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Original Article

Effect of plant growth regulators on indirect shoot organogenesis of *Ficus religiosa* through seedling derived petiole segments

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ABSTRACT

Ficus religiosa is known as a long-lived multipurpose forest tree. The tree plays an important role for religious, medicinal, and ornamental purposes. However, the propagation rate of *Ficus religiosa* is low in natural habitat so the plant tissue culture techniques are an applicable method for multiplication of this valuable medicinal plants. Thus, the aim of this study is to understand the effect of different auxin/cytokinin ratios on indirect shoot organogenesis of this plant. According to our results, the maximum callus induction frequency (100%) was obtained on Murashige and Skoog (MS) medium supplemented with 0.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) plus 0.05 mg/l 6-benzylaminopurine (BAP) from petiole segments. For shoot induction purpose, the yellow-brownish, friable, organogenic calli were inoculated on shoot induction medium. On MS medium supplemented with 1.5 mg/l BAP and 0.15 mg/l Indole-3-butyric acid (IBA), 96.66% of the petiole-derived calli responded with an average number of 3.56 shoots per culture. The highest root formation frequency (96.66%), root number (5.5), and root length (4.83 cm) were achieved on MS medium containing 2.0 mg/l IBA plus 0.1 mg/l Naphthaleneacetic acid (NAA). The rooted shoots were successfully transferred to field condition and the substrate with the mixture of cocopeat and perlite (1:1) had the highest survival rate (96.66%). This is the first report of an effective *in vitro* organogenesis protocol for *F. religiosa* by indirect shoot organogenesis through axenic seedling derived petiole explants, which can be efficiently employed for conservation of this important medicinal plant species as well as the utilization of active biomolecules.

1. Introduction

Ficus L. that belonged to the Moraceae family is known as the largest genus of vascular plants by including 750–1000 species. This genus is being derived from southeastern Asia, and it is broadly distributed in subtropical and tropical regions [7]. *Ficus religiosa* L. as an important tree in South Asia belonged to the genus of *Ficus*, and it possesses various common name such as Peepal, Bodhi, Bo tree, and Asvattha [35]. Furthermore, this species as a traditional tree is broadly planted as a roadside tree, and it plays an important role as a medicinal tree in various diseases such as asthma [43], stomatitis, diabetes [20], inflammations [18], glandular swelling disorders [1], and wound healing [9]. Since ancient time, this three has been strongly associated with the major religions of India such as Jainism, Hinduism, and Buddhism [7,35,37]. Thus, *Ficus religiosa* L. has become symbols of Buddhism and being broadly planted near Buddhist temples [7]. *Ficus religiosa* has a long petiole (over 9 cm) and long-acuminated leaves and also it is known as a heavily branched tree with long tipped, heart-shaped, and leathery leaves [35]. *Ficus religiosa* L. is naturally propagated via seeds. However, the rate of seed germination in this species is limited to 50%, and seeds cannot be stored for a long time [31,35]. Additionally, the vegetative propagation of this plant is not effective in various climate conditions [31]. Thus, the diversity of this valuable plant is threatened, and there is a dire need of introducing an alternative method for rapid multiplication of this plant in order to preserve its germplasm and for massive production of active compounds. Plant tissue culture is an effective and applicable techniques for plant propagation [37]. The plantlets that derived from tissue culture are season independent, Superior, uniform, and require smaller space in comparison with the seed grown plants [2]. However, plant tissue culture had limited

