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In vitro propagation of caprifig and figs (*Ficus carica* L.) through various explants

Rahma Labidi[1], Chokri Bayoudh[2]*, Afifa Majdoub[3], Margarita Lopez-Corrales[4] and Messaoud Mars[1]

*[1]LR21AGR02-Agrobiodiversity and Ecotoxicology, High Agronomic Institute of Chott-Mariam, University of Sousse, 4042, Sousse, Tunisia. [2]LR21AGR03-Production and Protection for a Sustainable Horticulture, Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB), 4042 Chott-Mariem, IRESA, Tunisia. [3]Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB), IRESA-University of Sousse; 4042 Chott-Mariem, Sousse, Tunisia. [4]Centro de Investigaciones Científicas y Tecnológicas de Extremadura. Km. 372; 06187 Guadajira (Badajoz)-Spain.*E-mail: chokribayoudh@gmail.com*

Abstract

Fig (*Ficus carica* L.) cultivation is affronting serious problems caused by biotic and abiotic stresses. Application of *in vitro* techniques became necessary for plant material sanitation, rapid propagation and conservation. The present study aims the regeneration of *in vitro* plants of local fig cultivars using different explant types. For that purpose, shoot tips, meristems and leaf segments of 3 local fig cultivars (Soltani, Zidi, Bither Abiadh) and a caprifig (Assafri) were cultivated *in vitro*. MS media with different concentrations of growth hormones: α-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), 6-Benzylaminopurine (BAP), Kinetin (Kin), N6-(2-Isopentenyl)adenine (2iP), Gibberellic acid (GA_3) and Thidiazuron (TDZ) and various antioxidants were tested for explants initiation, multiplication and rooting. The combination of 0.2 mg L^{-1} BAP, 0.1 mg L^{-1} GA_3 and 0.1 mg L^{-1} NAA was the most appropriate for the best meristem establishment rates. The highest establishment rates were obtained with explants taken in spring for Zidi (62.3%) and Bither Abiadh (96.7%) and in autumn for Assafri (100%). From shoot-tip explants, Zidi gave the highest shoot number during the multiplication step. The rates of leaf fragments regenerating adventitious shoots, reached 75.6% and 57.2%, respectively, for Soltani and Assafri on media enriched by TDZ and IBA. MS medium with 1 mg L^{-1} IBA allowed the best rooting rates for Bither Abiadh and Assafri explants. Vitroplants *in vivo* rooting was more effective due to their high success rates and the simplicity of the method. Three months after acclimatization, the survival and success rates of the vitroplants were 80-90%.

Key words: *Ficus carica; in vitro* regeneration; leaf segments; meristems; shoot-tips

Introduction

Fig tree (*Ficus carica* L.) is one of the oldest fruit species cultivated in the Mediterranean basin. It presents a multitude of varieties with interesting agronomic performances, allowing its adaptation to different conditions. This fruit crop is of great interest essentially for the nutritional value of its fruits (Lachtar *et al.*, 2022). However, it is facing serious limitations related to biotic and abiotic constraints. Nowadays, the use of *in vitro* tissue culture techniques for this species is widely required for viral sanitation of cultivars and caprifigs affected by Fig Mosaic Disease (FMD), for rapid propagation of healthy plant material intended for the installation of new intensive plantations (Bayoudh *et al.*, 2017), for genetic resources conservation and improvement (Chatti *et al.,* 2010). Fig tree plantlets are actually micropropagated from apex, meristems and leaf fragments (Hong *et al.*, 2020). Regeneration of genetically modified fig plants was possible from vitro leaflets (Soliman *et al.*, 2010). However, with *in vitro* propagation techniques, the fig tree was considered as a species with a recalcitrant morphogenesis (Dhage, 2015), causing explant oxidation and necrosis (Al-Shomali *et al.*, 2017), which lead to serious regeneration problems. In Tunisia, no clear strategy for fig local varieties sanitation has been adopted to face the fig mosaic disease (FMD) and to preserve our heritage from genetic erosion (Bayoudh *et al.*, 2014), which urges the establishment and the optimization of rapid fig *in vitro* propagation protocols. Thus, the present work aims to establish and study the *in vitro* regeneration protocols of local fig cultivars and caprifig through meristems, shoot-tips and vitroleaf segments.

