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An optimized protocol for *in vitro* propagation of *Pyrus communis* and *Pyrus syriaca* using apical-bud microcuttings

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ABSTRACT

Purpose: In Tunisia, pear cultivars are widely threatened by the attack of fire blight disease. Cultivation of tolerant cultivars is an effective control strategy for disease control. For this purpose, a reliable protocol was established for micropropagation of local *Pyrus communis* and *Pyrus syriaca* L. and for large-scale production of high-quality plantlets. **Research method:** Using apical explants, different media and hormones were tested to establish a micropropagation procedure for local Tunisian *Pyrus communis* cultivars 'Arbi', 'Malti', 'Mahdia 6' and 'Moknine 10' and for *Pyrus syriaca*. Disinfection with 4% $HgCl_2$ treatment for 20 minutes showed the highest percentage of plant survival. Successful initiation of the cultures was achieved on MS basal medium supplemented with 0.25 mg L^{-1} BA. **Findings:** During the proliferation stage, optimal shoot multiplication was obtained on MS medium with a half concentration of NH_4NO_3 and KNO_3 supplemented with 0.1 mg L^{-1} IBA and 2 mg L^{-1} BA, but for maximum shoot length the BA concentration needed to be lowered to 1 mg L^{-1}. A rooting rate of 100% and the highest root length and root number were attained on Cheng medium supplemented with 1.0 mg L^{-1} IBA. Pear vitroplants were successfully acclimatized on S_2 substrate, composed by peat moss. **Research limitations:** Vitroplants acclimatization step needs to be well studied for the improvement of the acclimatized vitroplant survival rates by reducing the symptoms of crown rot. **Originality/Value:** This efficient optimized *in vitro* protocol will be successfully applied for large multiplication of high quality of Tunisian *Pyrus* vitroplants and cultivars.

INTRODUCTION

Pear, belonging to the genus *Pyrus*, subtribe *Malinae* (corresponding to the former *Maloideae*), family Rosaceae (Zheng et al., 2014), is one of the oldest temperate fruit crops. It is considered as a worldwide fruit tree, belonging mainly to Asian countries and the Indian subcontinent (Sharma & Pramanick, 2012). Pear fruits are an excellent source of vitamins, sugars, and important phytochemicals (Xia et al., 2016). In Tunisia, local low chilling cultivars of *Pyrus communis* L. are cultivated in coastal regions and classical European cultivars in continental areas (Mars et al., 1994). *Pyrus syriaca* Boiss., growing spontaneously in north Tunisia and considered to be very resistant to drought and calcareous soils, was tested as potential rootstock for common pear (Brini et al., 2008). However, both wild and local pear cultivars have not been subjected to much research despite their interesting characteristics. Main threats for local pear cultivars and rootstocks in Tunisia are urbanization, generalized use of introduced cultivars, climatic variations, and fire blight (Rhouma et al., 2013; Gaaliche et al., 2018).

Traditional vegetative methods to propagate pear plants are cutting and grafting, but they do not ensure disease-free plants and have low multiplication rates (Mars et al., 1994). Micropropagation has proven to be an efficient way to overcome these problems from many species and it enables rapid multiplication of disease-free plants at a commercial scale (Bahmani et al., 2009; Ayed et al., 2018). However, the pear is considered as one of the most recalcitrant dicotyledonous species for tissue culture manipulations (Reed et al., 2013; Aygun & Dumanoglu, 2015) with low shoot multiplication rates, hyperhydricity, tissue oxidation, lack of consistent adventitious rooting, and loss during acclimatization as the major bottlenecks. Nevertheless, micropropagation of *P. communis* OHF 333, 'Old Home× Farmingdale 87,' 'Horner 51,' 'Winter Nelis', and OHF 51 have been reported (Cheng, 1979; Nacheva et al., 2009; Reed et al., 2013) and *P. syriaca* (Shibli et al., 1997).

Since no reports are available on *in vitro* micropropagation of local Tunisian pear cultivars, this study was undertaken to develop a reliable *in vitro* propagation protocol for *P. communis* cultivars 'Arbi', 'Malti', 'Mahdia 6' and 'Moknine 10' and *P. syriaca* .