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application in trees in comparison with other plants [34]. Indirect regeneration can be ensured the quality and uniformity and rapidly production of plantlets [17]. Meanwhile, the response of plant growth regulators change during plant tissue culture can be varied from species to species and also this response depends on the ability of tissues to response and the types of plant growth regulators [34]. For instance, auxins and cytokinins play an essential role in indirect regeneration in the procedure for plant propagation such as shoots development and stimulation and callus formation and induction [40]. The require levels of plant growth regulators in these two steps may be vary from species to species, so the auxins/cytokinins ratio play an important role in plant tissue culture [4,41]. Although complete plant organogenesis reported in *Ficus religiosa* by direct shoot organogenesis [6,11,12,37,38] and indirect shoot regeneration [15], there is no report according to plant growth regulators ratios in indirect organogenesis via immature explant of *Ficus religiosa*. Deshpande et al. [6] indicated that the maximum bud break frequency via axillary buds of a mature tree *Ficus religiosa* was obtained in MS medium supplemented with 5 mg/l BAP plus 0.2 mg/l IBA. Furthermore, Hassan et al. [11] reported that the best shoot induction of *Ficus religiosa* via apical and axillary buds was reached in MS medium consisting of 0.5 mg/l BAP and 0.1 mg/l IAA. Also, Siwach and Gill [37] demonstrated that the maximum bud frequency of *Ficus religiosa* through nodal segments was observed in MS medium containing 1 mg/l BAP plus 0.5 mg/l IAA. Same as the previous study, Siwach and Gill [38] reported that the maximum regeneration frequency of *Ficus religiosa* via leaf explant was achieved in MS medium along with 5 mg/l BAP. In another study, Jaiswal and Narayan [15] determined the MS medium supplemented with 2 mg/l BAP as a best medium for achieving the maximum number of shoots per callus of stem segments of *Ficus religiosa.* Hesami et al. [12] reported that the maximum shoot multiplication of *Ficus religiosa* was obtained on MS medium containing 1 mg/l TDZ in combination with 0.1 mg/l IBA.

The age of the explant could play a significant role in plant tissue culture, and physiologically younger segments had a better performance than the older one. One way to eliminate the effect of plant's age is to use different parts of *in vitro*-grown seedlings [2]. Naturally, explants from seedlings employed for standardization of *in vitro* propagation in plants [24,33]. Therefore, the present study introduces a useful and effective protocol for *in vitro* propagation of *Ficus religiosa* by using petiole segments that derived from seedlings. Given the importance of micropropagation of trees, the aim of this study is to establish indirect regeneration protocol for mass production of this valuable species.

2. Materials and methods

2.1. Seed germination and explant preparation

The fruits of *Ficus religiosa* were reached from 45–50 years old *F. religiosa* mother plants in a field grown on the campus of Ramin Agriculture and Natural Resources University, Ahwaz, Iran. For 30 min, the fruits were washed under running water then they cleaned with a liquid soap solution followed by washing with tap water. Further surface sterilization treatment was set out in a laminar air flow chamber. The seeds were surface sterilized with 70% aqueous ethanol for 10 s, and then dipped into 10% (v/v) NaOCl solution for 5 min followed by washing with 3 times in sterilized distilled water. The sterilized seeds were incubated on one-tenth strength MS medium. After 8–10 days, seeds were germinated, and the petiole segment from *in vitro* germinated plant was employed as a source of explant for the latter experiment.

2.2. Media and culture condition

The basic culture medium consists of Murashige and Skoog (MS) medium was reinforced with 30 g/l sucrose and gelled with 0.6% agar plus 2 g/l activated charcoal, and the pH of the medium was regulated to 5.8 ± 0.2 with 0.1 N KOH or 0.1 N HCl after adding plant growth regulators. The medium was distributed in a culture tube and autoclaved at 121 °C, for 30 min. All the cultures were kept in a sterilized culture room at 26 ± 2 °C, under 16 h photoperiods that provided by cool white fluorescent light (65 μmol m^{-2} s^{-1}) with 55–60% relative humidity.

2.3. Callus induction

The petiole segment that derived from the 3-week-old single seedling (Fig. 1a) was employed as explant for callus formation. The petiole was excised into sections of 1–2 mm in length. The explants were placed horizontally on MS medium containing 2,4-D in combination with BAP in the absence of light. One explant was used for each concentration of PGRs and the 2,4-D/BAP ratio were 1:0, 10:1, 2:1, and 1:1 in the media. Data of frequency (%) of callus induction was measured after 4 weeks of culture.

2.4. Morphogenesis from callus

The regenerative calli were shifted on MS medium containing various ratios (1:0, 10:1, 2:1, and 1:1.) between BAP and IBA in the presence of light. One explant was used for each concentration of PGRs and organogenic calluses that obtained from petiole explant were subcultured every 3 weeks on fresh MS medium at the same composition. The shoot organogenesis frequency (%) of callus and the average number of shoots per inoculum were measured on the 60th day after shifting the callus on shoot organogenesis medium.

2.5. Shoot elongation and rooting

The 1–2 cm long individual shoots were separated from the multiple shoots bunch and transferred to elongation MS medium consisting of 0.5 mg/l BAP and 0.5 mg/l GA_3. After 4 weeks, elongated shoots were transferred to rooting medium, containing various concentrations and combinations of IBA and NAA or no PGRs (Table 3). After 5 weeks, the percentage of root formation, the roots lengths, and a number of roots per shoot were recorded.