Materials and methods

Plant material: The study was carried out on 3 female fig cultivars: Zidi (ZDI), Smyrna type, colored fruits; Soltani (SNI), Smyrna type, colored fruits; Bither Abiadh (BA), San Pedro type with non-colored fruits and a caprifig: Assafri (ASF). As initial explants, 3 to 4 cm shoots were taken from old fig trees maintained in the varietal collection of the High Agronomic Institute of Chott-Mariem (Sousse). Shoots for apex explants were collected in early summer (June) and for meristems were harvested in autumn (October-November) and in early spring (February-March).

The shoots were washed under running tap water for 25 minutes to remove impurities and dipped in an antioxidant solution containing 150 mg L^{-1} of ascorbic acid and 100 mg L^{-1} of citric acid to avoid media and explants oxidations. Then, they were soaked in ethanol (70° for 30 s) and kept for 30 minutes in sodium

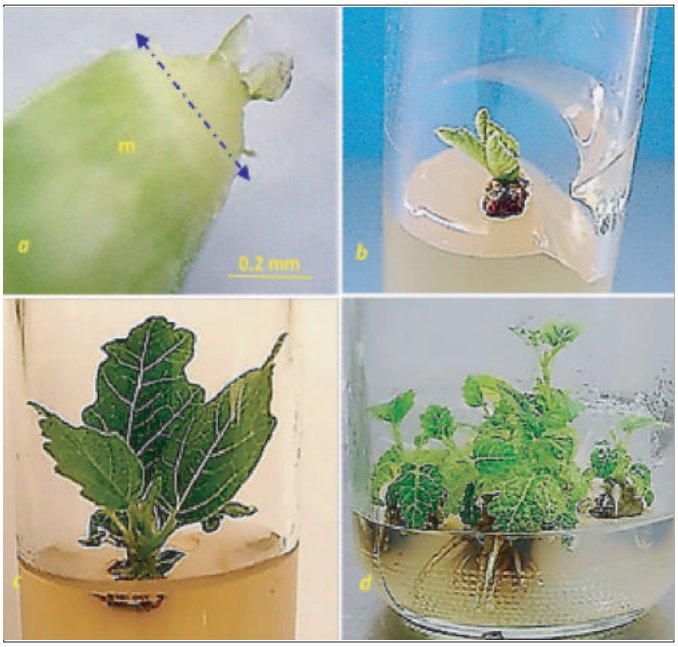

Fig. 1. Fig meristem (m) under binocular magnifier (X 32) (a); fig meristem established on medium M_3 (X 4) (b); plantlet issued from fig meristem on multiplication medium M_8 (c); rooting of vitroplants (Assafri cultivar) on medium M_9 (d).

chloride (10%) (v/v) by adding 1 to 2 drops of Tween 20. They were rinsed three times with sterile distilled water (15 minutes) under a laminar airflow bench. Meristems of 0.5 mm and apices of 2 mm size, with 2 primordial leaves, were cut (Fig. 1a) and placed on an appropriate MS (Murashige and Skoog, 1962) basal media.

Culture media composition: For meristem initiation, four culturing media were evaluated: M_1: 0.2 mg L^{-1} BAP + 0.1 mg L^{-1} Kin + 0.1 mg L^{-1} NAA; M_2: 0.2 mg L^{-1} BAP + 0.1 mg L^{-1} 2iP + 0.1 mg L^{-1} NAA; M_3: 0.2 mg L^{-1} BAP + 0.1 mg L^{-1} GA_3 + 0.1 mg L^{-1} NAA and M_4: 0.2 mg L^{-1} BAP + 0.1 mg L^{-1} 2,4-D. In addition, three media containing antioxidants were tested: M_5 (M_3 + 0.2 g L^{-1} PVP-40); M_6 (M_3 + 0.5 g L^{-1} activated charcoal) and M_7 (M_3 + 0.5 g L^{-1} ascorbic acid). After meristem development, obtained plantlets were subcultured on M_8 multiplication medium: Thiamine (0.3 mg L^{-1}) + BAP (0.5 mg L^{-1}) + IBA (0.1 mg L^{-1}) + GA_3 (0.1 mg L^{-1}) + Phloroglucinol (PG) (90 mg L^{-1}).

For apex initiation, only M_8 medium was used for both initiation (during 4 months) and multiplication (during 2 months) steps. Vigorous regenerated vitroplants were rooted on two media: M_9 (1 mg L^{-1} IBA) and M_{10} (½MS macroelements and ½MS microelements + 0.2 mg L^{-1} IBA). This step lasted 5 weeks for vitroplants issued from apices and 9 weeks for those from meristems.

