the best clonal micropropagation protocol based on shoot and root regeneration that will produce a large number of plants by optimizing various parameters under in vitro conditions for the *Ficus carica* L. cv. Sarılop. For this purpose, experiments of different basic media (MS, WPM, DKW and B5) that do not contain PGRs were carried out to determine the appropriate media composition for clonal micropropagation of node explants. After determining the appropriate basal medium, different auxins (IBA and IAA) and cytokinins (BAP and KIN) were added to the medium at various doses (0.5, 1 and 2 mg/L) to observe organ regeneration potentials. Then, the appropriate auxin and cytokinins were tested in combination and the appropriate medium composition for shoot propagation in the fig plant was decided. In order to determine the appropriate culture vessel and lighting conditions for regeneration, different culture vessel containing the determined medium will be cultured under photoperiod conditions (16 h day/8 h night) under LED lighting and 2:1 red-blue (RB) LED lighting.

2 Materials and Methods

2.1 Materials

In this study, single-node explants prepared by removing leaves from the shoots of *Ficus carica* L. cv. Sarılop plantlets taken from in vitro donor stocks available in the Plant Cell, Tissue and Organ Culture Laboratory of Ege University Faculty of Engineering, Department of Bioengineering, were used as material in all experiments (Fig. 1).

2.2 Methods

2.2.1 Determination of Basic Media For Clonal Micropropagation

In this study, MS [37], WPM [43], DKW [45] and B5 [39] media were used to determine composition of the appropriate medium and also combination at different doses of various PGRs for clonal micropropagation (Table 1). Node explants were cultured in hormone-free MS, WPM, DKW and B5 media. After observations were made as a result of 30-day culture, the most suitable basic medium composition was decided. Root regeneration was also examined. After determining the best shoot propagation medium for shoot regeneration, the obtained shoots were transferred to glass tubes containing this medium and subcultured 4 times in 4-week periods.

2.2.2 Effects of Auxins and Cytokinins on Shoot Regeneration

In the study, experiments were carried out in media containing different auxins (IBA and IAA) and cytokinins (BAP and KIN) in order to determine the effects of PGRs. DKW medium determined in the first stage was used as the basic medium and PGRs were added at different doses (0.5, 1 and 2 mg/L). After observations were made as a result of 30 days of culture, the most appropriate auxin and cytokinin dose in terms of shoot and root regeneration was decided. The same doses of the determined auxins and cytokinins were combined to determine the most suitable medium composition (Table 2).

2.2.3 Effects of Culture Vessel and Culture Condition on Shoot Regeneration

To determine the most suitable culture vessel for micropropagation, Vitrovent, Magenta GA-7 vessels, 55 mL glass tubes, glass jars of different volumes (170 mL, 300 mL and 265 mL) were used as culture vessels (Fig. 2). The medium composition determined in the second stage of the study was used for plant growth. Culture vessels maintained under photoperiod

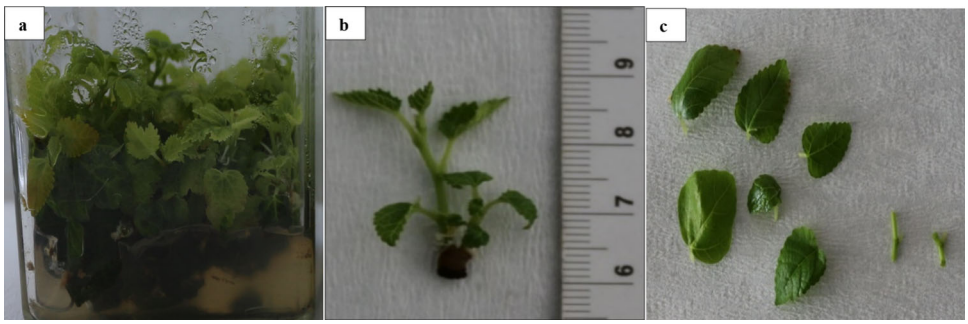

Fig. 1 In vitro*Ficus carica* L. cv. Sarılop plantlets **a** Donor stock plantlets, **b** shoot material taken from donor stock plantlets, **c** single-node explants prepared by removing leaves from the shoot

Table 1 Basic media (mg/L) used throughout all experiments[a]

Compounds	Doses of compounds in media (mg/L)			
	MS	DKW	WPM	B5
Macrocompounds				
KNO_3	1900	–	–	2500
K_2SO_4	–	1560	1700	–
NH_4NO_3	1650	1416	400	–
$(NH_4)_2SO_4$	–	–	–	134
$NaH_2PO_4.H_2O$	–	–	–	150
$MgSO_4$	–	–	–	250
$MgSO_4.7H_2O$	370	740	370.1	–
$CaCl_2.2H_2O$	440	147	96	150
$Ca(NO_3)_2.4H_2O$	–	1960	471.26	–
KH_2PO_4	170	259	170	–
Microcompounds				
$MnSO_4.H_2O$	16.9	33.5	22.3	10
KI	0.83	–	–	0.75
H_3BO_3	6.2	4.8	6.2	3.0
$ZnSO_4.7H_2O$	8.6	17	8.6	2.0
$CuSO_4.5H_2O$	0.025	0.25	0.25	0.025
$Na_2MoO_4.2H_2O$	0.25	0.39	0.25	0.25
$CoCl_2.6H_2O$	0.025	–	–	0.025
$FeSO_4.7H_2O$	27.8	33.4	27.9	27.8
Tritriplex (Na_2.EDTA)	37.3	44.7	37.3	37.3
Organic compounds				
Nicotinic acid	0.5	2.0	0.5	1.0
Pyridoxine–HCl	0.5	–	0,5	1.0
Thiamine-HCl	0.1	2.0	1.0	10
Myo-inositol	100	100	100	100
Other compounds				
Glycine	2.0	2.0	2.0	–

[a]30 g/L sucrose was added to all experiments and 3 g/L gelrite was used as a gelling agent (pH: 5.8). Glass tubes were used as culture vessels and the experiments were allowed to grow at 24 ± 2 °C, 16/8 h light/dark photoperiod conditions and 4500 lx light intensity with white LED illumination

Table 2 The content of the shoot regeneration media in which the fig plant was cultured[a]

Medium code	Doses of PGRs (mg/L)
DS1	0.5 mg/L BAP
DS2	0.5 mg/L IBA
DS3	0.5 mg/L IAA
DS4	0.5 mg/L KIN
DS5	1 mg/L BAP
DS6	1 mg/L IBA
DS7	1 mg/L IAA
DS8	1 mg/L KIN
DS9	2 mg/L BAP
DS10	2 mg/L IBA
DS11	2 mg/L IAA
DS12	2 mg/L KIN
DS13	0.5 mg/L BAP + 0.5 mg/L IBA
DS14	1 mg/L BAP + 0.5 mg/L IBA
DS15	2 mg/L BAP + 0.5 mg/L IBA

[a]30 g/L sucrose was added to all experiments and 3 g/L gelrite was used as a gelling agent (pH: 5.8). Glass tubes were used as culture vessels and the experiments were allowed to grow at 24 ± 2 °C, 16/8 h light/dark photoperiod conditions and 4500 lx light intensity with white LED illumination

(16 h light/8 h dark) at 24 ± 2 °C under white light-LED (4500 lx) and 2:1 RB light-LED (600 lx) illumination conditions for 4 weeks. At the end of the four-week culture period, different physiological changes in the plantlets will be observed.

