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Optimization of In Vitro Propagation of Pear (*Pyrus communis* L.) 'Pyrodwarf®(S)' Rootstock

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Abstract: Pears are among the most economically important fruits in the world that are grown in all temperate zones. *Pyrus communis* L., 'Pyrodwarf®(S)' rootstock is one of the gene sources used to improve fruit productivity, rootstock resistance, and tolerance to biotic and abiotic stresses. Traditional propagation of *P. communis* L. is time-consuming and limited by a short growing season and harsh winter conditions. Therefore, in vitro propagation is a suitable alternative. Murashige and Skoog medium (MS) and woody plant medium (WPM) supplemented with different concentrations of 6-benzyladenine (BA) and kinetin (Kin), individually or in combination, were used for in vitro shoot proliferation. Nodal segments were used as explants. MS medium augmented with indole-3-butyric acid (IBA) or indole-3-acetic acid (IAA) was then used for rooting of microshoots. A combination of 2 mg·L^{-1} BA and 1 mg·L^{-1} Kin in MS medium resulted in a significant improvement in shoot proliferation. This combination produced the highest number of shoots (4.352 per explant) and leaves (10.02 per explant). The longest shoots (4.045 cm) were obtained in WPM enriched with 1 mg·L^{-1} BA. However, these shoots were not suitable for multiplication and rooting steps. The largest number of roots (5.50 per microshoot) was obtained on MS medium augmented with IAA at 1 mg·L^{-1}. The produced plantlets were cultivated in pots filled with perlite and cocopeat (in a ratio of 1:3) and acclimatized gradually in a greenhouse, recording an even 90% survival rate.

Keywords: fruit trees; micropropagation; plant growth regulators; rooting; shoot proliferation

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1. Introduction

Pear trees and shrubs belong to the genus *Pyrus*, in the family Rosaceae, and are native to coastal and mildly temperate regions of Europe, North Africa, and Asia. About 3000 known cultivars of pears are grown worldwide, with fruits varying in both shape and taste. European pear (*Pyrus communis* L.) is the main species of commerce in Europe, North America, South America, Africa, and Australia [1]. In northern regions, the cultivation of *P. communis* is limited by the short growing season and harsh winter conditions [2]. Some species of the *Pyrus* genus are suitable gene sources used to improve rootstock tolerance and resistance to biotic (e.g., pathogens) and abiotic (e.g., drought and salinity) stresses [3,4]. The selection of clonal rootstocks from dwarf or semi-dwarf genotypes is important in pear breeding programs [4].

Pyrodwarf, a hybrid of 'Old Home' and 'Gute Luise' cultivars, is particularly tolerant to winter cold temperatures, calcareous soils, and has a highly significant compatibility with the other pear cultivars. Moreover, it is characterized by high precocity, good productivity, and uniformity in the size of fruits produced [5]. Increasing market demand for this stress-tolerant rootstock offers an opportunity to investigate alternative methods for the efficient production of pyrodwarf [6].

Propagation of pears is possible by vegetative means, i.e., cutting, layering, and tissue culture techniques. Among these methods, in vitro propagation allows fruit breeders

to quickly multiply a new rootstock in a short time. Micropropagation helps overcome limitations found with traditional propagation, such as variability and seasonal availability of raw materials or fresh material losses [4].

The type of basal culture medium, type and concentration of plant growth regulators (PGRs), and explant parameters are the most important factors for the success of each in vitro propagation approach. Murashige and Skoog (MS) [7], Lepoivre (LP) [8], Driver-Kuniyuki Walnut (DKW) [9], and Woody Plant Medium (WPM) [10] have most frequently been applied for tissue culture of different pear species, cultivars, and genotypes [4,11–14]. As for PGRs, α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), N^6 benzyladenine (BA), 6-benzylaminopurine (BAP), 1-phenyl-3-(1,2,3-thidiazol-5-yl)urea (thidiazuron; TDZ), 2-isopentenyladenine (2-iP), zeatin (Zt), and gibberellic acid (GA_3) have mostly been used with this genus [1,4,11–15]. The most commonly used explants for the establishment and in vitro multiplication of pear are shoot tips, axillary buds, and single nodes, particularly those obtained from grafted plants grown in the greenhouse [1,16].