Plant regeneration capacity from leaf fragments was studied on media containing IBA and TDZ. Vitroleaves from apex shoots were isolated and divided into 1 cm^2 fragments with perpendicular wounds to the veins on their abaxial side. Two MS basal initiation media were tested: M_{11} (0.5 mg L^{-1} TDZ + 1 mg L^{-1} IBA) and M_{12} (1 mg L^{-1} TDZ + 1 mg L^{-1} IBA) enriched with 0.3 mg L^{-1} Thiamine (Kim *et al.*, 2007). Leaf fragments were darkened for a week before being exposed to light. Shoots regenerated from adventive leaf buds (0.5 cm long) were transplanted on M_8 multiplication medium.

All pH media was adjusted to 5.7 and autoclaved for 20 minutes at 121°C. Vitrocultures were placed in a growth chamber at 26±1°C, lightened by cool white fluorescent lamps under 16 hours photoperiod and 35 $\mu mol.m^{-2}.s^{-1}$ light intensity.

The regenerated plantlets were transferred to a greenhouse (25 °C) on a sterilized peat substrate (Hepaksoy and Aksoy, 2008). After root development period (4 weeks), the plants were watered and fertilized 1 time/week with a nutrient solution containing the macro-elements of Hoagland and KH_2PO_4 (1M).

Data Analysis: All experiments were carried out in a factorial completely randomized design with three replicates and 30 samples per each experimental unit (n = 90). The analysis of variance and mean comparisons analysis was done using Duncan's test (at $P \leq 0.05$) by SPSS (Version 13.0 for windows Inc., Chicago, IL, USA).

Results and discussion

Regeneration from meristem explants

Initiation step: On initiation media, meristems of the three cultivars: Zidi, Bither Abiadh and Assafri evolved 10 days after their cultivation. A highly significant difference was noted between the establishment rates, which ranged from 56.7% for Zidi to 93.1% for Assafri (Table 1). The average rate of explant callogenesis varied considerably from 39.3% (Assafri) to 63.9% (Zidi). Although no significant differences were noticed, the percentage of tissue necrosis varied from 44.2% (Bither Abiadh) to 55.5% (Zidi). In addition, media composition affected the meristem evolution rate. Meristem development (Fig. 1b) ranged from 40% (on medium M_2) to 66.8% (on M_4) for Zidi, from 56.7% (on M_1) to 80% (on M_2) for Bither Abiadh and from 86.3% (on M_2) to 100% (on M_3) for Assafri. Similar results were reported by Chalak *et al.* (2015) with meristem development of Aswad fig cultivars grown on MS medium with mainly BAP, GA_3 and IBA.

Significant effects of culture media on explant callus and necrosis rates were noticed only for Assafri. In fact, the lowest callus rate (15.7%) was recorded on M_4 medium, while the highest average (86.3%) was recorded on M_2. This process varied, also, from 46.7% on M_1 to 83.3% on M_2 for Zidi and from 40% on M_3 to 60% on M_1 for Bither Abiadh. Explant callusing phenomenon was reported to be mainly due to the combination effects of BAP with any auxin (Dhage *et al.*, 2015; Nur and Zarina, 2020). For the Assafri necrosis rate, this parameter has extended from 33.3% on M_1 to 75.1% on M_3 (Table 1).

Effects of antioxidants: Antioxidants have the unique ability to prevent browning and necrotic effects on *in vitro* cultures. (Fatima *et al.*, 2012). M_3 medium supplemented with antioxidants (activated charcoal, ascorbic acid and PVP) allowed meristem development. With activated charcoal, 100% of Bither Abiadh meristems, 90% of Assafri meristems and only 45.7% of Zidi meristems were developed. Also, the PVP supplemented to medium allowed development rates of 96.7%, 75% and 62.3%, respectively, for Bither Abiadh, Assafri and Zidi meristems. On the medium enriched with ascorbic acid, explant development rates were lower. In fact, cultivars Bither Abiadh, Assafri and Zidi recorded 75.7%, 70% and 34.8% development rates, respectively (Table 2). Similar results were reported by Toma and Tamer (2015) who noted the best survival rate of fig meristems on media enriched with activated charcoal (2 g L^{-1}) and Singh *et al.* (2016), who reported the positive effects of activated charcoal (0.5 to 2 g L^{-1}) on fig explants development.