2.6. Acclimatization and transplantation

Well-developed plantlets in term of shoots and roots were removed carefully from the culture, and the roots were washed with tap water for removing any traces of medium sticking to the roots. Afterward, roots of each plantlet were treated with 0.5% Bavistin before placing into plastic pots filled with sterile planting substrates viz. sterile perlite and cocopeat individually or in combinations (1:1). In order to ensure high humidity, the pots were covered with transparent polyethylene bags, and the pots were irrigated with diluted MS liquid sucrose-free medium (0.1) for every alternate day for two weeks. The potted plantlets were initially kept in the controlled environment at 25 ± 2 °C with 80–90% relative humidity and photoperiod of 16 h and 65 μmol m^{-2} s^{-1} light intensity for two weeks. After 2 weeks, Polythene bags were gradually opened to acclimatize plants to field conditions. Later on, they were shifted to the greenhouse. The success rate in acclimatization was recorded after 2 months transferring into the greenhouse.

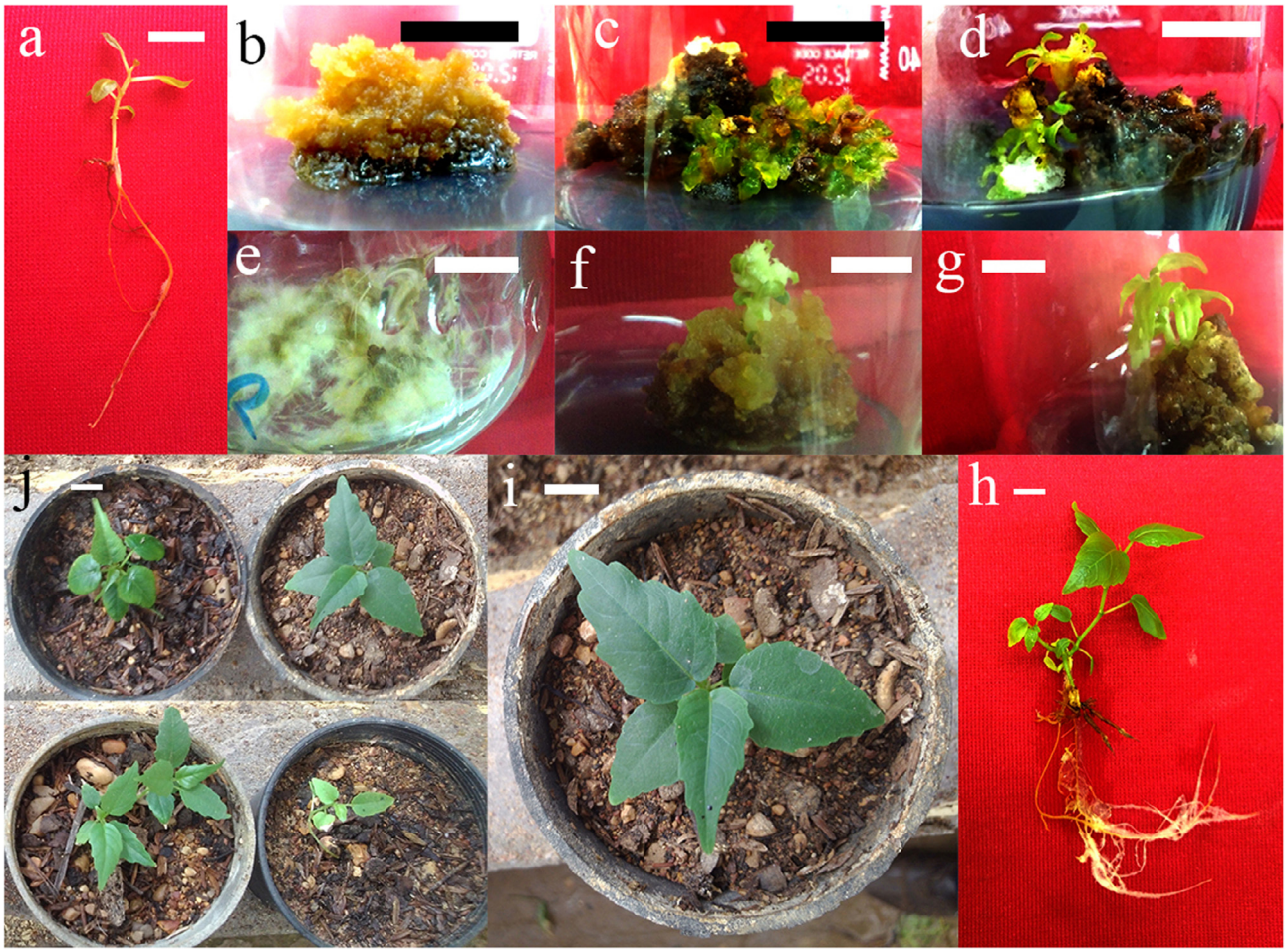

Fig. 1. *In vitro* shoot regeneration through indirect organogenesis from seedling derived petiole segments of *Ficus religiosa* L. (a) Seedling from *in vitro* seed germination; (b) Yellow-brownish and friable callus induction on MS + 0.5 mg/l 2,4-D + 0.05 mg/l BAP + 2 g/l activated charcoal; (c,d) Shoot buds formation in the surface of callus on MS + 1.5 mg/l 2,4-D + 1.5 mg/l BAP2 + 2 g/l activated charcoal (e) Root formation in callus surface on MS + 1 mg/l BAP + 1 mg/l IBA; (f,g) Shoot regeneration from callus on MS + 1.5 mg/l BAP + 0.15 mg/l IBA2 + 2 g/l activated charcoal after 2 and 6 weeks, respectively; (h) *In vitro* root formation on MS + 2.0 mg/l IBA + 0.1 mg/l NAA; (i,j) Acclimatized regenerated plants after four weeks, *bar* 0.5 cm.

2.7. Statistical analysis

The experiments were conducted in completely randomized design (CRD) and there were 10 replicates per treatment and each treatment was repeated in three sets. The data were analyzed by Analysis of Variance (ANOVA) followed by Duncan's multiple range test ($P < .05$). Data analysis was carried out by using SAS version 9.3.

3. Result and discussion