Unlike many herbaceous species, in vitro propagation, especially root induction, is difficult in most woody plants including *Pyrus* spp. [17]. Micropropagation protocols are available for major *Pyrus communis* L. cultivars [17]. Some studies have been carried out on wild and domestic *Pyrus* spp. [4,13,14,18–27]. However, publications on the in vitro culture of *Pyrus communis* L. 'Pyrodwarf®(S)' rootstock are limited [28]. Ruzić et al. [28] tested the effect of various cytokinins, added in the MS medium singly and combined with auxin IBA, on the in vitro multiplication of this semi-dwarfing rootstock. Nonetheless, other basal media types and PGRs should also be included in the micropropagation process [29]. Therefore, the objective of the present study was to improve the protocol for in vitro shoot proliferation (by using MS and WPM media, as well as BA and/or Kin) and root formation (by using MS medium and IAA or IBA) of *P. communis*, 'Pyrodwarf®(S)' rootstock. Developing a complete micropropagation protocol for this rootstock cultivar will enable not only its mass proliferation but is also a key technology for breeding purposes and long-term storage under cryogenics.

2. Materials and Methods

2.1. Plant Material

The plant material was prepared from the National Center of Genetic Resources, Karaj, Iran. The experiments were set in a plant tissue culture laboratory in Rasht city, Iran. A two-year-old, pot-grown, healthy rootstock of *P. communis* L., 'Pyrodwarf®(S)' was used as the mother plant. Semi-hardwood portions of shoots with lateral buds were dissected from the rootstocks in April–May 2022. The leaves were removed from plant samples and they were cut into about 2–3 cm-length pieces with 2 or 3 buds (Figure 1A).

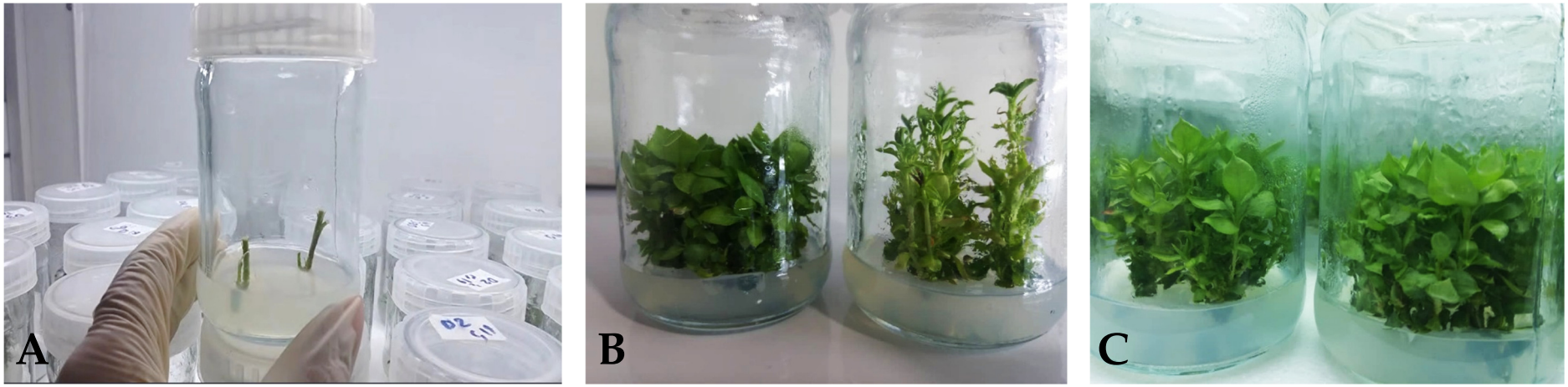

Figure 1. In vitro propagation of pear (*Pyrus communis* L.) 'Pyrodwarf®(S)' rootstock. (**A**) Nodal segments containing two lateral buds used as primary explants, (**B**) Microshoots produced in the MS medium enriched with 2 mg·L^{-1} BA together with 1 mg·L^{-1} Kin, after 30–35 days (left jar), and microshoots produced in the WPM medium with 2 mg·L^{-1} BA and 1 mg·L^{-1} Kin (right jar), (**C**) Microshoots produced in the MS medium enriched with 2 mg·L^{-1} BA together with 1 mg·L^{-1} Kin, after 30–35 days of the second sub-culture (left and right jars).