MATERIALS AND METHODS

Plant material preparation

Four cultivars ('Arbi', 'Malti', 'Mahdia 6' and 'Moknine 10') of *P. communis* and an accession of *P. syriaca* were used. *In vitro* stock cultures were established from apical buds collected in the spring from 20-year-old trees located at the Higher Agronomic Institute of Chott-Mariem, Tunisia. Apical segments (3 cm-long) were washed under running tap water for 1 hour and were surface-sterilized with 70% ethanol for 1 min, followed by treatment with 0.6% Benomyl for 2 min. Then, the explants were dipped in an antioxidant solution (ascorbic and citric acid) at 0.2% and 0.1%, respectively, and washed under running tap water to remove all residues. Prior to culturing, the explants were sterilized by submersion in commercial bleach sodium hypochlorite ($NaOCl_2$; 10, 12, 15 and 20%) (w/v) or mercuric chloride ($HgCl_2$; 1, 2 and 4%) (w/v) for 5, 10, 15, 20 and 30 min, adding a few drops of Tween-20. Finally, explants were rinsed three times with sterile distilled water and planted in initiation medium. Explant survival was scored 10 days after transfer to the medium.

Culture initiation and shoot proliferation

Three media were tested for culture initiation (M1, M2 and M3) and for proliferation and elongation (M4, M5, M6) of the *in vitro* shoots (Table 1). They all contained 3% sucrose

(w/v), myo-inositol (100 mg L^{-1}), thiamine-HCl (1 mg L^{-1}), nicotinic acid (1 mg L^{-1}), pyridoxine-HCl (1 mg L^{-1}), phloroglucinol (162 mg L^{-1}), and 0.7% (w/v) Difco Bacto-Agar. The pH was adjusted to 5.7 with KOH/HCl and the growth regulators were added before autoclaving at 121 °C for 20 minutes.

The cultures were kept at 25 ± 1°C under a photoperiod of 16 hours under fluorescent light (40 µmol m^{-2} s^{-1}). The explants of nodal segments (≈ 1.5 cm) were cultured in glass tubes (12 cm×2.5 cm) containing 10 ml of M1, M2 and M3. After 3 weeks, the percentage of explants forming shoots was recorded. After 3 weeks, when growth started (Fig. 1- a), the best-grown explants were shifted to multiplication medium M4, M5 and M6. Subculture was done on a fresh medium with the same compositions every 4 weeks. The number of shoots per explant and the shoot length (mm) were measured monthly with a digital caliper at the end of the fourth subculture.

Rooting

The rooting experiments were conducted on four rooting media (M7-M10; Table 1) under *in vitro* conditions with micropropagated shoots (approximately 1.5 cm long) obtained from the fourth subcultures. All media were supplemented with 3% sucrose (w/v), thiamine-HCl (400 mg L^{-1}), inositol (250 mg L^{-1}), phloroglucinol (162 mg L^{-1}) and 0.6% of (w/v) Difco Bacto-Agar. After 3 days, shoots were transferred to growth regulator-free medium under standard growth room conditions for 3 weeks (24±1 °C under 16-h photoperiod with 40 µmol m^{-2} s^{-1} fluorescent light). The percentage of rooted shoots, the number of roots and average root length per rooted shoot (mm) were measured with a digital caliper and recorded after 14 days.

Acclimatization

The roots of the *in vitro* regenerated pear plants were rinsed with tap water to eliminate culture medium and immersed in 0.1% fungicide solution (Pelt 500 SC®) for 3 min. The plants were transferred into trays containing one of two substrates: S1, 1/2 perlite and peat or S2, peat moss, and kept under a tunnel at 24 ± 2°C, 16-h photoperiod. After 4 weeks, new leaves emerged and acclimated plants were transferred to a shaded greenhouse at 26/20 °C (day/night), under 70% of relative humidity for hardening.

Table 1. Medium composition for *Pyrus* micropropagation

	Medium	BA Mg L^{-1}	IBA mg L^{-1}	NAA mg L^{-1}
Initiation				
M1	MS*	0.25	-	-
M2	MS**	0.25	-	-
M3	MS**	1	-	-
Proliferation/Elongation				
M4	MS**	-	-	-
M5	MS**	1	0.1	-
M6	MS**	2	0.1	-
Rooting				
M7	Cheng***	-	1	-
M8	Cheng***	-	-	1
M9	MS*	-	1	-
M10	MS*	-	-	1

* Original MS (Murashige & Skoog, 1962)

** MS with half the concentration of NH_4NO_3 and KNO_3

*** Cheng medium (Cheng, 1979)

Statistical analyses

All experiments were conducted in a completely randomized design with three replicates and 20 samples per each experimental unit (n = 60). The data were presented as means ± standard error (SE). The standard factorial analysis of variance and mean comparisons analysis, using Duncan's test (at P ≤0.05), were done using SPSS (Version 20.0 for windows Inc., Chicago, IL, USA).