3.1. Effect of plant growth regulators on callus induction

In vitro plant regeneration from different parts of *in vitro* grown seedlings have received notable attention and many researchers have used various parts of *in vitro* grown seedling as explants [14,23,24,28] in order to improve the micropropagation method for many plant species.

The majority of woody plants and some herbaceous species made the medium browning. If this browning was so extreme, the explants turn its color to brown/black and become necrotic and finally become die [21]. The browning of the medium is due to releasing phenol by the explants which get oxidized, and this oxidation product could be phytotoxic. Thus, it needs a scrutinized investigation before incubating explants in the culture medium [5]. The degree of browning is different from species to species and depend on the age of the tissue (old tissues show more browning than the younger one), the season of culture initiation (more in winters and autumn), and composition of the medium [30]. In the situation that the number of explant discoloration was high in the medium, some absorbent material such as activated charcoal (1–3 g/l) was used [2]. Thomas [42] indicated that activated charcoal could promote regeneration by absorbing inhibitory compounds and also employed for alleviating toxic metabolites, phenolic exudation, and its accumulation. Activated charcoal could promote growth by releasing substances that are naturally present in the charcoal. It also immediately adsorbed PGRs and vitamins from medium and gradually release them again in the medium. Regarding our study, activated charcoal at 2 g/l concentration were applied for reducing and controlling lethal browning.

The callus initiation from the cut end of petiole in *Ficus religiosa* was obtained in MS medium containing various 2,4-D/BAP ratios, and also the whole explant's surface was covered with callus after 2 weeks. However, there was no callus initiation in the control treatment. Various 2,4-D/BAP ratios had a significant influence on growth and morphology of the callus. The low ratio between 2,4-D and BAP induced more callus in petiole explant. In another

Table 1
Effect of 2,4-D in combination with BAP in MS medium on callus induction of *F. religiosa* from petiole explant.