2.2. Explant Disinfection

The samples (50 per baker with a size of 500 mL) were thoroughly washed under running tap water with 1–2 drops of dishwashing liquid for 20 min. After defoliating, nodal segments (1–2 cm long) were cut and surface sterilized with 70% (*v*/*v*) ethyl alcohol for 45 s. Thereafter, nodal sections were washed with sterilized distilled water followed by a 20% (*v*/*v*) sodium hypochlorite solution (NaOCl, containing 5% active chlorine) for 15 min together with a few drops of Tween-20 under aseptic conditions of a laminar airflow cabinet. Finally, the explants were thoroughly washed three times (2, 3, and 5 min, respectively) with sterilized distilled water.

2.3. Establishment of In Vitro Culture

Nodal segment explants were placed vertically into sterilized MS [7] or WPM [10] media containing 3% (*w*/*v*) sucrose and 0.7% (*w*/*v*) agar without PGRs. The content of 30 mL medium was poured into each glass jar. The media pH was adjusted to 5.7–5.8 and the media were then autoclaved for 20 min at 121 °C and 105 kPa. The cultures were incubated in a culture room at 24 ± 2 °C with a 16-h photoperiod at a photosynthetic photon flux of 50–60 µmol m^{-2} s^{-1}, provided by cool daylight fluorescent lamps. After two weeks, newly formed shoot segments (Figure 1A) were used as secondary explants for subsequent experiments (Figure 1B,C).

2.4. Shoot Multiplication and Root Induction

Two cytokinins, BA (0, 1, and 2 mg·L^{-1}, i.e., 0, 4.44, and 8.88 µM) and Kin (0, 0.5, and 1 mg·L^{-1}, i.e., 0, 2.32, and 4.65 µM) were added to the MS and WPM basal media, alone or in combination with each other. Media with PGRs were autoclaved. The microshoots produced in the multiplication stage were suitable for root induction, but to produce more shoots, explants were subcultured on the fresh medium of the same composition three times with a 30-day subculture duration (Figures 1B,C and 3A–F).

Fast-growing shoot cultures were selected for root production. Two auxins, IAA and IBA, both in concentrations of 0, 0.5, and 1 mg·L^{-1} (i.e., 0, 2.85, and 5.71 µM for IAA or 0, 2.46, and 4.92 µM for IBA) were added to the MS basal medium. The cultures were incubated in a culture room at 24 ± 2 °C with a 16-h photoperiod at a photosynthetic photon flux of 50–60 µmol m^{-2} s^{-1}, provided by cool daylight fluorescent lamps.

This factorial experiment was conducted in a completely randomized block design with 10 repetitions. Each experimental block consisted of 5 jars with 3 explants inoculated in each jar.

2.5. Acclimatization Process

The rooted microshoots (Figure 3A–D) were removed from the culture jars and the roots were washed well but gently under running tap water to remove traces of agar adhering to the roots. The lower leaves of the plantlets were sniped using a pair of scissors so that the establishment of the plantlets in the pot bed would not be disturbed. Plantlets were then transferred to plastic pots (6 cm diameter) containing a mixture of perlite and cocopeat (in a ratio of 1:3) in September–October 2022 and watered with distilled water. A transparent plastic cup was placed over each plantlet to prevent excessive water loss. The pots were moved to an acclimatization room at an air temperature of 25 ± 2 °C with 70 ± 5% relative humidity under a 16-h photoperiod with irradiation of 100 µmol m^{-2} s^{-1} provided by cool-white fluorescent tubes for 20 days. Primary hardened plantlets were then transferred to larger plastic pots and transferred to the greenhouse for further acclimatization in October–November 2022 (Figure 3E,F). Plantlets were fed every two weeks with NPK liquid fertilizer in a ratio of 20:20:20. All rooted plantlets of each treatment, except for the control, were transferred to the greenhouse for acclimatization.