RESULTS

Efficacy of sterilizing agents for *in vitro* culture establishment

$NaOCl_2$ and low concentrations of $HgCl_2$ were efficient to remove bacterial and fungal contaminations, but later, all the explants were lost. $HgCl_2$ at 4% for 20 min yielded the highest sterilization and survival rates for *P. communis* and *P. syriaca* explants (Table 2). Extending the exposure time to 30 min was detrimental for the explants, whereas reducing the exposure time resulted in increased explant loss due to contamination (Table 3).

Effect of medium composition on tissue browning and culture initiation

Three different media were tested to obtain an optimized *in vitro* initiation of the sterilized explants. As shown in Table 3, the medium composition had a significant impact on the response of the explants. M1 medium, a full-strength MS with 0.25 mg L^{-1} BA, was superior and gave a 100% explant establishment and completely prevented tissue browning in all the cultivars tested. Decreasing the NH_4NO_3 and KNO_3concentration (M2 and M3) as well as increasing the BA concentration (M3) stimulated necrosis and had a negative impact on initiation, both in the *P. communis* cultivars and in *P. syriaca* (Table 3). Although the trends in the responses were comparable for all cultivars, there was a significant interaction between cultivar and medium composition (Table 3).

Table 2. Effect of different exposure times to $HgCl_2$ (4%) on explant survival (%) of different *Pyrus* sp.

Species/cultivar		Exposure time				
		5 min	10 min	15 min	20 min	30 min
Pyrus communis	'Arbi'	0±0 ^{e}	22 ±1.22 ^{c}	31±1.30 ^{b}	45±1.14 ^{a}	6±1.14 ^{d}
	'Malti'	4 ± 1.30 ^{d}	12±1.14 ^{c}	17±0.83 ^{b}	30±0.83 ^{a}	1±0.70 ^{e}
	'Mahdia 6'	10±0.70 ^{d}	19±1.14 ^{c}	31±1.22 ^{b}	45±1.14 ^{a}	0±0 ^{e}
	'Moknine 10'	7±1.14 ^{d}	14±0.89 ^{c}	24±0.70 ^{b}	40±1.58 ^{a}	3±0.83 ^{e}
Pyrus syriaca		34±1.30 ^{d}	52±1.22 ^{c}	67±1.58 ^{b}	85±1.11 a	7±1.14 ^{e}
Significance of exposure duration effect		**	**	**	**	**
Significance of interaction 'CVS×Duration'		**	**	**	**	**

The values are compared horizontally. Means with a different letter in a row are statistically different (Duncan, $P \leq 0.01$).

Table 3. Effect of medium composition on tissue browning and *Pyrus* explant establishment during culture initiation

Species /cultivar		Tissue browning (%)			Healthy explant establishment (%)		
		M1	M2	M3	M1	M2	M3
Pyrus communis	'Arbi'	0±0 ^{c}	5±0.22 ^{b}	20±0.41^{a}	100±0 ^{a}	90±0.30 ^{b}	85±0.36 ^{c}
	'Malti'	0±0 ^{c}	5±0.22 ^{b}	25±0.44 ^{a}	100±0 ^{a}	90±0.30 ^{b}	80±0.41 ^{c}
	'Mahdia 6'	0±0 ^{c}	10±0.30 ^{b}	15±0.36 ^{a}	100±0 ^{a}	90±0.30 ^{b}	85±0.36 ^{c}
	'Moknine 10'	0±0 ^{c}	15±0.36 ^{b}	25±0.44 ^{a}	100±0 ^{a}	70±0.47 ^{b}	60±0.50 ^{c}
Pyrus syriaca		0±0 ^{c}	10±0.30 ^{b}	15±0.36 ^{a}	100±0 ^{a}	90±0.30 ^{b}	85±0.36 ^{c}
Medium effect		**	**	**	**	**	**
interaction 'Cultivars × Medium'		**	**	**	**	**	**

The values are compared horizontally. Means with a different letter in a row are statistically different (Duncan, $P \leq 0.01$).

Table 4. Effect of culture media on multiplication rate and shoot length in different *Pyrus* sp. at the end of the fourth subculture.