Plant growth regulator (mg/l)		Auxin: Cytokinin ratio	Callus formation frequency (%)	Callus fresh weight (g)
2,4-D	BAP			
0.0	0.0	0:0	0.00 g	0.00 f
0.5	0.0	1:0	73.33 bc	1.83 a
0.5	0.05	10:1	100.00 a	2.08 a
0.5	0.25	2:1	76.66 b	1.96 a
0.5	0.5	1:1	73.33 bc	1.86 a
1.0	0.0	1:0	66.66 bcd	1.26 b
1.0	0.1	10:1	90.00 a	1.10 bc
1.0	0.5	2:1	63.33 cde	0.90 cd
1.0	1.0	1:1	60.00 de	0.70 de
1.5	0.0	1:0	26.66 f	0.46 e
1.5	0.15	10:1	53.33 e	0.52 e
1.5	0.75	2:1	53.33 e	0.43 e
1.5	1.5	1:1	33.33 f	0.36 e

Means in each column followed by same letters are not significantly different according to DMRT at $P < .05$.

word, the low concentration of 2,4-D within BAP had the high callus formation rate from petiole explant (Table 1). One of the most important synthetic auxin, 2,4-D, plays an important role in tissue culture and embryogenic cell systems, and callus induction in plant tissue culture studies [8]. Also, 2.4-D had a positive impact on the molecular and physiological process of callus by inducing specific proteins, regulates the endogenous IAA metabolism, and control DNA methylation [25]. Based on our results, the maximum callus formation frequency (100%) as well as callus fresh weight (2.08 g) were obtained from MS medium supplemented with 0.5 mg/l 2,4-D along with 0.05 mg/l BAP. As the same as our results, Siwach et al. [39] indicated that MS medium containing 0.5 and 1.0 mg/l 2,4-D had better callus induction from shoot apices, intermodal, and nodal explants of *Ficus religiosa* but they did not use a cytokinin in their research. According to another study, Jaiswal and Narayan [15] reported that the high frequency of callus induction through internode explant of *F. religiosa* was reached in MS medium supplemented with 0.5 mg/l NAA in combination with 1.0 mg/l BAP. It seems that 2,4-D at the high concentration may serve as an herbicide that suppressed callus formation [2]. Furthermore, our results indicated that by increasing the 2,4-D concentration, the callus formation decreased significantly. In the other study, Parasharami et al. [27] demonstrated that MS medium supplemented with 2.4 mg/l 2,4-D plus 1.0 mg/l BAP had the highest callus formation from fruit segments of *F. religiosa.* Ultimately, the difference results that obtained from those studies maybe due to the age, source, and the internal PGRs of explants [2,22]. The calli that obtained from various concentrations of auxins and cytokinins possessed a friable type with yellow-brownish color (Fig. 1b). The concentration of 2,4-D and BAP at the ratio of 10:1 had the better callus formation in comparison with other treatments. Zhao [45] reported that the ratio between auxins and cytokinins play an important role in *in vitro* culture that initiates the develops events. Our results showed that MS medium containing 0.5 mg/l 2,4-D plus 0.05 mg/l BAP (10:1) known as the best treatment for callus induction (100%) that coincides with Su et al. [41] who indicated that the high ratio of auxin/cytokinin is critical for callus formation. Other previous studies also proved that the callus could be induced in the high auxin/cytokinin ratio [4,40]. Růžička et al. [29] and Jones et al. [16] indicated that during callus induction, the external auxins could increase the expression of ARABIDOPSIS HISTIDINE KINASE4 (AHK4), and also the auxin biosynthetic YUCCA (YUC) genes and the expression of the auxin efflux carriers PINFORMED (PIN) can be regulated by external cytokinin. Furthermore, in order to maintain SAM, auxin can regulate the cytokinin response by the negative regulation of A ARABIDOPSIS RESPONSE REGULATOR15 (ARR15) by ADP Ribosylation Factor 5 (ARF5)/MONOPTEROS [46].

The three concentration of 2,4-D and BAP, namely; 1.0 mg/l 2,4-D plus 1.0 mg/l BAP (1:1 ratio), 1.5 mg/l 2,4-D plus 0.75 mg/l BAP (2:1 ratio), and 1.5 mg/l 2,4-D plus 1.5 mg/l BAP (1:1 ratio) produced novel shoot buds from the surface of callus accompany with shoot primordia (Fig. 1c and d) maybe due to the high concentration of cytokinin in the medium [4,41].