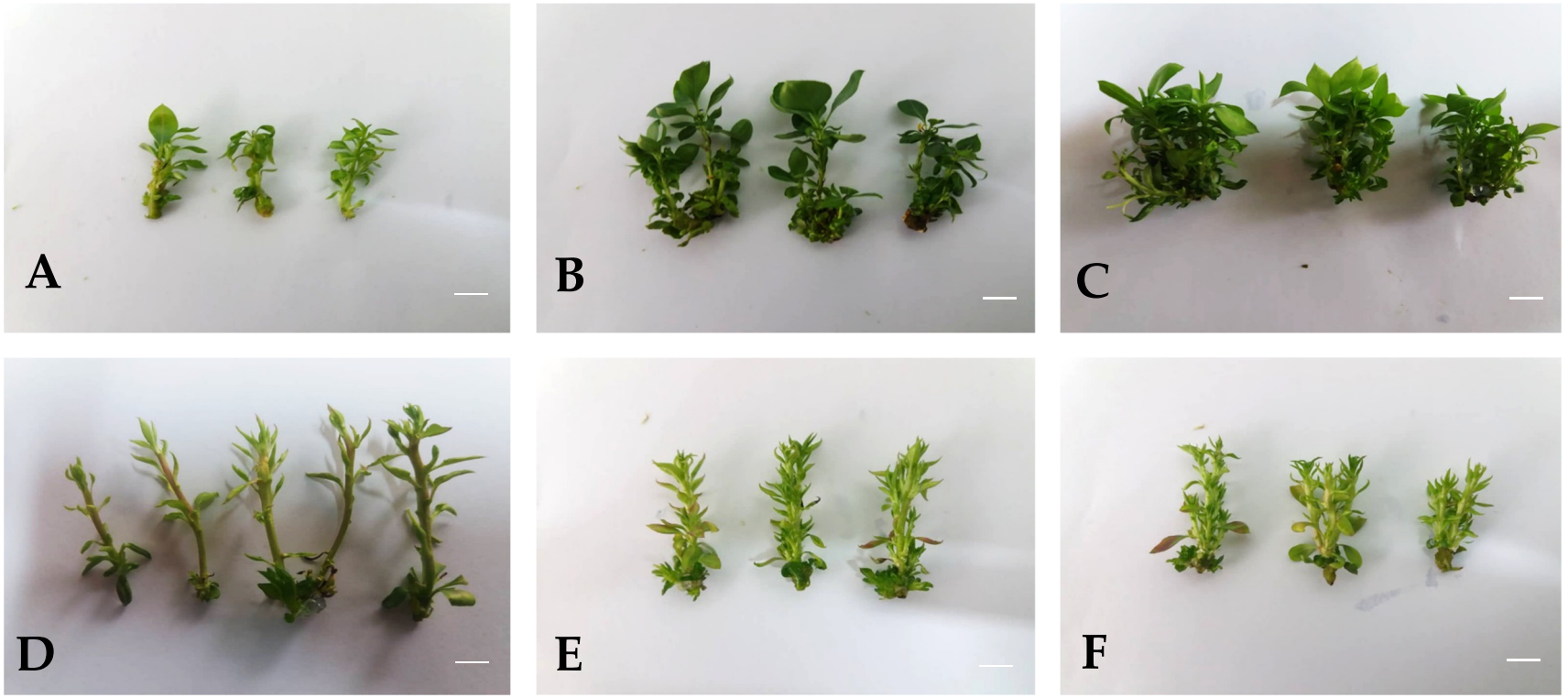

Figure 2. In vitro root induction in pear (*Pyrus communis* L.) 'Pyrodwarf®(S)' rootstock. (**A**) Rooted microshoots in the MS medium with 0.5 mg·L^{-1} IAA, (**B**) Rooted microshoots in the MS medium with 1 mg·L^{-1} IAA, (**C**) Rooted microshoots in the MS medium with 0.5 mg·L^{-1} IBA, (**D**) Rooted microshoots in the MS medium with 1 mg·L^{-1} IBA, (**E**) Hardening of plantlets in plastic dishes filled with perlite and cocopeat (in a ratio of 1:3) in the acclimatization room, (**F**) Plantlets transferred to plastic pots (after 20 days) for further acclimatization in the greenhouse.