Table 1. Rates of meristem establishment, callus and necrosis on initiation media and significat level differences between cultivars (Zidi, Bither Abiadh and Assafri)

	Cultivar	Medium	Average rate (%)	F calculated
Establishment	Zidi	M_1	60	1.61 ^{NS}
		M_2	40	
		M_3	60	
		M_4	66.8	
	B. Abiadh	M_1	56.7	1.36 ^{NS}
		M_2	80	
		M_3	60	
		M_4	69.8	
	Assafri	M_1	90	0.79 ^{NS}
		M_2	86.3	
		M_3	100	
		M_4	96.3	
Callus	Zidi	M_1	46.7	2.14 ^{NS}
		M_2	83.3	
		M_3	60	
		M_4	65.5	
	B. Abiadh	M_1	60	1.06 ^{NS}
		M_2	50	
		M_3	40	
		M_4	40.2	
	Assafri	M_1	26.7	33.78 **
		M_2	86.3	
		M_3	28.5	
		M_4	15.7	
Necrosis	Zidi	M_1	46.7	1.38 ^{NS}
		M_2	40	
		M_3	70	
		M_4	65.2	
	B. Abiadh	M_1	63.3	1.63 ^{NS}
		M_2	43.3	
		M_3	36.7	
		M_4	33.6	
	Assafri	M_1	33.3	10.83**
		M_2	34,1	
		M_3	75.1	
		M_4	65.2	

Low effects of various antioxidants on media oxidation and explant necrosis were registered. However, the medium supplemented with ascorbic acid resulted in the lowest necrosis levels during the meristem initiation of the three cultivars. On medium supplemented with activated charcoal, necrosis rates were the highest (Table 2). Activated charcoal is, in fact, an adsorbent and has a dehydrating effect on plant tissues, especially on small ones such as meristems (Kim *et al.*, 2007)

Shoot multiplication: Meristems of cultivars Bither Abiadh, Zidi and Assafri initiated on medium M_3 and transplanted on multiplication medium M_8 for 8 weeks, offered large shoot proliferation and high length plantlets (Table 3). These results were similar to those reported by Ling *et al.* (2022) for fig shoot regeneration. Bither Abiadh plantlets reached 13 mm length with the highest average number of leaves (13 leaves/plantlet).

Table 2. Effects of antioxidants added to medium M_3 on establishment and necrosis rates of Zidi, Bither Abiadh and Assafri meristems

Anti-oxidant		PVP	Activated charcoal	Ascorbic acid	F calculated
Zidi	ER	62.3±16.1	45.7±14.4	34.8±21.7	0.73 NS
	NR	66.7±7.6	100±0.0	65.9±14.3	12.93 NS
Bither Abiadh	ER	96.7±5.8a	100±0.0a	75.7±5.1b	26.25 **
	NR	58.3±17.6	100±0.0	44±13.8	4.01 NS
Assafri	ER	75±15.3	90±10	70±20.0	1.27 NS
	NR	96.7±5.8	100±0.0	90±17.3	0.7 NS

NR=Necrosis rate (%). ER=Establishment rate (%). **: Highly significant differences (Duncan, $P \leq 0.01$); ^{NS}: not significant differences. Values

Plantlets of the two other cultivars developed less leaf number. Concerning the average number of new developed lateral shoots and leaves per shoot, Bither Abiadh plantlets gave the best results (2.3 shoots and 2.1 leaves/shoot). Zidi plantlets showed moderate length and developed few lateral shoots. Assafri plantlets extended well and developed an average of 1.3 lateral shoots and 1.5 leaves/shoot (Table 3) (Fig. 1c). This may be due to a specific characteristic of the Assafri caprifig having a typical *in vivo* morphological character that is mainly the high length of the internodes (Gaaliche *et al.*, 2017).