Species/cultivar		Medium	Multiplication rate	Shoot length (mm)
Pyrus communis	'Arbi'	M_4	1±0 ^{c}	7.46±0.33 ^{c}
		M_5	6.6±0.59 ^{b}	23.05±0.42 ^{a}
		M_6	11.7±0.97 ^{a}	18.76±0.52 ^{b}
	'Malti'	M_4	1±0 ^{c}	7.09±0.36 ^{c}
		M_5	5.85±0.36 ^{b}	21.68±0.47 ^{a}
		M_6	9.05±0.82 ^{a}	18.74±0.59 ^{b}
	'Mahdia 6'	M_4	1±0 ^{c}	7.30±0.48 ^{c}
		M_5	6.35±0.48 ^{b}	22.34±0.69 ^{a}
		M_6	9.65±0.58 ^{a}	15.48±0.81 ^{b}
	'Moknine 10'	M_4	1±0 ^{c}	9.57±0.51 ^{c}
		M_5	6.45±0.51 ^{b}	20.04±0.35 ^{a}
		M_6	9.35±0.48 ^{a}	15.11±0.63 ^{b}
Pyrus syriaca		M_4	1±0 ^{c}	10.08±0.26 ^{c}
		M_5	7.25±0.44 ^{b}	22.67±0.33 ^{a}
		M_6	10.4±0.50 ^{a}	12.46±0.83 ^{b}
Media effect			**	**
Cultivar effect			**	**
Interaction 'Cultivars × Media'			**	**

The values are compared vertically. Means with a different letter in a row are statistically different (Duncan. $P \leq 0.01$).

Effect of hormone concentrations on shoot proliferation

After three weeks, the healthy shoots were excised from the initiation media (M1) and transferred on to multiplication medium. Shoot proliferation was assessed on three different MS media with half the concentration of NH_4NO_3 and KNO_3 and different concentrations of BA and IBA (Table 4). A significant impact of medium composition and pear variety was noted. Without hormones (M4), none of the genotypes multiplied and the shoot length remained constant after the first subculture (Table 4). When 1 mg L^{-1} BA and 0.1 mg L^{-1} IBA were supplemented to the medium (M5), the number of shoots per subculture increased considerably and the shoots were longer for all cultivars (Table 4, Fig. 1- b). When the BA concentration was increased to 2 mg L^{-1} (M6), the multiplication rate increased with each subculture for all tested cultivars. However, under these conditions, the shoot length decreased and leaves turned narrow (Table 4), which made more difficult the further handling of the shoots multiplication and rooting.

Rooting of *in vitro* propagated *Pyrus* plantlets

Multiple shoots of high quality were produced on M5 then placed on M4 (without plant growth regulator) for one week to improve shoot elongation and leaf size prior to rooting. As shown in Table 5, both medium composition and variety significantly affected these parameters. Overall, Cheng medium with IBA (M7) resulted in the best rooting response for all tested genotypes, whereas, MS medium with NAA (M10) gave the worst results (Table 5). Additionally, the presence of IBA led to the highest root number and length in all cultivars (M7, M9) (Table 5). Although NAA also stimulated rooting to some extent (M8, M10) (Table 5), the roots were short and fleshy and developed from excessive brown, spongy and friable callus at the stem base.