2.2.4 Acclimatization

In in vitro conditions, 20 rooted plantlets obtained from DS-15 medium were selected and acclimatized under 4500 lx white LED light. The roots of the fig shoots, which were carefully removed from 265 mL, 300 mL glass jars and Magenta culture vessels, were washed with pure water to remove the solidifying agent residues on them. The roots were carefully placed between the rock (stone) wool, which was soaked and softened with pure water, and then placed in plastic cups with needle-pierced bottoms. They were placed in a styrofoam box prepared as a mini greenhouse environment and covered with stretch film. The transferred shoots were kept under 16 h light/8 h dark photoperiod, 4500 lx light intensity and 24 ± 2 °C conditions. Astretch film was covered over the box to ensure adaptation to *ex vitro* conditions; on the 2nd day 5 min, on the 5th day 15 min, on the 10th day 25 min, on the 18th day 45 min, on the 20th day 2 h, on the 19th day it was increased to 5 h, and the plants were ventilated and watered with some pure water. On the 20th day, the stretch was completely removed and the plants were kept completely open for 10 days in laboratory conditions. After the 30-day acclimatization period, the shoots were transferred to pots containing peat and survival rates (%) were observed after 4 weeks.

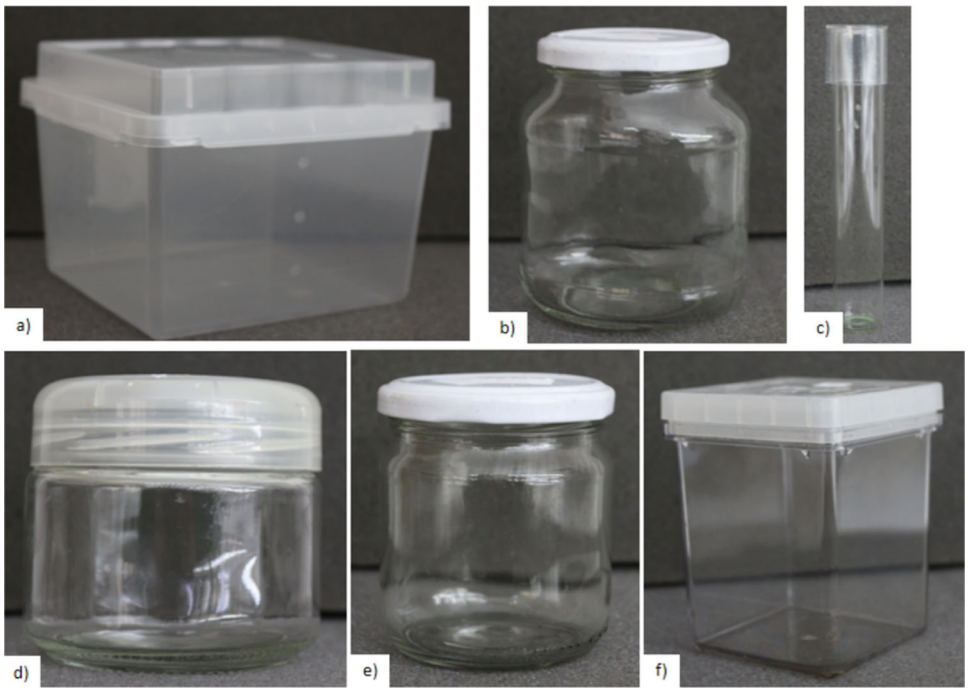

Fig. 2 Culture vessels used in the study. **a** Vitrovent (12 × 11 × 9.5 cm), **b** 300 mL jar (8 × 6.5 × 10 cm), **c** Tube (2 × 2 × 14.5 cm), **d** 265 mL jar (8 × 8 × 8.4 cm), **e** 170 mL jar (6.4 × 6.4 × 7.6 cm), **f** Magenta (7.4 × 7.4 × 9.6 cm)

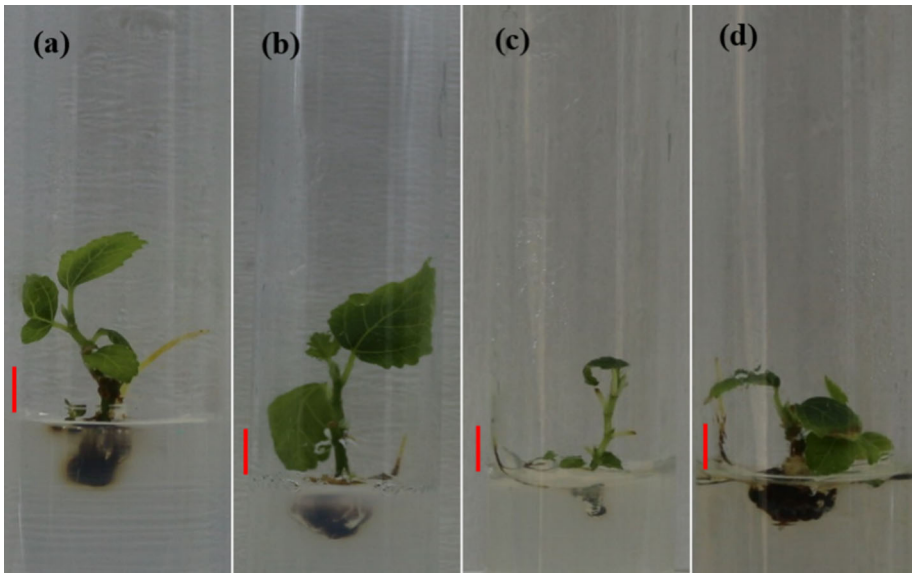

Fig. 3 Shoots regeneration in hormone-free shoot propagation media; **a** WPM, **b** DKW, **c** MS, **d** B5 (Bar: 0.5 cm)

2.2.5 Observation Parameters

Four weeks after culture; shoot regeneration percentage (%), average number of shoots (number), average shoot length (cm), average number of nodes (number), average number of leaves (number), average leaf length (cm), rooting percentage (%), average number of roots (number), average root length (cm), and multiplication coefficient and in addition to all these, physiological properties such as hyperhydricity, darkening, yellowing, necrosis were also examined as observation parameters.