Table 3. Plantlets average rate of necrosis and multiplication parameters of cultivars Zidi, Bither Abiadh and Assafri during 8 weeks of culturing on medium M_8

Cultivar	Highest plantlet length (mm)	Average leaf number/ plantlet	Average shoot height/ explant	Average shoot number/ explant	Average leaf number/ shoot
Zidi	8.3 ± 2.7	2.6 ± 0.6	2.5 ± 0.9	0.5 ± 0.1	1.2 ± 1.0
Bither Abiadh	13.0 ± 3.1	13.0 ± 8.7	3.8 ± 1.5	2.3 ± 1.1	2.1 ± 0.5
Assafri	16.2 ± 3.4	4.4 ± 1.8	5.6 ± 2.5	1.3 ± 0.8	1.5 ± 0.2

Rooting: Medium M_9 (MS + 1 mg L^{-1} IBA) offered the best average rates of *in vitro* plantlets rooting for all the three cultivars. In fact, 100% of Bither Abiadh plantlets, 86.9% of Soltani plantlets and 88.2% of Assafri plantlets gave roots (Table 4). These rates were similar to those recorded by El-Dessoky *et al.* (2016) for *in vitro* rooting of fig vitroplantlets on media enriched with IBA. Also, for all cultivars, medium M_{10} showed important average rooting rates, higher than those found by Hepaksoy and Aksoy (2008). The best results of root number and root length for both cultivars Soltani (7.7 roots and 8.2 mm) and Bither Abiadh (15 roots and 19.6 mm) were obtained on M_{10} medium. The obtained roots were thin, filiform and quite numerous.

Table 4. Rooting rate, vitroplants root length and number of cultivars Soltani, Bither Abiadh and Assafri on media M_9 and M_{10}

Medium		M9: MS + 1 mg L^{-1} IBA	M10: ½MS + 0,2 mg L^{-1} IBA
Soltani	Rooting rate (%)	86.9	85.7
	Average root number	5.8 ± 4.6	7.7 ± 5.6
	Average root length (mm)	6.2 ± 5.7	8.2 ± 6.5
Bither Abiadh	Rooting rate (%)	100	96.4
	Average root number	5.0 ± 1	15.0± 2
	Average root length (mm)	14.0 ± 3.3	19.6 ± 8.1
Assafri	Rooting rate (%)	88.2	80
	Average root number	5.9 ± 3.6	5.2 ± 3.3
	Average root length (mm)	20.3 ± 11.3	5.5 ± 2.2

For Assafri plantlets, M_9 medium induced less numerous but longer roots (5.9 roots with 20.3 mm long) (Table 4) (Figure 1d), but they seemed very fragile. These results were comparable to those reported by Bayoudh *et al.* (2015), but higher than those obtained by Shahcheraghi and Shekafandeh (2016) for the cultivar Bargchenari on medium with 1.5 mg L^{-1} IBA. In addition, the induction of numerous aerial roots was noticed on Soltani and Assafri plantlets on rooting media M_9 and on M_{10}, respectively. This reminded particularity of some species of the genus *Ficus* to develop aerial roots (Jain and Khan, 2015). Medium M_9 induced rapid rhizogenesis after two weeks of culturing. On medium M_{10}, it took 9 weeks to have roots, since this medium was less rich in macro and oligo-elements (½MS with 0.2 mg L^{-1} IBA compared to medium M_9 (MS + 1 mg L^{-1} IBA).

Regeneration from shoot-tips

Initiation step: All Assafri shoot-tips initiated on medium M_8 supplemented with PG were established without any necrosis. They developed white calli at their bases with a callusing rate of 18%. For Zidi shoot-tips, their establishment rate was 94.4%, and callus rate was 25%. Whereas Bither Abiadh shoot-tips showed an establishment rate of 90.5% (Fig. 2a) and the lowest callusing rate (6.7%) (Table 5).

Shoot-tip browning was observed on 100% of Bither Abiadh, 97.2% of Zidi and 90.3% of Assafri explants, which is reported to be caused by cells exudation released from the wounded tissues (Sophia *et al.*, 2021). Different cultivar behavior with relation to explants browning was noticed. Also, the antioxidant PG added to M_8 medium would reduce the explants browning, prevented them from total necrosis and enhanced their evolution (Siwach and Gill, 2011; Bayoudh *et al.*, 2015). The plantlets issued from shoot-tips of all cultivars were elongated and developed leaves that were sometimes quite large (Fig. 2b). More than one third (35% to 39%) of the shoots presented more than three leaves. A slight superiority of leaf number was noticed with Zidi compared to other cultivars (Table 5).