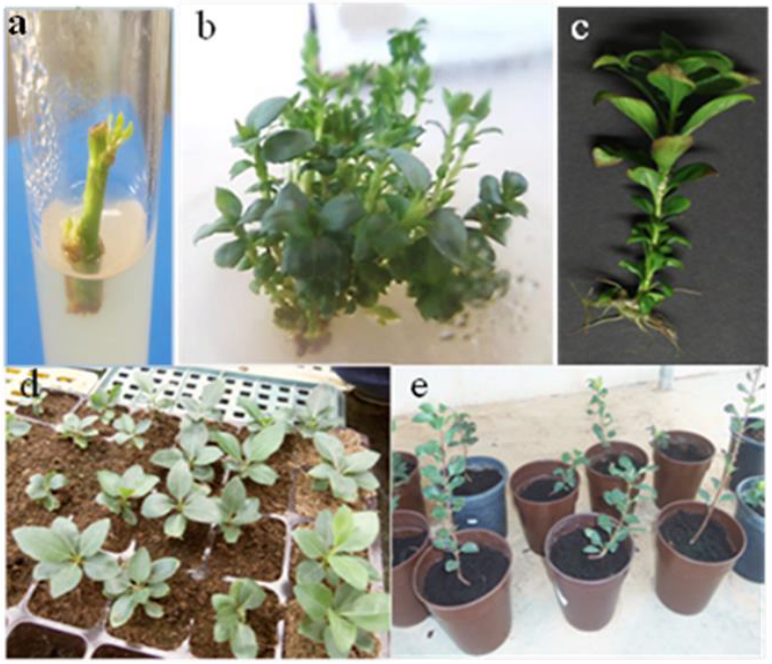

Fig. 1. Overview of the complete procedure for micropropagation of pear. **(a)** *In vitro* establishment of explants. **(b)** The proliferation of pear shoots on medium containing MS with half the concentration of NH_4NO_3 and KNO_3 and 1 mg L^{-1} BA + 0.1 mg L^{-1} IBA. **(c)** *In vitro* rooting, on Cheng medium with 1 mg L^{-1} IBA. **(d)** First-stage acclimatization of pear plantlets after two months. **(e)** Hardening of acclimatized pear vitroplants under insect-proof greenhouse conditions (picture taken after 10 months).

Table 5. Effect of medium composition on rooting rate number and length in different *Pyrus* sp.

Species/cultivar		Medium	Rooting rate (%)	Root number	Root length (mm)
Pyrus communis	'Arbi'	M_7	100±0 ^{a}	13.71±0.48 ^{a}	29.72±0.94 ^{a}
		M_8	78.57±0.41 ^{b}	8.71±1.11 c	5.76±0.97 ^{c}
		M_9	100±0 ^{a}	10.71±0.48 ^{b}	21.78±0.55 ^{b}
		M_{10}	50±0.50 ^{c}	9.28±1.79 ^{c}	4.06±0.84 ^{d}
	'Malti'	M_7	60.71±0.49 ^{a}	6.57±0.53 ^{a}	12.73±0.26 ^{a}
		M_8	14.25±0.35 b	3.42±0.78 ^{c}	5.16±0.46 ^{c}
		M_9	46.42±0.50 ^{a}	4.24±0.48 ^{b}	9.98±0.53 ^{b}
		M_{10}	10.71±0.31 ^{b}	2.85±0.89 ^{c}	4.28±0.39 ^{d}
	'Mahdia 6'	M_7	42.85±0.50 ^{a}	9.42±0.53 a	18.73±0.92 ^{a}
		M_8	21.42±0.41 ^{ab}	5±0.81 ^{c}	5.37±1.02 ^{c}
		M_9	25±0.44 ^{ab}	6.85±0.69 ^{b}	15.27±0.42 ^{b}
		M_{10}	7.14±0.26 ^{b}	3.57±0.78 ^{d}	4.81±0.34 ^{c}
	'Moknine 10'	M_7	39.28±0.49 ^{a}	6.85±0.69 a	9.77±0.88 ^{a}
		M_8	21.42±0.41 ^{ab}	3.42±0.53 ^{c}	5.91±0.57 ^{c}
		M_9	10.71±0.31 ^{b}	4.57±0.78 ^{b}	7.11±0.47 ^{b}
		M_{10}	3.57±0.18 ^{b}	2.71±0.48 ^{d}	4.36±0.59 ^{d}
Pyrus syriaca		M_7	50±0.50 ^{a}	7.71±0.75 a	26.82±0.87 ^{a}
		M_8	17.85±0.39 ^{b}	5±0.57 b	13.25±0.41 ^{c}
		M_9	28.71±0.46 ^{ab}	5.42±0.53 ^{b}	20.32±0.75 b
		M_{10}	21.42±0.41 ^{b}	2.57±0.53 ^{c}	6.73±0.54 ^{d}
Media effect			**	**	**
Interaction 'Cultivars × Media'			**	**	**

The values are compared vertically. Means with a different letter in a row are statistically different (Duncan, $P \leq 0.05$).

Effect of substrates on acclimatization and hardening

In vitro Well-rooted pear plantlets (Fig. 1- c) were transferred to a substrate with peat and perlite (S1) or peat moss (S2) for acclimatization. During the first four weeks, there were 100% survivals for all accessions on both substrates. Later during the acclimatization process, a fungal infestation strongly affected the plants, except for *P. communis* 'Arbi' of which 5% survived on S1 and 18% on S2 substrate (Fig. 1- d). On S2 substrate the surviving plants achieved a better growth, plant height and branching number, whereas on S1 substrate the highest leaf number was recorded; on S1: plant height: 7.5 cm; branching number: 1.3; leaf number: 9; on S2: plant height: 5.5 cm; branching number: 1; leaf number: 14; recorded after 10 months. The acclimatized plants were transferred to an insect-proof greenhouse and subsequently showed good growth and formed thick trunks and new leaves (Fig. 1- e) without any variation in morphological characteristics.