2.2.6 Evaluation of Data

All in vitro applications performed throughout the study were handled in 3 replicates, with 10 explants in each experiment, according to the randomized plot design. The data obtained from the applications were evaluated with Minitab 17 (Minitab®, LLC, Pennsylvania, USA, 2015) Statistical Software program and one-way and two-way ANOVA analyzes were performed using Tukey HSD and Fisher LSD methods with a 95% confidence interval.

3 Results

3.1 Effect of Different Media on Clonal Micropropagation

Node explants were cultured in glass tubes containing hormone-free WPM, DKW, MS and B5-based media to evaluate shoot regeneration. Shoots provided from all media after 4 weeks of culture are shown in Fig. 3. The shoot regeneration percentage (%) obtained from node explants was achieved with 100% success in all media. It has been observed that newly developed shoots arise from the bottom parts of the explants and mostly 1 or 2 shoots are formed. In all experiments, physiological problems especially hyperhydricity, darkening, yellowing and necrosis, were not observed in the plantlets produced. However, it was determined that the leaves of some explants in B5 medium turned yellow. As a result of the variance analysis conducted based on the data obtained from different media experiments, "mean number of shoots" and "mean number of nodes" were found to be significant according to statistical analysis (Table 3). When the data is examined; the highest mean number of shoots per explant (1.23) and the highest mean leaf length per explant (1.27 cm) were obtained in DKW. When the highest mean number of nodes per explant values were examined, the highest result was obtained as 1.32 in the DKW medium. Since

Table 3 Some physiological parametres of culture

Media	Mean number of shoots (number) ± SE	Mean number of nodes (number) ± SE	Mean leaf length (cm) ± SE
WPM	1.03 ± 0.03^{b}	1.60 ± 0.11^{a}	0.69 ± 0.07^{b}
DKW	1.23 ± 0.07^{a}	1.62 ± 0.04^{a}	1.27 ± 0.13^{a}
MS	1.00 ± 0.00^{b}	1.23 ± 0.09^{ab}	0.95 ± 0.04^{ab}
B5	1.03 ± 0.03^{b}	1.17 ± 0.09^{b}	0.78 ± 0.09^{b}
p Value	0.01*	0.01*	0.009**

*Significant at $p < 0.05$ level, **Significant at $p < 0.01$ level. *SE* Standard Error
Different letters in the column show the level of signifcance

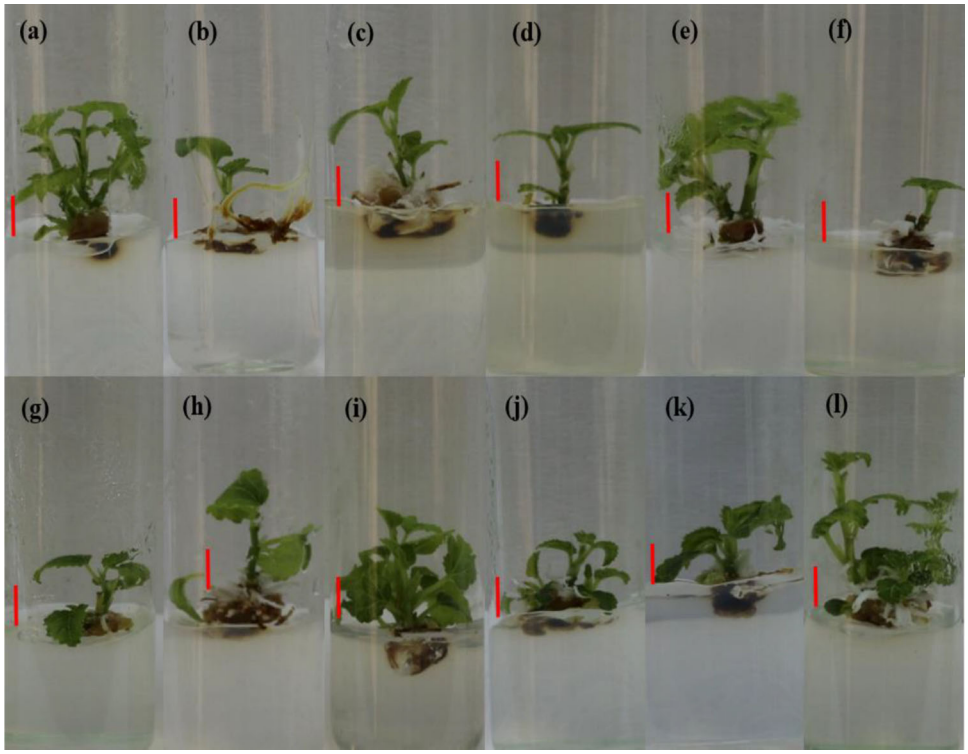

Fig. 4 Elongation of shoots in DKW media containing PGRs at different doses; **a**–**d** DKW media containing 0.5 mg/L BAP, IBA, IAA and KIN resp., **e**–**h** DKW media containing 1 mg/L BAP, IBA, IAA and KIN resp., **i**–**l** DKW media containing BAP, IBA, IAA and KIN, resp., (Bar 0.5 cm)

the mean number of regenerated shoots reached the highest rate in the DKW medium, it was decided to continue the next stages of the study with this medium.

3.2 Effects of Auxin and Cytokinin Experiments on Shoot Regeneration

In order to determine the effects of PGRs on shoot regeneration, node explants were subjected to shoot propagation containing different ddoses of BAP, KIN, IBA and IAA (0.5, 1 and 2 mg/L), using DKW as the basal medium that were cultured in glass tubes in medium (DS) (Table 2). As a result of the observations made 4 weeks after the culture, the elongation of regenerated shoots obtained from DKW media containing PGRs at different doses is shown in Fig. 4., the mean values are shown in Table 4. The shoot regeneration percentage (%) obtained from cultured node explants was achieved with 100% success in all media. It was also observed that the newly developed shoots emerged from the bottom parts of the explants and mostly 1 or 2 shoots were formed. As a result of the statistical analysis performed, many parameters were found to be statistically significant (Table 4). Since the highest rate (2.67 number) was reached in the DS-9 (2 mg/L BAP) medium as the mean number of regenerated shoots, it was decided to continue with the DKW medium containing 2 mg/L BAP in the following stages of the study. As the mean number of regenerated roots, the highest rate (2.17 number) was reached in DS-2 (0.5 mg/L IBA), in the following stages of the study, it was also decided to continue with DKW medium containing 0.5 mg/L IBA as auxin.

3.3 Effects of PGRs Combination on Shoot Regeneration

Node explants were examined using combinations of PGRs, IBA (0.5 mg/L) and BAP (0.5, 1 and 2 mg/L) at different doses, in which DKW was used as the basal medium to evaluate its effect on shoot regeneration. As a result of the observations made 4 weeks after culture, the mean values are shown in Table 5. (Fig. 5.). The shoot regeneration percentage (%) obtained from cultured node explants was achieved with 100% success in all media. The formation of multiple shoots (3–5 numbers) was achieved in DKW medium containing 2 mg/L BAP and 0.5 mg/L IBA. As the mean number of regenerated shoots, the highest rate (3.2 number) was reached in DS-15 (2 mg/L BAP and 0.5 mg/L IBA) medium. In the next stages of the study, it was decided to continue with the DKW medium composition containing the combination of 2 mg/L BAP and 0.5 mg/L IBA.