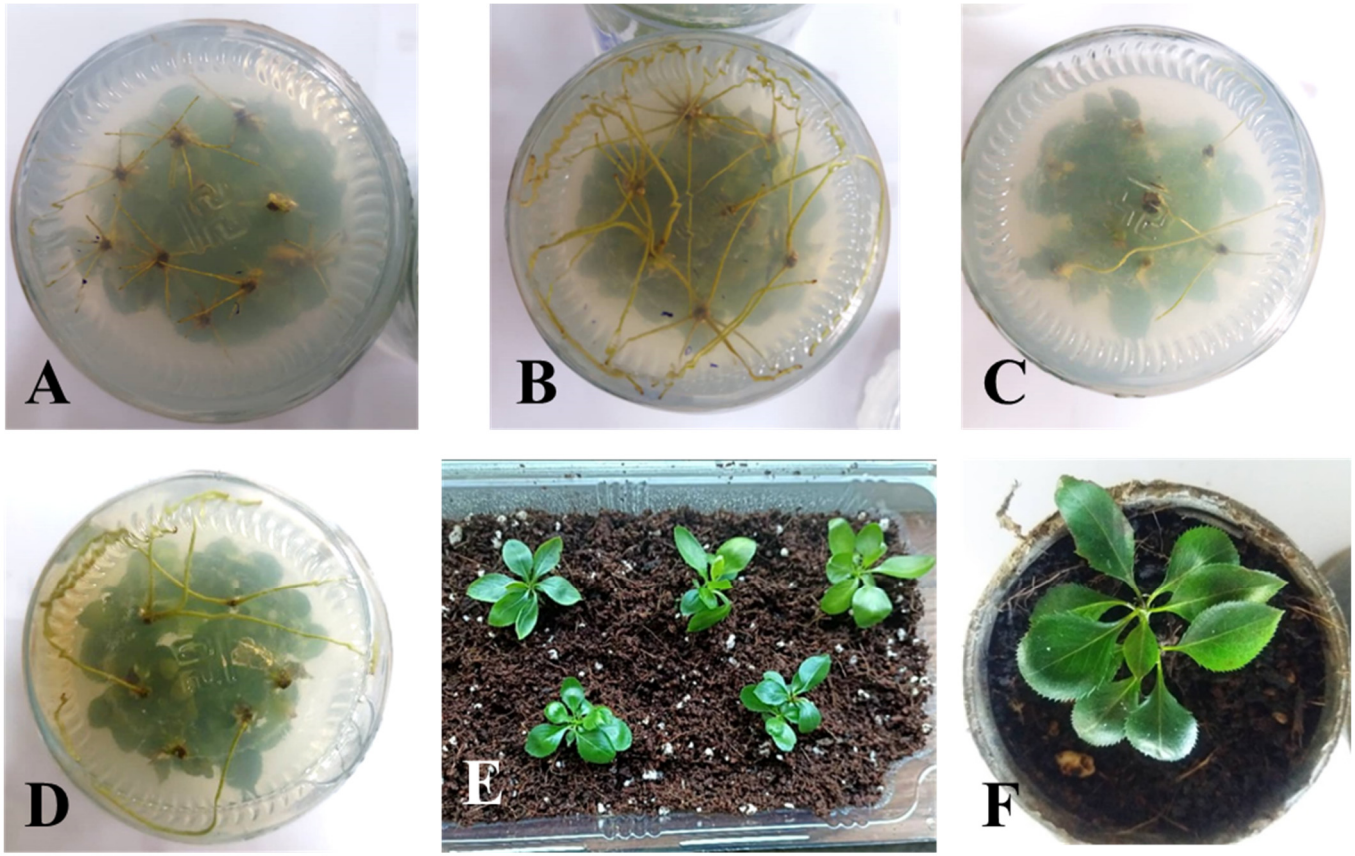

Figure 3. In vitro shoots of pear (*Pyrus communis* L.) 'Pyrodwarf®(S)' rootstock, after the last sub-culture. (**A**) Microshoots produced in the MS medium supplemented with 1 mg·L^{-1} BA, (**B**) Microshoots produced in the MS medium with 2 mg·L^{-1} BA and 0.5 mg·L^{-1} Kin, (**C**) Microshoots produced in the MS medium with 2 mg·L^{-1} BA and 1 mg·L^{-1} Kin, (**D**) Microshoots produced in the WPM medium with 1 mg·L^{-1} BA, (**E**) Microshoots produced in the WPM medium with 2 mg·L^{-1} BA and 0.5 mg·L^{-1} Kin, (**F**) Microshoots produced in the WPM medium with 2 mg·L^{-1} BA and 1 mg·L^{-1} Kin. Scale bar = 10 mm.

2.6. Measured Traits and Data Analysis

The cultures and hardened plantlets were observed periodically at regular intervals. For the shoot multiplication stage, the shoot length (cm), shoot number, and leaf number were evaluated as the basis of comparison after 30 days of culture (at the end of the third sub-culture). After 30 days of in vitro rooting, the rooting percentage, root number, and root length (cm) were measured. The survival rate of plantlets was recorded 30 days after transplantation from culture jars to the greenhouse.

The results were analyzed statistically using SAS version 9.1 software [30]. The significance of differences among means was compared using the LSD test at $p < 0.05$.

3. Results

3.1. Microshoot Establishment

The disinfection efficiency reached 90%. Next, the primary clean explants were transferred to the initiation culture medium, on which 95% of shoots were successfully established (Figure 1A). No callus development was observed throughout the experiment.

3.2. Shoot Proliferation

The composition of the culture medium affected the in vitro proliferation of shoots from axillary buds in *P. communis* 'Pyrodwarf®(S)' rootstock (Table 1, Figures 1 and 2). Significantly more shoots and leaves were produced using the MS medium containing BA and Kin (Figures 1C and 2B,C). The highest number of microshoots (4.352 per explant) was obtained in the MS medium augmented with 2 mg·L^{-1} BA together with 1 mg·L^{-1} Kin (Table 1, Figures 1C and 2C). This combination of PGRs in the WPM also stimulated the growth of many microshoots (3.64) (Figures 1B and 2F), although this number was significantly lower compared to the MS medium. The lowest rates of microshoot proliferation (0.955 and 1.025) were found in WPM and MS media without cytokinins.

Table 1. Effect of different concentrations of BA and Kin, and culture medium type on shoot proliferation and development in pear (*Pyrus communis* L.) 'Pyrodwarf®(S)' rootstock grown in vitro.