DISCUSSION

In this study, we established an efficient protocol for tissue culture initiation and propagation for local *P. communis* cultivars and *P. syriaca* accession. Yeo and Reed (1995) reported that explants from field-grown pear trees are usually difficult to disinfect and better results were obtained with explants taken from actively growing plants. Nevertheless, 20-minute incubation with 4% $HgCl_2$ was highly efficient to disinfect the apical explants from old pear trees. The effectiveness of $HgCl_2$ as a disinfectant is in agreement with previous studies on other pear cultivars (Bahri-Sahloul et al., 2005), other Rosales (Assareh & Sardabi, 2005) and others species (Qin et al., 2017). Further, in accordance with Mihaljevic et al. (2013), $NaOCl_2$ proved to be inadequate as a disinfectant in our experiments. In contrast, Shibli et al. (1997) obtained 95% survival with $NaOCl_2$ for *P. syriaca*, but the explants were harvested from a phytotron which might explain the discrepancy with our results.

Optimal culture initiation was accomplished on M1 medium consisting of full-strength MS medium supplemented with 0.25 mg L^{-1} BA for all tested cultivars, although growth medium composition and genotype influenced explant establishment. These findings agree well with those of Leite et al. (1997) and Karimpour et al. (2013) who studied other European and Iranian pear cultivars. The inclusion of phloroglucinol in all our media likely prevented excessive phenolic browning, as reported for the *in vitro* establishment of apple rootstock (Sharma et al., 2007). Additionally, as observed by Mamaghani et al. (2010) for roses, lowering the total nitrogen content by halving the NH_4NO_3 and KNO_3 concentration led to increased tissue necrosis.

Concerning propagation, in the absence of cytokinins, no multiplication occurred. Cytokinins are reported to be essential for all pear species proliferation (Karimpour et al., 2013; Lotfi et al., 2019). On medium with plant growth regulators, the multiplication rate for the selected pear cultivars increased with each subculture and was the highest on M6 medium containing MS medium with half the concentration of NH_4NO_3 and KNO_3 supplemented with 2 mg L^{-1} BA and 0.1 mg L^{-1} IBA. However, shoot elongation was best on M5 medium, which only has 1 mg L^{-1} BA. In *Bacopa monnieri*, the optimal biomass yield with particular combinations of BA and IBA has been attributed to the synergistic effect of auxin and cytokinin on the growth of tissues, cell expansion and cell division (Sakharam et al., 2017). Overall, our observations are in agreement with those reported for *in vitro* propagation of other *P. communis* varieties and pear species (Dimitrova et al., 2016; Lizarraga et al., 2017; Hassan & Zayed, 2018).

Optimal rooting of the plants micropropagated on M5 medium was achieved on M7 medium consisting of Cheng medium with 1 mg L^{-1} IBA. Compared to MS medium, Cheng medium has a lower concentration of ammonium and nitrate ions. A reduced salt concentration has been reported to improve adventitious rooting in diverse plants for commercial exploitation, increasing the root number and the root length (Moncousin, 2012). The superior rooting response of the selected pear varieties with IBA as compared to NAA is in concordance with the findings of Shibli et al. (1997), Reed (1995) and Thakur & Kanwar, (2008) for other pears. In contrast, Al-Maarri et al. (1994) obtained the best *in vitro* rooting for the pear cultivars 'Passe Crassane' and 'Williams' with NAA at 0.2 mg L^{-1}, indicating the occurrence of a genotype-dependent response. Unfortunately, concerning the acclimatization, our results are inconclusive due to fungal rots that eradicated most of our plants.

CONCLUSIONS

An efficient tissue culture protocol was established for the *P. communis* cultivars 'Arbi', 'Malti', 'Mahdia 6', and 'Moknine 10' and for *P. syriaca*. An overview of the protocol is given in Figure 1. The recommended procedure consists of 20 minutes explant disinfection with 4% $HgCl_2$, followed by transfer to MS medium supplemented with 0.25 mg L^{-1} BA for initiation and to MS basal medium with half the concentration of NH_4NO_3 and KNO_3 supplemented with 2 mg L^{-1} BA and 0.1 mg L^{-1} IBA for optimal shoot multiplication; rooting of the propagated shoots should be executed on Cheng medium with 1 mg L^{-1} IBA. Pending the optimization of the acclimatization and hardening process and the molecular validation of the genetic stability of the regenerated plants, this protocol can be applied for a large-scale production of good quality and healthy plants to be used for the establishment of a successful commercial production of local Tunisian pear cultivars.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