Multiplication step: Two weeks after explant culturing on medium M_8, plantlets showed well growth and vigor (main stem) and developed axillary shoots. Zidi plantlets were the most vigorous and long (40.6 mm) and their evolution and elongation were faster than those of Assafri (33.1 mm) and Bither Abiadh (27.3 mm) plantlets. In addition, they developed a high number of leaves (13.5 leaves/plantlet) compared to those of Assafri (8.4 leaves) and Bither Abiadh (2.4 leaves) (Fig. 2b) (Table 6). Zidi plantlets produced more shoots (3.8 shoots, 7.6 mm in length and 2 leaves), followed by Bither Abiadh (2.8 shoots, 3.6 mm in length and 0.26 leaves), then those of Assafri with an average of 2.5 shoots with 4.8 mm length and 1.2 leaves (Table 6). The results showed that using medium M_8 for shoot multiplication was adequate. It contained mainly BAP and IBA, which were the best hormonal combination for the multiplication step of *Ficus* species such as *Ficus carica* (Parab *et al.*, 2021), *Ficus benjamina* and *Ficus Anastasia* (Al Malki and Elmeer, 2010).

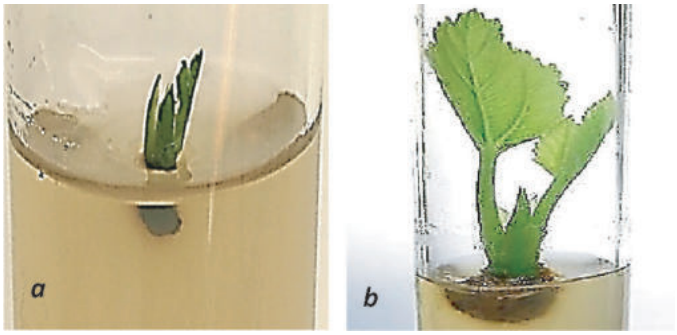

Fig. 2. Fig shoot-tip initiation on medium M_8 (a); shoot-tip evolution on M_8 and induction of large leaves (Bither Abiadh cultivar) (b).

Table 5. Establishment, callus, oxidation and plantlet leaf induction rates of shoot-tips of Zidi, Bither Abiadh and Assafri cultivars during 8 weeks of initiation on M_8

Cultivar	Establishment rate (%)	Callus rate (%)	Plantlet browning rate (%)	Rate of plantlets (%) with 3 leaves or more	Rate of plantlets (%) with less than 3 leaves
Zidi	94.4±9.6	25.0±8.3	97.2±19.4	39.0±16.0	61.0±4.2
B. Abiadh	90.5±16.5	6.7±1.5	100.0	35.0±18.8	65.0±15.2
Assafri	100.0	18.1±13.5	90.3±14.2	36.0±18.6	64.0±9.4

Table 6. Shoot-tip responses of cultivars Zidi, Bither Abiadh and Assafri during their multiplication on M_8 (8 weeks) and rooting on M_9 (5 weeks)

Cultivar	Principal plantlet		Regenerated shoots/explant		Roots	
	Average length (mm)	Average leaf number	Average number	Average length (mm)	Average number	Average length (mm)
Zidi	40.6	13.5	3.8	7.6	0.0	0.0
B. Abiadh	27.3	2.4	2.8	3.6	7.0	27.1
Assafri	33.1	8.4	2.5	4.8	4.7	19.9

Rooting step: Plantlets derived from all cultivar shoot-tips failed to root on M_{10} (½MS + 0.2 mg L^{-1} IBA). On the other hand, M_9 medium (MS + 1 mg L^{-1} IBA) was able to induce rhizogenesis five weeks after the explant cultivation of two cultivars. Bither Abiadh plantlets developed the highest average number of roots (7 roots/plant) with the highest average length (27.1 mm). These roots were thick, long but fragile and easily breakable. Assafri showed less extensive rhizogenesis with the average of 4.7 roots/plant and 19.9 mm long. No roots were induced on medium M_9 for Zidi plantlets (Table 6). This may be due to the excessive formation of spongy and friable calli at the base of the stems that prevented rhizogenesis. The use of IBA auxin to induce *in vitro* rooting of many *Ficus* species has been reported by several authors (El-Homosany and Hossam, 2019; Sriskanda *et al.*, 2021). Hepaksoy and Aksoy (2008) found that excessive callus formation, due to a high dose of IBA (2 mg L^{-1}), was unfavourable to rooting and negatively affected the survival rate of the plants, by absence or lack of roots.