3.4 Effect of Culture Vessel and Culture Condition on Shoot Regeneration

Node explants were cultured in DKW medium containing a combination of 2 mg/L BAP and 0.5 mg/L IBA (DS-15),

Table 4 Different parameters observed at different doses of BAP, IBA, IAA and KIN

PGRs	Doses (mg/L)	Mean number of shoots (number) ± SE	Mean shoot length (cm) ± SE	Mean number of nodes (number) ± SE	Mean number of leaves (number) ± SE	Mean leaf length (cm) ± SE	Mean root regeneration percentage (%) ± SE	Mean number of roots (number) ± SE	Mean root length (cm) ± SE
BAP	0.5	1.91 ± 0.16^{bcd}	1.38 ± 0.02^{ab}	2.08 ± 0.02^{b}	5.48 ± 0.10^{bc}	0.92 ± 0.04^{abcd}	56.67 ± 17.6^{abc}	0.56 ± 0.18^{b}	0.18 ± 0.04^{bc}
	1	1.97 ± 0.12^{bc}	1.16 ± 0.044^{bcd}	2.13 ± 0.03^{ab}	5.93 ± 0.29^{b}	0.86 ± 0.02^{abcd}	10.00 ± 5.77^{c}	0.10 ± 0.06^{b}	0.04 ± 0.03^{c}
	2	2.67 ± 0.09^{a}	1.29 ± 0.07^{abc}	2.16 ± 0.00^{ab}	8.20 ± 0.43^{a}	0.85 ± 0.01^{abcd}	30.00 ± 10^{bc}	0.33 ± 0.07^{b}	0.09 ± 0.03^{c}
IBA	0.5	1.00 ± 0.00^{e}	0.95 ± 0.07^{de}	1.37 ± 0.167^{d}	2.76 ± 0.07 e	0.76 ± 0.01^{d}	100 ± 0.00^{a}	2.17 ± 0.27^{a}	0.63 ± 0.07^{a}
	1	1.27 ± 0.07^{de}	0.84 ± 0.03^{e}	1.75 ± 0.06^{bcd}	4.47 ± 0.18^{bcd}	0.84 ± 0.05^{bcd}	46.67 ± 8.82^{abc}	0.46 ± 0.09^{b}	0.21 ± 0.04^{abc}
	2	1.13 ± 0.07^{e}	0.84 ± 0.05^{e}	1.82 ± 0.02^{bcd}	4.32 ± 0.10^{cd}	0.75 ± 0.01^{d}	73.3 ± 6.67^{ab}	0.73 ± 0.07^{b}	0.38 ± 0.07abc
IAA	0.5	1.37 ± 0.09^{cde}	1.07 ± 0.02^{cde}	1.78 ± 0.07^{bcd}	4.23 ± 0.26^{cde}	0.89 ± 0.04^{abcd}	83.33 ± 12ab	0.83 ± 0.12^{b}	0.33 ± 0.06^{abc}
	1	1.20 ± 0.06^{e}	1.04 ± 0.02^{cde}	1.93 ± 0.06^{bc}	4.03 ± 0.03^{cde}	0.80 ± 0.03^{cd}	76.67 ± 8.82 ab	1.00 ± 0.10^{b}	0.52 ± 0.04^{ab}
	2	1.13 ± 0.03e	0.95 ± 0.01^{de}	1.93 ± 0.06^{bc}	3.50 ± 0.03^{de}	0.94 ± 0.02^{abcd}	70.00 ± 10^{abc}	1.20 ± 0.06^{a}	0.54 ± 0.05^{ab}
KIN	0.5	1.40 ± 0.06^{cde}	0.90 ± 0.04^{de}	1.57 ± 0.06^{cd}	3.37 ± 0.06^{de}	1.06 ± 0.04^{ab}	83.33 ± 12^{ab}	0.83 ± 0.12^{b}	0.30 ± 0.07^{abc}
	1	1.50 ± 0.20^{cde}	1.25 ± 0.03^{abc}	2.11 ± 0.08^{ab}	4.03 ± 0.46^{cde}	1.07 ± 0.06^{a}	56.67 ± 8.82^{b}	0.77 ± 0.42^{b}	0.41 ± 0.16^{abc}
	2	2.50 ± 0.17^{ab}	1.48 ± 0.09^{a}	2.56 ± 0.12^{a}	5.40 ± 0.27^{bc}	0.98 ± 0.04^{abc}	30.0 ± 25.2^{bc}	0.30 ± 0.25^{b}	0.06 ± 0.04^{c}
P Value:									
PGRs		0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Dose (D)		0.00**	0.087	0.00**	0.00**	0.543	0.00**	0.001**	0.163
PGR*D		0.00**	0.00**	0.00**	0.00**	0.046*	0.338	0.00**	0.001**

*Significant at $p < 0.05$ level, **Significant at $p < 0.01$ level. *SE* Standard Error
Different letters in the column show the level of signifcance

Table 5 Different parametres ere observed at different BAP doses

PGRs	BAP		
Doses (mg/L)	0.5	1	2
Mean number of shoot (number) ± SE	2.5 ± 0.36	2.5 ± 0.06	3.2 ± 0.11
Mean shoot lenght (cm) ± SE	1.43 ± 0.04	1.27 ± 0.05	1.01 ± 0.47
Mean number of nodes (number) ± SE	2.53 ± 0.10	2.33 ± 0.04	2.38 ± 0.04
Mean number of leaves (number) ± SE	2.73 ± 0.19	6.10 ± 0.33	6.06 ± 0.11
Mean leaf lenght (cm) ± SE	0.91 ± 0.03	0.85 ± 0.00	0.84 ± 0.01
Mean root regeneration percentage (%) ± SE	40.00 ± 0.00	36.37 ± 8.82	53.33 ± 3.33
Mean root number (number) ± SE	0.40 ± 0.00	0.37 ± 0.09	0.53 ± 0.03
Mean root lenght (cm) ± SE	0.17 ± 0.03	0.14 ± 0.03	0.15 ± 0.02

SE Standard Error

in order to determine the effects of the culture vessel and light condition on shoot regeneration. Vitrovent, Magenta GA-7 vessels, 55 mL glass tubes, glass jars of different volumes (170 mL, 300 mL and 265 mL) were used as culture vessels (Fig. 6). In illuminating the cultures, 4500 lx white LED light and 2:1 RB LED light (600 lx) were tried. As a result of the observations made 4 weeks after the culture, the mean values are shown in Table 6 (Fig. 7). The shoot regeneration percentage (%) was achieved with 100% success in all media. As a result of the study, although culture vessels and light were found to be statistically effective parameters separately, their interaction was also determined to be an effective factor (Table 6). The highest mean number of shoot (unit) was observed at white LED light, in Vitrovent and 265 mL jar (2.30 and 2.33 respectively). Additionally, the highest mean number og node was achieved glass tube*RB LED light experiment as 2.68 unit.