REFERENCES

Al-Maarri, K., Arnaud, Y., & Miginiac, E. (1994). Micropropagation of *Pyrus communis* cultivar 'Passe Crassane' seedlings and cultivar 'Williams': factors affecting root formation *in vitro* and *ex vitro*. *Scientia Horticulturae, 58*, 207-214. https://doi.org/10.1016/0304-4238(94)90152-x

Assareh, M.H., & Sardabi, H. (2005). Macropropagation and micropropagation of *Ziziphus spina-christi*. Pesquisa Agropecuaria Brasileira, *40*, 459-465. https://doi.org/10.1590/s0100-204x2005000500006

Ayed, C., Bayoudh, C., Rhimi, A., Mezghani, N., Haouala, F., & Al Mohandes Dridi, B. (2018). *In vitro* propagation of Tunisian local garlic (*Allium sativum* L.) from shoot-tip culture. *Journal of Horticulture and Postharvest Research*, *1*(2), 75-86. https://doi.org/10.22077/jhpr.2018.1457.1016

Aygun, A., & Dumanoglu, H. (2015). *In vitro* shoot proliferation and *in vitro* and *ex vitro* root formation of *Pyrus elaeagrifolia* Pallas. *Frontiers in Plant Science*, *6*, 225. https://doi.org/10.3389/fpls.2015.00225

Bahmani, R., Karami, O., & Gholami, M. (2009). Influence of carbon sources and their concentrations on rooting and hyperhydricity of apple rootstock MM106. *World Applied Sciences Journal*, *6*, 1513-1517.

Bahri-Sahloul, R., Mtar, R., Msallem, A., & Ammar, S. (2005). Micropropagation of three "*Pyrus*" rootstocks. *Advances in Horticultural Science, 19*, 1000-1008.

Brini, W., Mars, M., & Hormaza, I. (2008). Genetic diversity in local Tunisian pears (*Pyrus communis* L.) studied with SSR markers. *Scientia Horticulturae*, *115*, 337-341. https://doi.org/10.1016/j.scienta.2007.10.012

Cheng, T.V. (1979). Micropropagation of clonal fruit tree rootstocks. *Compact Fruit Trees, 12*, 127-137.

Dimitrova, N., Nacheva, L., & Berova, M. (2016). Effect of meta-topolin on the shoot multiplication of pear rootstock OHF-333 (*Pyrus communis* L.). *Hortorum Cultus-Acta Scientiarum Polonorum*, *15*, 43-53. https://doi.org/10.17660/actahortic.2009.839.23

Gaaliche, B., Chehimi, S., Dardouri, S., & Hajlaoui, M.R. (2018). Health status of the pear tree following the establishment of Fire blight in Northern Tunisia. *International Journal of Fruit Science*, *18*, 85-98. https://doi.org/10.1080/15538362.2017.1377670

Hassan, S.A.M., & Zayed, S.N. (2018). Factor controlling micropropagation of fruit trees: A review. *Science International*, *6*, 1-10. https://doi.org/10.17311/sciintl.2018.1.10

Karimpour, S., Davarynejad, G.H., Bagheri, A., & Tehranifar, A. (2013). *In vitro* establishment and clonal propagation of Sebri pear cultivar. *Journal of Agricultural Sciences and Technology*, *15*, 1209-1217.

Leite, G.B., Finardi, N.L., & Fortes, G.R.L. (1997). The effect of BAP and NAA concentration on multiplication "*in vitro*" of pears cv. Bartlett and clone OH × F 97. *Ciencia-e-Agrotecnologia*, *21*, 436-441.

Lizarraga, A., Fraga, M., Ascasibar, J., & Gonzalez, M.L. (2017). *In Vitro* propagation and recovery of eight apple and two pear cultivars held in a germplasm bank. *American Journal of Plant Sciences*, *8*, 2238-2254. https://doi.org/10.4236/ajps.2017.89150

Lotfi, M., Mars, M., & Stefaan, W. (2019). Optimizing pear micropropagation and rooting with light emitting diodes and trans-cinnamic acid. *Plant Growth Regulation*. 1-8. https://doi.org/10.1007/s10725-019-00498-y

Mamaghani, A.B., Ghorbanli, M., Assareh, M.H., & Ghamari, Z.A. (2010). *In vitro* propagation of three Damask Roses accessions. *Iranian Journal of Plant Physiology*, *1*, 85-94.

Mars, M., Carraut, A., Marrakchi, M., Gouiaa, M., & Gaaliche F. (1994). Ressources génétiques fruitières en Tunisie (poirier, oranger, figuier, grenadier). *Plant Genetic Ressources Newsletter*, *100*, 14-17.