Regeneration from leaf fragments

Initiation: The rate of leaf fragments that could regenerate adventitious shoots on medium M_{11} (MS + 0.5 mg L^{-1} TDZ + 1 mg L^{-1}IBA), reached 75.6% with an average of 3.6 shoots per explants for Soltani cultivar. For Assafri, only 57.2% of the explants reacted and regenerated an average of 1.9 shoots / explant. On M_{12} medium (MS + 1 mg L^{-1} TDZ + 1 mg L^{-1} IBA), 60% of Soltani and 55.8% of Assafri leaf fragments initiated their development (Fig. 3a and 3b) and regenerated, respectively, 1.4 and 1.2 shoots/ explant (Table 7). These regeneration rates were close to those mentioned by other researchers (Soliman *et al.*, 2010; Dhage *et al.*, 2015).

Medium M_{11} tended to regenerate a higher number of adventitious shoots compared to medium M_{12}. In addition, all Soltani explants induced calli irrespective of the medium on which the leaf fragments were initiated (Fig. 3c). This could be related to the

potential effects of TDZ combination with auxins on cell division (Abdolinejad, 2020). For Assafri explants, medium M_{11} induced less callogenesis (65%) (Table 7). Similar results were reported by Kim *et al.* (2007) who obtained 67.9% of callogenesis on leaf fragments of the cultivar Seungjung Dauphine initiated on media enriched with various TDZ concentrations. Thus, organogenesis by adventitious bud formation was essentially favored by culturing leaf fragments on medium M_{11} for Soltani and on medium M_{12} for Assafri explants. However, the Soltani cultivar showed better responses. This reflected the inter-cultivar differences in the ability of tissues to regenerate fig buds (Al-Shomali *et al.*, 2017).

Table 7. Leaf fragment responses of cultivars Soltani and Assafri during 8 weeks on initiation media M_{11} and M_{12}

Cultivar	Initiation medium	Leaf fragments regenerating shoots (%)	Average of shoots/ leaf fragment	Callus induction rate (%)
Soltani	M_{11}	75.6	3.6	100.0
	M_{12}	60.0	1.4	100.0
Assafri	M_{11}	57.2	1.9	65.0
	M_{12}	55.8	1.2	95.8

M_{11} (0.5 mg L^{-1} TDZ + 1 mg L^{-1} IBA, M_{12} (1 mg L^{-1} TDZ + 1 mg L^{-1} IBA)

Shoot multiplication: Adventive buds induced on leaf fragments evolved and regenerated shoots up to 5 mm long, with well-structured stems and small green leaves. They were separated and subcultured on medium M_8 (without PG). Four weeks after their multiplication, the average of the highest plantlets was registered with both Soltani (15.3 mm) and Assafri (15.6 mm) cultivars initiated on medium M_{11}. Assafri plantlets developed, on medium M_{12}, the highest average number of leaves (41.7 leaves) and their multiplication offers the highest average number of shoots formed (13.7 shoots/plantlets) (Table 8). In fact, medium M_{12} contained a relatively high dose of cytokinin TDZ (1 mg L^{-1}), perfectly adequate to the formation of new lateral vegetative buds (Singh *et al.*, 2016). Whereas medium M_{11} allowed to regenerate Soltani plantlets to develop a high average number of leaves (32 leaves) and a considerable average number of shoots (9.8 shoots/

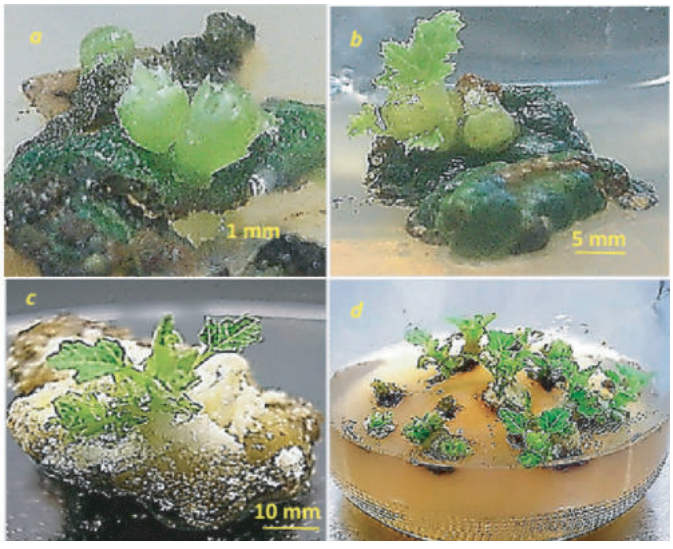

Fig. 3. Induction of lateral buds on leaf fragments on medium M_{11} (x 4) (a and b); plantlet on a spongy callus issued from a leaf lateral bud (x 4) (c); plantlets issued from leaf fragments (Soltani cultivar) on the multiplication medium M_8 (d).

plantlets) after their culture on the multiplication medium (Fig. 3d) (Table 8).