3.5 Effect of Number of Subcultures on Multiplication Coefficient and Shoot Regeneration

Node explants obtained from shoot regeneration experiments were subcultured 4 times in DS-15 medium with 4-week intervals and multiplication coefficients calculated (Fig. 7). In all experiments, physiological problems such as hyperhydricity, darkening, yellowing and necrosis were not observed in the produced plantlets. As a result of all the data obtained, it was shown that the number of subcultures was statistically significant. The highest multiplication coefficient (3.27) was obtained in the 3rd subculture. In addition, different physiological parameters were examined in the study and these values were found to be statistically significant. As a result of the study, it was determined that the number of subcultures was not only an effective factor on the root regeneration percentage (Table 7).

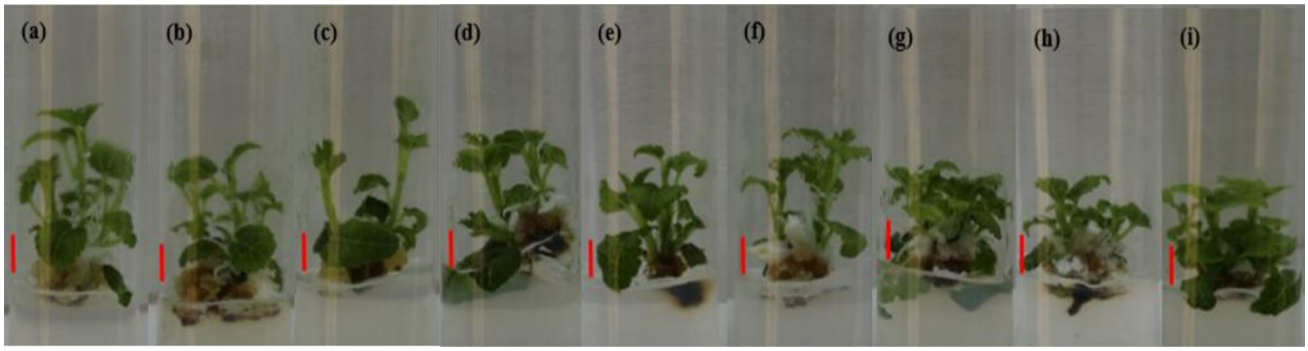

Fig. 5 Elongation of regenerated shoots obtained from DKW media containing combinations of 0.5 mg/L IBA and BAP at different doses **a–c** Shoot lengths in combination of 0.5 mg/L BAP and 0.5 mg/L IBA, **d–f** Shoot lengths in combination of 1 mg/L BAP and 0.5 mg/L IBA, **g–i** 2 shoot lengths on combination of mg/L BAP and 0.5 mg/L IBA (Bar 0.5 cm)

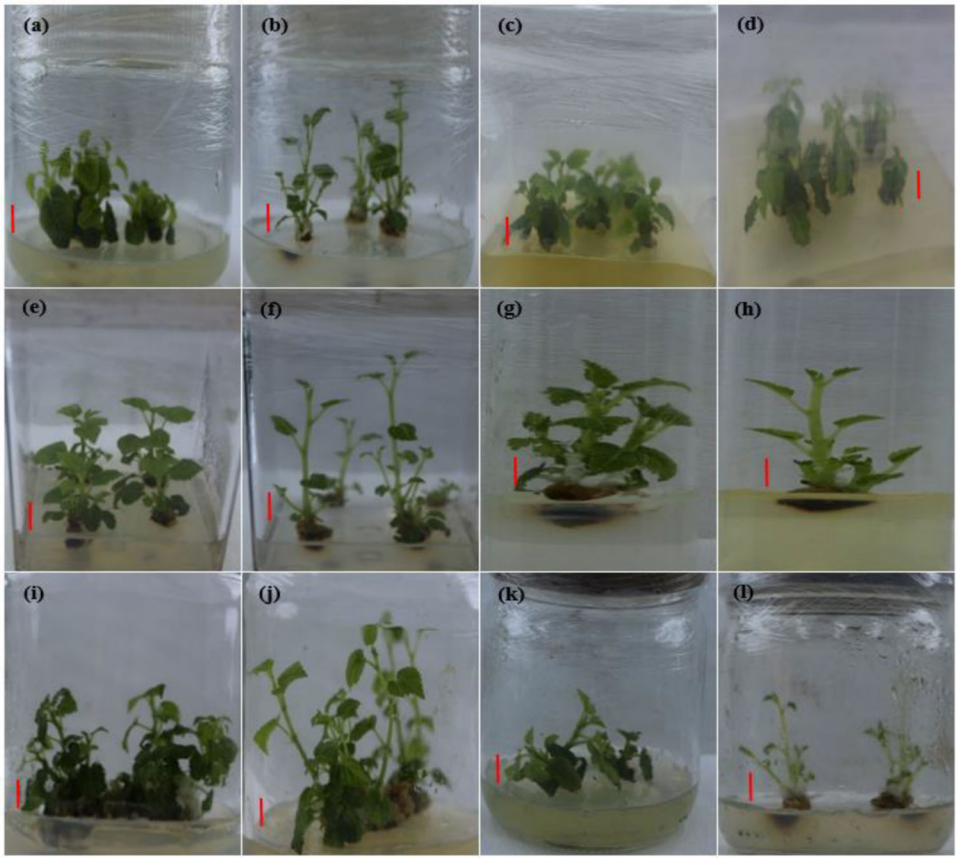

Fig. 6 Regenerated shoots grown in in different culture vessel and light conditions; **a–b** In 265 mL glass jars, under white LED light and RB LED light, resp., **c–d** In Vitrovent culture vessels, under white LED light and RB LED light, resp., **e–f** In magenta culture vessels, under white LED light and RB LED light, resp. under RB LED light, **g–h** in 55 mL glass tubes under white LED light and RB LED light, resp., **i–j** in 300 mL glass jars under white LED light and RB LED light, resp., **k–l** in 170 mL glass jars under white LED light and RB LED light, resp. (Bar: 0.5 cm)

3.6 Acclimatization

Under 4500 lx white LED light and from the medium coded DS-15 (2 mg/L BAP + 0.5 mg/L IBA), 20 rooted plantlets were selected and acclimatized after 4 weeks. On the 20th day, the stretch on the mini greenhouse was completely removed and the plants were kept completely open for 10 days in laboratory conditions at a light intensity of 4500 lx, a 16 h light/8 h dark photoperiod and a temperature of 24 ± 2 °C. At the end of 30 days, 14 shoots from pots containing peat were successfully acclimatized. Acclimatization success was recorded as 70% after 3 moths (Fig. 8). Damage to plant roots during the acclimatization process due to their very long and thin roots are factors that reduce acclimatization success. In this study, it is thought that working with plants with thicker and shorter roots will increase acclimatization success.