Mihaljevic, I., Dugalic, K., Tomas,V., Viljevac, M., Pranjic, A., Cmelik, Z., Puskar,B., & Jurkovic, Z. (2013). *In vitro* sterilization procedures for micropropagation of 'Oblacinska' sour cherry. *Journal of Agricultural Sciences, Belgrade*, *58*, 117-126. https://doi.org/10.2298/jas1302117m

Moncousin, C. (2012). Rooting of *in vitro* cuttings. In *High-tech and micropropagation I (17).* (Bajaj Y.P ed.). Springer Science & Business Media, Berlin, 231-261. https://doi.org/10.1007/978-3-642-76415-814

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, *15*, 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nacheva, L.R., Gercheva, P.S., & Dzhuvinov, V.T. (2009). Efficient shoot regeneration system of pear rootstock OHF 333 (*Pyrus communis* L.) leaves. *Acta Horticulturae*, *839*, 195-201. https://doi.org/10.17660/actahortic.2009.839.23

Qin, J., Wang, Y., He, G., Chen, L., He, H., Cheng, X., Xu, K., & Zhang, D. (2017). High-efficiency micropropagation of dormant buds in spine base of red pitaya (*Hylocereus polyrhizus*) for industrial breeding. *International Journal of Agriculture and Biology*, *19*, 193-198. https://doi.org/10.17957/ijab/15.0264

Reed, B.M., Wada, S., De Noma, J., & Niedz, R.P. (2013). Mineral nutrition influences physiological responses of pear *in vitro*. *In Vitro Cellular & Developmental Biology - Plant*, *49*, 699-709. https://doi.org/10.1007/s11627-013-9556-2

Reed, B.M. (1995). Screening *Pyrus* germplasm for *in vitro* rooting response. *HortScience, 30*, 1292-1294. https://doi.org/10.21273/hortsci.30.6.1292

Rhouma, A., Helali, F., Chattaoui, M., Hajjouji, M., & Hajlaoui, M.R. (2013). First report of fire blight caused by *Erwinia amylovora* on pear in Tunisia. *Plant Disease, 98*, 158. https://doi.org/10.1094/pdis-05-13-0542-pdn

Sakharam, N.P., Rameshwar, A.P., & Bhagyashree, B.A. (2017). Effect of different concentrations of IBA in combination with BA on biomass yield of *Bacopa monnieri* (Brahmi). *International Journal of Current Microbiology and Applied Sciences, 6*, 3301-3306. https://doi.org/10.20546/ijcmas.2017.609.407

Sharma, T., Modgil, M., & Thakur, M. (2007). Factors affecting induction and development of *in vitro* rooting in apple rootstocks. *Indian Journal of Experimental Biology*, *45*, 824- 29.

Sharma, Y.P., & Pramanick, K.K. (2012). Utilization of plant genetic resources for the improvement of temperate fruit crops. *Indian Journal of Genetics and Plant Breeding*, *72*, 130-135.

Shibli, R.A., Ajlouni, M.M., Jaradat, A., Aljanabi, S., & Shatanawi, M. (1997). Micropropagation of wild pear (*Pyrus syriaca*). *Scientia Hortuculturae*, *68*, 237-242. https://doi.org/10.1016/s0304-4238(96)00972-7

Thakur, A., & Kanwar, J.S. (2008). Micropropagation of "Wild pear" *Pyrus pyrifolia* (Burm F.) Nakai. II. Induction of Rooting. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 36*, 104-111. https://doi.org/10.15835/nbha36278

Xia, L., Xuejiao, L., Wang, T., & Gao, W. (2016). Nutritional composition of pear cultivars (*Pyrus* spp.). In *Nutritional Composition of Fruit Cultivars* (Preedy V.R. & Simmonds M.S.J. ed.). Elsevier, USA, 573-608. https://doi.org/10.1016/b978-0-12-408117-8.00024-6

Yeo, D.Y., & Reed, B.M. (1995). Micropropagation of three *Pyrus* rootstocks. *HortScience, 30*, 620-623. https://doi.org/10.21273/hortsci.30.3.620

Zheng, X., Cai, D., Potter, D., Postman, J., Liu, J., & Teng, Y. (2014). Phylogeny and evolutionary histories of Pyrus L. revealed by phylogenetic trees and networks based on data from multiple DNA sequences. *Molecular Phylogenetics and Evolution*, *80*, 54-65. https://doi.org/10.1016/j.ympev.2014.07.009