Table 8. Average of height, leaf and shoot numbers of plantlets Soltani and Assafri regenerated from leaf fragments on M_{11} and M_{12} and evolved during 4 weeks on multiplication medium M_8

Cultivar	Initiation medium	Plantlet length (mm)	Leaf number	Shoot number
Soltani	M_{11}	15.3 ± 6.9	32 ± 20.4	9.8 ± 5.4
	M_{12}	10.9 ± 2.9	25 ± 16.4	7.2 ± 3.4
Assafri	M_{11}	15.6 ± 2.3	23.7± 1.5	5.7 ± 1.5
	M_{12}	15.2 ± 1.5	41.7± 19.1	13.7 ± 6.8

M_{11} (0.5 mg L^{-1} TDZ + 1 mg L^{-1} IBA, M_{12} (1 mg L^{-1} TDZ + 1 mg L^{-1} IBA)

Acclimatization step: Three months after their acclimatization, the success rate of fig vitroplant emerging from Soltani meristems and rooted *in vitro* was 80.2%. These rates were in concordance with those found by Fraguas *et al.* (2012) for the acclimatization of *cv.* Roxo de Valinhos. *In vivo* rooted Soltani vitroplants achieved a survival rate of 95% (Table 9). *In vivo* rooting during the acclimatization phase was followed by the elongation of the plant stems. We registered the highest stem length (10.3 mm) and branching number (2 branches/plant). Thus, *in vivo* rooting seems to be an efficient and interesting procedure because of the simplicity of this method and reducing time and costs of vitroplants (Bayoudh *et al.*, 2015) by avoiding passage through the rooting medium. Singh *et al.* (2016) also obtained the best acclimatization success rate of plants (95.5%) by proceeding with rhizogenesis *in vivo*. The survival rate of Assafri plants rooted *in vivo* was 90%, with good elongation of the stems (13.5 mm) and a high number of leaves (8.4 leaves/plant) (Table 9).

Table 9. Growth of acclimatized vitroplants with *in vitro* and *in vivo* rooting

Parameter	Soltani with *in vitro* rooting	Soltani with *in vivo* rooting	Assafri with *in vivo* rooting
Success rate (%)	80.2	95.0	90.0
Stem length increase (mm)	9.6	10.3	13.5
Number of induced new leaves	8.1	7.4	8.4
Brunching number	0.5	2.0	0.9

The regenerated fig plants remained very susceptible to dehydration. The major loss of plants was observed during the first transplantation. The success of this step was dependent on the maintainance of a relative humidity higher than 80%, a temperature of 25 °C, a constant light intensity and the initial state of the plants at their exit (Fraguas *et al.*, 2012; Boliani *et al.*, 2019). For successful *in vivo* rooting and acclimatization, fig vitroplantlets must show a good vegetative state, having at least two nodes and reaching a height beyond 2.5 cm. The rooted plants had a normal appearance, good vigor and vegetation, continuous growth in length and development of new leaves observed from the third week of acclimatization.

In conclusion, the regeneration of fig vitroplants from three explant types was a feasible process. The hormonal composition of the medium had important effects on the response of fig tissues. During their initiation, meristems initiated better on medium M_4 containing BAP, and on M_1 medium (BAP + Kin). Globally, Assafri cultivar has shown the best responses to initiation media for meristems and shoot-tips establishment. Induction rates of adventitious shoots from leaf fragments on media with TDZ and IBA were considerable and led to an average of 3.6 shoots

per explant. During their multiplication on medium M_8, shoot-tips evolved better than meristems and leaf segments for all cultivars. The highest average number of shoots, leaves and roots developed per explant were registered with shoot-tips compared to meristems. *Ex vitro* rooting during acclimatization seems more interesting, taking into account the obtained high success rates and the simplicity of the technique.

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