4 Discussion

The most important factor affecting success in plant tissue cultures is the determination of the appropriate basic medium composition. In the study, experiments were carried out for this purpose and as a result of the four-week culture period, the highest mean number of shoots per explant (1.23 number), the highest mean number of leaves (2.7 number) and leaf length (1.27 cm) values were obtained in DKW medium. Therefore, it was decided to use DKW as the basal medium in the remaining part of the study. In all nutrient medium experiments in the study, 100% shoot regeneration was achieved and it was determined that all new shoots were in the bottom parts of the node explants. In other studies in the literature [106], this situation was different and it was thought that the reason for this difference could be genotype. A research conducted by Das et al. (1998), histological studies have been shown that shoot buds differentiate from cortical cells. It has been understood that the pH of the regeneration medium has a significant effect on the regeneration efficiency. In the study carried out on *Vigna mungo* L. plant, it was determined that newly developing shoot buds were formed away from the base of the explant and were associated with the calluses on the explants [108].

In the study conducted to determine the effects of various PGRs at different doses on shoot regeneration, the highest mean number of shoots (2.67 number) and the highest mean number of leaves (8.20 number) per explant were those containing 2 mg/L BAP; the highest mean shoot length (1.48 cm) and the highest mean number of nodes (2.56 numbers) containing 2 mg/L KIN; the highest mean leaf length (1.07 cm)

Table 6 Parametres observed at different culture vessels and light conditions

Culture vessel	Light condition	Mean number of shoots (number) ± SE	Mean shoot length (cm) ± SE	Mean number of nodes (number) ± SE	Mean number of leaves (number) ± SE	Mean leaf length (cm) ± SE	Mean root regeneration percentage (%) ± SE	Mean number of roots (number) ± SE	Mean root length (cm) ± SE
Glass tube	White LED light	2.23 ± 0.14^{ab}	1.27 ± 0.27^{ab}	2.44 ± 0.05^{ab}	4.89 ± 0.19^{ab}	0.81 ± 0.03^{abc}	56.67 ± 6.67^{abc}	0.63 ± 0.09^{bcde}	0.18 ± 0.02^{bcd}
	RB LED light	1.87 ± 0.12^{bc}	1.57 ± 0.19^{a}	2.68 ± 0.11^{a}	4.09 ± 0.20^{bc}	0.54 ± 0.01^{fg}	53.33 ± 6.67^{abc}	0.70 ± 0.11^{abcd}	0.18 ± 0.01^{bcd}
170 mL jar	White LED light	2.03 ± 0.18^{abc}	1.03 ± 0.07^{b}	2.32 ± 0.05^{ab}	4.56 ± 0.11^{ab}	0.79 ± 0.03^{abcd}	33.33 ± 12^{c}	0.33 ± 0.12^{ef}	0.14 ± 0.06^{bcd}
	RB LED light	2.13 ± 0.19^{abc}	1.44 ± 0.19^{a}	2.02 ± 0.25^{ab}	2.93 ± 0.03^{d}	0.48 ± 0.03^{g}	46.67 ± 13.3^{bc}	0.47 ± 0.13^{def}	0.16 ± 0.04^{bcd}
300 mL jar	White LED light	2.10 ± 0.11^{abc}	1.33 ± 0.07^{ab}	2.18 ± 0.02^{ab}	4.52 ± 0.11^{abc}	0.87 ± 0.04^{ab}	43.33 ± 6.67^{bc}	0.43 ± 0.07^{def}	0.15 ± 0.03^{cd}
	RB LED light	2.07 ± 0.17^{abc}	1.55 ± 0.05^{a}	2.09 ± 0.02^{ab}	4.49 ± 0.10^{abc}	0.64 ± 0.01^{ef}	30.00 ± 0.00^{c}	0.30 ± 0.00^{f}	0.11 ± 0.01^{d}
265 mL jar	White LED light	2.33 ± 0.07^{a}	1.33 ± 0.07^{a}	2.33 ± 0.21^{ab}	4.96 ± 0.10^{a}	$0.75 \pm 0.04b^{cde}$	80.00 ± 5.77^{a}	0.80 ± 0.06^{abc}	0.24 ± 0.04bc
	RB LED light	2.10 ± 0.06^{abc}	1.51 ± 0.12^{a}	2.14 ± 0.17^{ab}	4.04 ± 0.20^{c}	0.68 ± 0.01^{de}	86.67 ± 23.3^{a}	0.90 ± 0.10^{a}	0.34 ± 0.05a
Magenta	White LED light	2.30 ± 0.15^{a}	1.29 ± 0.07^{ab}	2.62 ± 0.03^{ab}	4.81 ± 0.08^{a}	0.89 ± 0.02^{a}	43.33 ± 8.82^{bc}	0.43 ± 0.09^{def}	0.13 ± 0.04^{cd}
	RB LED light	1.73 ± 0.22^{c}	1.44 ± 0.01^{a}	1.85 ± 0.30^{b}	4.65 ± 0.21^{a}	0.87 ± 0.00^{ab}	70.00 ± 5.77^{ab}	0.83 ± 0.19^{ab}	0.25 ± 0.03^{ab}
Vitrovent	White LED light	2.30 ± 0.06^{a}	1.52 ± 0.02^{a}	2.20 ± 0.16^{ab}	4.48 ± 0.20^{abc}	0.80 ± 0.02^{abcd}	53.33 ± 14.5^{abc}	$0.53 \pm 0.14b^{cdef}$	0.15 ± 0.03^{cd}
	RB LED light	2.27 ± 0.07^{ab}	1.56 ± 0.06^{a}	2.29 ± 0.20^{ab}	4.10 ± 0.34bc	0.74 ± 0.00^{cde}	50.00 ± 5.77^{abc}	0.50 ± 0.06^{cdef}	0.18 ± 0.06^{cd}
P Value:	Light condition (LC)	0.026*	0.010*	0.081	0.00**	0.00**	0.929	0.164	0.113
	Culture vessel (CV)	0.368	0.366	0.159	0.00**	0.00**	0.074	0.001*	0.009**
	LC*CV	0.194	0.806	0.071	0.00**	0.00**	0.256	0.251	0.284

*Significant at $p < 0.05$ level, **Significant at $p < 0.01$ level. *SE* Standard Error
Different letters in the column show the level of signifcance