3.2. Effect of plant growth regulators on morphogenesis from callus

According to our results, the high regeneration frequency was observed in MS medium containing cytokinin/auxin with the ratio of 10:1. The regeneration frequency was decreased by increasing in the cytokinin/auxin ratio. The highest regeneration frequency (96.66%) as well as shoot number (3.56) were observed in MS medium supplemented with 1.5 mg/l BAP and 0.15 mg/l IBA (Fig. 1f and g). No shoot formation was observed in the control and MS medium containing 0.5 mg/l BAP. The previous studies indicated that the high and low ratio of auxin/cytokinin promote root and shoot induction, respectively [4,40,46]. Therefore, it seems that auxin and cytokinin have a cross-talk and play an important role in *in vitro* organogenesis [45]. However, the molecular mechanism of auxin and cytokinin interaction in the meristem formation during *in vitro* condition remains unknown [46]. During *in vitro* shoot proliferation via cultured root explants of Arabidopsis, cytokinin triggers the promotion of ectopic WUSCHEL (WUS) expression within the callus. Therefore, this expression is adequate for promoting shoot organogenesis in *in vitro* culture [10]. During callus formation stage, the pretreatment of auxin on root explants of Arabidopsis promotes to the up-regulation of AHK4 expression (a cytokinin receptor gene). In order to activate WUS during shoot formation, it is necessary to increase AHK4 transcription [10]. In the study that conducted by Buechel et al. [3], the roles of A ARABIDOPSIS RESPONSE REGULATOR7 (ARR7) and ARR15 in shoot organogenesis have been characterized and they found that the overexpression of ARR7 and ARR15 can be limited shoot organogenesis. However, loss of function of ARR7 and ARR15 highly stimulates callus induction on callus-inducing medium (CIM) that enriched with auxins and induced shoot formation on shoot-inducing medium (SIM) that enriched with cytokinin [3]. Furthermore, the previous studies showed that external auxin leads to the up-regulation of AHK4 expression on CIM. Afterward, when callus is transferred to SIM, AHK4 enhances the response to external cytokinin. High cytokinin response induced WUS expression, which is critical to promote shoot induction [3,4]. Based on our

results, the root formation was observed in some callus surface (Fig. 1e) of three treatments, namely, 1 mg/l BAP + 1 mg/l IBA (1:1), 1.5 mg/l BAP + 0.75 mg/l IBA (2:1), and 1.5 mg/l BAP + 1.5 mg/l IBA (1:1) maybe due to the high concentration of auxin in the medium [4,46].

3.3. Effect of plant growth regulators on shoot elongation and root formation

After 3–4 subculture on MS medium, the shoots were transferred to MS medium containing 0.5 mg/l GA_3 in combination with 0.5 mg/l BAP (elongation medium). After 3–4 weeks of culture, shoots showed a significant increase in length (2–3 cm) on elongation medium. According to our study, MS medium supplemented with 0.5 mg/l BAP had the longest shoot length per explants. In general, the longest shoots (1.86 cm) were obtained on MS medium fortified with 0.5 mg/l BAP plus 0.05 mg/l IBA in comparison with other treatments (Table 2). The positive impact of BAP on shoot length was also indicated by Siwach and Gill [37] in *Ficus religiosa*. Xiao et al. [44] reported that GA_3 had a massive impact on shoot elongation and cell division. Also, many studies proved the positive effect of GA_3 on the elongation of *in vitro* shoots in the medium [13,39,44]. However, some studies figured out that transferring shoots into MS medium in combination with low PGRs or free PGR medium is the successful way for shoot elongation [36]. According to our study, shoot elongation (2–3 cm) were obtained from both the above-mentioned strategies.

Since PGRs free MS medium did not produce roots from the *in vitro* regenerated shoots, the elongated shoots were shifted into rooting MS medium containing a various combination of IBA and NAA (Table 3). MS medium consisting of 2.0 mg/l IBA in combination with 0.1 mg/l NAA possessed the maximum frequency of root induction (96.66 %) and number of roots per shoot (5.50) as well as root length (4.83 cm) (Fig. 1h). Siwach and Gill [37] reported that some Auxins such as IBA and NAA play an important role in root induction and Jaiswal and Narayan [15] demonstrated that MS medium supplemented with 1.0 mg/l NAA had a possible root induction in *Ficus religiosa*. In other studies, the most effective root induction for *Ficus religiosa* was found in MS medium containing 2 mg/l IBA in combination with 0.1 mg/l NAA [6,11].

3.4. Acclimatization and transplantation

Plantlets that have well-developed roots were transferred successfully into small pots consisting of various types of substrates in different proportions (Fig. 1i and j). Plants survived were analyzed, and the results demonstrate different response (Fig. 2). As of the three substrates that used for acclimatization, a mixture of sterile perlite: cocopeat had the better performance than other substrates in *Ficus religiosa* plantlets with the survival rate of 96.66%. Similar kind of results was reached in *Salix tetrasperma* [19], *Semecarpus anacardium* [26] and *Ficus religiosa* [37]. Shukla et al. [32] reported that the mixture of perlite: cocopeat (1:1) enhance the growth of roots of plantlets during primary hardening stage.

Table 2
Effect of BAP in combination with IBA in MS medium on shoot regeneration from callus of *F. religiosa*.

Plant growth regulator (mg/l)		Cytokinin:Auxin ratio	Regeneration frequency (%)	No. shoots per explant	Shoot length (cm)
BAP	IBA				
0.0	0.0	0:0	0.00 f	0.00 h	0.00 g
0.5	0.0	1:0	0.00 f	0.00 h	0.00 g
0.5	0.05	10:1	73.33 b	2.13 c	1.86 a
0.5	0.25	2:1	53.33 c	1.16 e	1.76 ab
0.5	0.5	1:1	53.33 c	1.06 e	1.73 ab
1.0	0.0	1:0	26.66 e	1.10 e	1.53 cd
1.0	0.1	10:1	93.33 a	2.13 c	1.66 bc
1.0	0.5	2:1	70.00 b	1.46 d	1.56 cd
1.0	1.0	1:1	36.66 d	0.66 g	1.50 d
1.5	0.0	1:0	66.66 b	2.20 c	0.83 f
1.5	0.15	10:1	96.66 a	3.56 a	1.23 e
1.5	0.75	2:1	76.66 b	2.70 b	1.16 e
1.5	1.5	1:1	43.33 cd	0.83 f	0.96 f

Means in each column followed by same letters are not significantly different according to DMRT at P < .05.

Table 3
Effect of auxins (IBA and NAA) in MS medium on *in vitro* root induction in regenerated shoots of *F. religiosa*.

Plant Growth Regulator (mg/l)		Rooting frequency (%)	Number of roots per explant	Roots length (cm)
IBA	NAA			
–	–	0.00 i	0.00 i	0.00 g
1.0	–	43.33 h	2.43 h	2.83 d
1.5	–	63.33 def	2.50 gh	2.56 d
2.0	–	80.00 b	4.06 c	2.83 d
1.0	0.1	56.66 efg	2.56 fgh	2.76 d
1.5	0.1	73.33 bcd	3.56 d	3.30 c
2.0	0.1	96.66 a	5.50 a	4.83 a
–	1.0	46.66 gh	2.33 h	1.40 f
–	1.5	60.00 ef	2.70 fg	1.96 e
–	2.0	66.66 cde	3.63 d	2.06 e
0.1	1.0	53.33 fgh	2.76 ef	1.40 f
0.1	1.5	73.33 bcd	2.96 e	2.60 d
0.1	2.0	76.66 bc	4.33 b	4.26 b

Means in each column followed by same letters are not significantly different according to DMRT at P < .05.

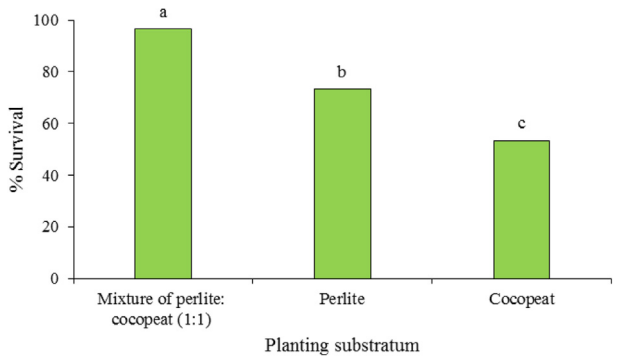

Fig. 2. Effect of planting substratum on survival rate of acclimatized regenerated plants of *Ficus religiosa* L.

4. Conclusions

The present study introduces an efficient and reproducible method for indirect shoot regeneration of *Ficus religiosa* using seedling derived petiole segments. Considering the medicinal and economic importance of the plant, this current protocol offers a potential system for rapid multiplication as well as germplasm conservation.

Conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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