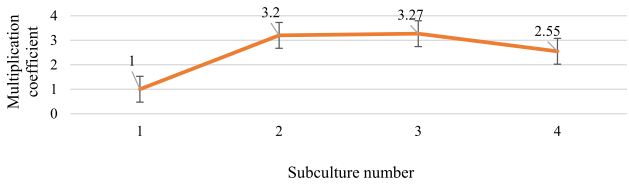

Fig. 7 Multiplication coefficients obtained after 4 weeks of subculture of node explants

was achieved in DKW medium containing 1.0 mg/L KIN. In fig plant, increasing BAP and decreasing IBA doses when used alone led to an increase in shoot regeneration, and increasing KIN doses when used alone led to an increase in shoot length. The results obtained are similar to the data in the literature [58–60, 62]. The addition of low doses of cytokinins or their combination with auxin to the medium isn't thought to promote the induction of fig shoots. BAP, an exogenous hormone, its role in the micropropagation stage is to induce the formation of shoot xylem tissue, which will facilitate the conversion of water and nutrients by leading to shoot growth [109]. In our study, shoot regeneration was achieved with a 100% success rate in all media of node explants cultured to determine the effects of combinations of BAP and IBA at different doses on shoot regeneration. The highest mean number of shoots per explant (3.2) was obtained with 2 mg/L BAP and 0.5 mg/L IBA; the highest mean shoot length (1.43 cm) and the highest mean number of nodes (2.53 number) were obtained from 0.5 mg/L BAP and 0.5 mg/L IBA; the highest mean number of leaves (6.10 number) was reached in DKW medium containing the combination of 1 mg/L BAP and 0.5 mg/L IBA. For this reason, it was decided to continue with DKW medium containing a combination of 2 mg/L BAP and 0.5 IBA. In another study, it was stated that culturing nodal explants of "Roxo de Valinhos" fig variety in WPM medium containing higher than 2.0 mg/L BAP dose resulted in vitrified shoots with yellowed leaves [110]. It has been stated that shoot and leaf development can be stimulated if the ratio of cytokinin in the medium is higher than auxin. Conversely, if the proportion of cytokinin used in the medium is lower than the proportion of auxin, then stimulation of root growth will result. It has been reported that when the ratio of cytokinin and auxin combinations in the medium is balanced, shoot, leaf and root growth will also be balanced [72]. The data obtained in the study were similar to the literature data [54, 55, 72].

In the current study, which was conducted to determine the effects of different culture vessel types and LED light conditions on shoot regeneration, node explants were cultured in DKW medium containing a combination of 2 mg/L BAP and 0.5 mg/L IBA. The highest mean number of shoots per explant (2.33) was observed in the interaction between a 265 mL glass jar culture vessel and 4500 lx white light-LED condition; the highest mean shoot length (1.57 cm) was observed in the interaction between a 55 mL glass tube culture vessel and 2:1 RB LED light (600 lx); the highest mean number of leaves (4.96 number) was obtained in the 265 mL glass jar culture vessel and the highest mean leaf length (0.89 cm) in interaction with the Magenta culture vessel under 4500 lx white LED light condition. In our study obtained better results than [111]. Although there are no studies on the effect of culture vessel and LED light condition interaction on shoot regeneration and shoot length in *Ficus carica* L., these studies conducted on different plant species [65, 72, 76] have shown that the use of various LED light conditions and culture vessel types can affect shoot regeneration, shoot length, its effect on the number and length of leaves was similar to the data in this study.

In micropropagation of plants, rooting is considered a critical stage as it determines the survival of the plant during acclimatization. In the current study, which was conducted to evaluate the effect of different media on root regeneration percentage, root number and length, node explants were cultured in 4 different hormone-free media (WPM, DKW, MS and B5). As a result of the observations, the highest root regeneration percentage (50%) and the highest mean number of roots (0.57 number) per explant were obtained in WPM and MS media, while the highest mean root length (0.37 cm) was observed in MS medium. However, the highest rooting percentage per explant (80.83%), the highest mean number of roots (2.17 number) and the highest mean root length (0.63 cm) were achieved in DKW medium containing 0.5 mg/L IBA. Borkehyli et al. [112] stated that in micropropagation studies carried out on woody plants, media containing lower doses of nitrogen and potassium salts such as WPM increased the number of roots. The results obtained in our study are consistent with [112], which stated that the use of WPM and MS media increased the number and length of roots. Studies were carried out on "Violette de Solliès" fig variety in WPM media containing different doses of IBA, and 6.8 root regeneration was observed in the addition of 3 mg/L IBA. However, the roots developed in two different ways. These were thin, hairy aerial roots formed above the medium and thick, brittle roots embedded in the medium [56]. In this study, rooting occurred in a similar manner.

Repeated subcultures make it possible to obtain a more stable and homogeneous culture in micropropagation of many woody plant species. The addition of PGRs to such stable shoot propagation media can result in higher shoot multiplication coefficients compared to previous subcultures [113]. In this study, in order to evaluate the effect of the number of subcultures on the multiplication coefficient, node explants obtained from shoot regeneration experiments were subcultured 4 times in DS-15 medium. Shoot regeneration of the cultured node explants was achieved with a 100% success

Table 7 Observed parameters according to the number of subcultures

SN[a]	Mean shoot length (cm) ± SE	Mean number of nodes (number) ± SE	Mean number of leaves (number) ± SE	Mean leaf length (cm) ± SE	Mean number of roots (number) ± SE	Mean root length (cm) ± SE
1	1.62 ± 0.11^{a}	1.55 ± 0.08^{c}	2.70 ± 0.15^{c}	1.30 ± 0.10^{a}	0.57 ± 0.09^{a}	0.28 ± 0.02^{a}
2	1.17 ± 0.03^{b}	2.39 ± 0.05^{a}	6.06 ± 0.11^{a}	0.85 ± 0.01^{b}	0.53 ± 0.03^{a}	0.15 ± 0.02^{b}
3	1.28 ± 0.07^{ab}	2.06 ± 0.08^{b}	4.89 ± 0.19^{b}	0.86 ± 0.03^{b}	0.41 ± 0.04^{ab}	0.21 ± 0.05^{ab}
4	1.24 ± 0.10^{ab}	2.30 ± 0.07^{ab}	5.28 ± 0.25^{ab}	0.90 ± 0.04^{b}	0.26 ± 0.02^{b}	0.12 ± 0.01^{b}
p Value:	0.02*	0.00**	0.00**	0.00**	0.01*	0.01*

[a]Subculture number, *Significant at $p < 0.05$ level, **Significant at $p < 0.01$ level. *SE* Standard Error
Different letters in the column show the level of signifcance

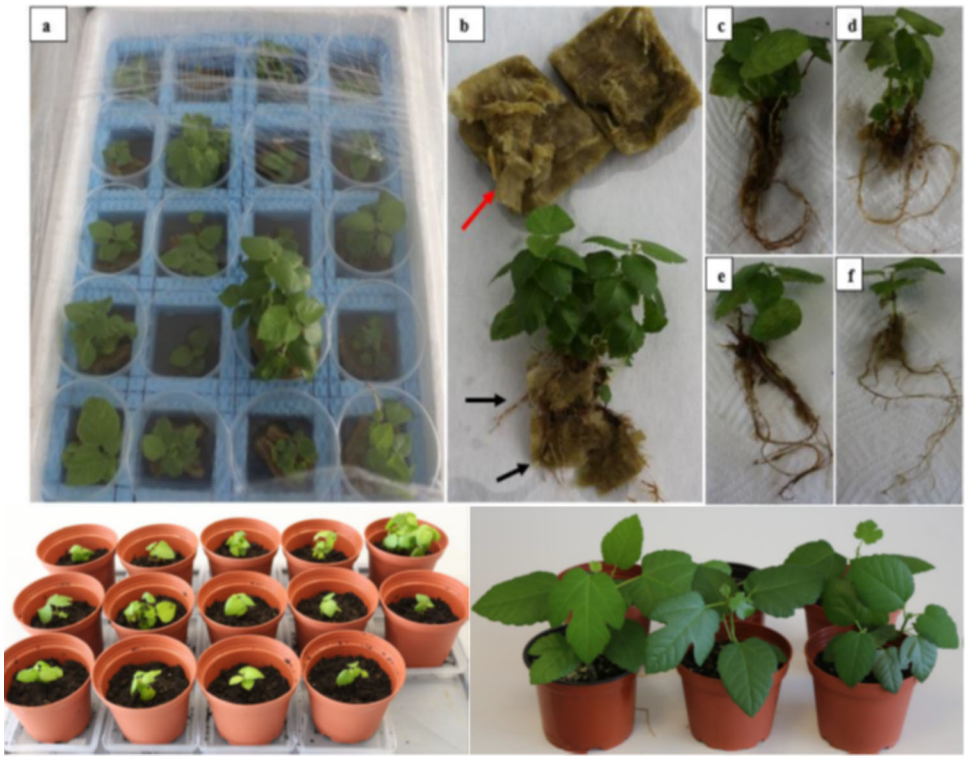

Fig. 8 Acclimatization **a** survivor of the shoots in a mini greenhouse environment, **b** roots cleared of rock wool (indicated by red arrow) before transfer to soil (indicated by black arrows), **c**–**f** shoots of fig plant ready to be transferred to soil free of rock wool

rate 4 weeks after culture in all media. The highest multiplication coefficient (3.27) was obtained in the 3rd subculture. In the 4th subculture, the multiplication coefficient decreased again. In addition, in order to evaluate the effect of the number of subcultures on shoot length, number of nodes, number and length of leaves, as a result of the data obtained from the shoot regeneration experiments, the highest mean shoot length per explant (1.62 cm) in the 1st subculture, the highest mean number of nodes per explant (2.39 number) in the 1st subculture, the highest mean number of leaves (6.06 number) was achieved in the 2nd subculture and the highest mean leaf length (1.30 cm) was achieved in the 1st subculture. The results we obtained in this study are consistent with [97].

As a result of culturing the node explants, no physiological problems were observed in the produced plantlets, especially hyperhydricity, darkening, yellowing and necrosis, in all experiments carried out during the study. However, it was determined that the leaves of some shoots on B5 medium turned yellow. In a study conducted for in vitro micropropagation of fig, the formation of small and hyperhydric buds was observed at a KIN dose of 2 mg/L. However, in WPM medium containing 0.5 mg/L KIN, hyperhydricity-free, rooted, longer shoots were obtained and the plantlets were visually in better condition. However, after adding GA_3 to the medium, the number of shoots decreased and hyperhydricity, yellowing, etiolation and apical necrosis were induced in the plantlets [100]. In another study, small hyperhydric shoots with yellowed leaves as well as excessive callus formation were detected when nodal explants of the "Roxo

de Valinhos" cultivar were cultured in WPM medium containing a BAP doses higher than 2.0 mg/L. Additionally, it was determined that when GA_3 was added to the medium, excessive elongation was induced along with hyperhydric, yellowed and burnt-tip shoots [113]. In another study, shoot tip necrosis was observed in full and ½ MS media from nodal bud explants cultured in different media in micropropagation of the "Golden Orphan" variety. On the other hand, in studies where full and ½ WPM medium was used, small and hyperhydric shoots were obtained and it was determined that the leaves aged rapidly [113]. In our study, better results were obtained than these studies in terms of the effect of physiological parameters on plantlets.

In order to evaluate the effect of DKW medium containing 2 mg/L BAP and 0.5 mg/L IBA on the acclimatization success of rooted shoots, data on the survival rate of shoots acclimatized in pots containing peat were observed 30 days after the acclimatization process. As a result of the data obtained, the rooted plantlets were acclimatized with a success rate of 70%. In the literature, acclimatization success was 80% for "Salti Kodari" variety [54], 90% for "Bursa Siyahı" variety [94] and 83.33% for "Golden Orphan"" variety [113]. When the rooted shoots obtained from the shoot tip and nodal explants cultured for in vitro micropropagation of the *F. carica* plant were kept in the greenhouse and then transferred to pots containing sterile soil (peat and sand), 100% acclimatization success was achieved [31]. The data we obtained in our study are similar to these studies.

5 Conclusion

Ficus carica L. is one of the oldest traditional fruit trees, which is considered sacred in the world and was brought into culture when people settled. In addition to our country being one of the important producers of fresh and dried figs in the world, Aydın province is also the place where the "*Sarılop*" variety, which has a large share in this trade, is produced the most. Sarılop is a registered fig variety with superior drying properties that increases our contribution to production and exports. Problems experienced in classical fig production have led to the use of modern cultivation methods. This study is the first successful in vitro clonal micropropagation study of node explants of the Sarılop fig variety belonging to the *Ficus carica* L. species, cultured in hormone-free DKW medium and sprouting from the bottom. All the data obtained in this study will lead to studies for the rapid, low-cost and clonally mass propagation of the registered Sarılop variety fig plant, which has superior quality and features, in a narrow area. In light of the information obtained as a result of the study, the appropriate basal medium (WPM) was determined for micropropagation studies in fig plants, the appropriate medium composition was decided for shoot multiplication and root regeneration (2 mg/L BAP and 0.5 mg/L IBA) and the use of Magenta was decided. These data can be said to be a complete production protocol suitable for commercialization.

In future studies, higher shoot and root regeneration values can be obtained with different plant growth regulators. Since fig is a commercially important plant, its mass production is very important. For this reason, there is a need for the production of node explants with different temporary immersion systems for shoot multiplication. In addition, studies on mass production can be carried out by producing somatic embryos in different bioreactors (e.g. air-lift bioreactors). Fig plants are also rich in secondary metabolite content. For this purpose, different plant cell culture studies such as the production of these valuable secondary metabolites by elicitation can be carried out.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This research did not receive any specific funding.

Data Availability The data that support this study will be shared upon reasonable request to the corresponding author.

Declarations

Conflict of Interest The authors declare no conflicts of interest.

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