Treatment			Shoot Number	Shoot Length	Leaf Number
Culture Medium	**BA (mg·L^{-1})**	**Kin (mg·L^{-1})**	**(cm)**		
MS	0	0	1.025 ^{ef} ± 0.124	1.190 ^{j} ± 0.313	2.535 ^{ij} ± 0.197
	1	0	1.668 ^{de} ± 0.203	2.903 $^{d–f}$ ± 0.762	7.345 ^{c} ± 0.573
	2	0	1.327 $^{d–f}$ ± 0.160	1.589 ^{hi} ± 0.419	3.769 ^{h} ± 0.292
	0	0.5	1.296 $^{d–f}$ ± 0.158	1.457 $^{h–j}$ ± 0.382	2.325 ^{jk} ± 0.181
	1	0.5	1.549 $^{d–f}$ ± 0.187	2.184 ^{g} ± 0.575	4.220 ^{g} ± 0.327
	2	0.5	3.017 ^{bc} ± 0.364	3.314 ^{bc} ± 0.870	8.348 ^{b} ± 0.651
	0	1	1.127 ^{ef} ± 0.135	1.355 ^{ij} ± 0.354	2.625 ^{i} ± 0.205
	1	1	2.905 ^{c} ± 0.353	2.267 ^{g} ± 0.596	5.122 ^{f} ± 0.397
	2	1	4.352 ^{a} ± 1.716	3.182 ^{cd} ± 0.834	10.02 ^{a} ± 0.780
WPM	0	0	0.955 ^{f} ± 0.114	1.702 ^{h} ± 0.448	1.655 ^{l} ± 0.131
	1	0	1.337 $^{d–f}$ ± 0.159	4.045 ^{a} ± 1.060	7.015 ^{d} ± 0.547
	2	0	1.657 ^{de} ± 0.200	2.955 ^{de} ± 0.772	2.215 ^{k} ± 0.172
	0	0.5	1.237 $^{d–f}$ ± 0.151	2.277 ^{g} ± 0.596	2.135 ^{k} ± 0.163
	1	0.5	1.357 $^{d–f}$ ± 0.168	2.607 ^{f} ± 0.686	4.130 ^{g} ± 0.319
	2	0.5	2.675 ^{c} ± 0.327	3.592 ^{b} ± 0.941	7.345 ^{c} ± 0.572
	0	1	1.127 ^{ef} ± 0.135	2.177 ^{g} ± 0.571	2.557 ^{ij} ± 0.200
	1	1	1.88 ^{d} ± 0.229	2.832 ^{ef} ± 0.744	3.790 ^{h} ± 0.294
	2	1	3.64 ^{b} ± 0.928	3.077 $^{c–e}$ ± 0.809	6.675 ^{e} ± 0.523

Means with different letters in the same column are significantly different ($p < 0.05$) based on the LSD test.

There was a significant difference between the number of leaves produced in various experimental blocks (Table 1). A maximum number of leaves (10.02 per explant) was obtained

in the MS medium supplemented with 2 mg·L^{-1} BA and 1 mg·L^{-1} Kin (Figures 1C and 2C). Many leaves (8.348 per explant) were also produced in the MS medium containing 2 mg·L^{-1} BA together with 0.5 mg·L^{-1} Kin (Figure 2B). The same combination of PGRs added into the WPM resulted in the production of a significantly lower number of leaves (6.675–7.345). The lowest number of leaves (1.655 per explant) was obtained in the WPM without PGRs (Table 1).

In terms of shoot length, there was a significant difference between different levels of PGRs and different culture media types. The longest microshoots (4.045 and 3.592 cm) were obtained in the WPM enriched with 1 mg·L^{-1} BA (Figure 2D), as well as 2 mg·L^{-1} BA together with 0.5 mg·L^{-1} Kin (Figure 2E, Table 1). These shoots were approximately two to three times longer than the controls without PGRs (1.190–1.702 cm). Likewise, shoot length (3.314 cm) was high in the explants cultured in the MS medium augmented with 2 mg·L^{-1} BA and 0.5 mg·L^{-1} Kin. These microshoots were suitable for multiplication and rooting.

3.3. Root Induction and Acclimatization

Microshoots produced in a sufficient number in the proliferation medium (2 mg·L^{-1} BA and 1 mg·L^{-1} Kin) were transferred to the MS rooting media (Figure 3A–D). Microshoots could not be rooted in the control block without auxins (Table 2). Root induction on the base of microshoots cultured in the media supplemented with IAA was significantly higher than that of those cultured in the media with IBA (Table 2, Figure 3). The highest rooting percentage (95%) was observed in the medium enriched with 1 mg·L^{-1} IAA, followed by the medium enriched with 0.5 mg·L^{-1} IAA (50%). IBA was less effective with the rhizogenesis efficiency reaching 20 to 45% (Table 2). The highest number of roots (5.50 per microshoot) was regenerated in the presence of 1 mg·L^{-1} IAA, followed by the medium containing 0.5 mg·L^{-1} IAA (3.50) (Figure 3A,B).

Table 2. Effect of various concentrations of IAA and IBA on the rooting and acclimatization of pear (*Pyrus communis* L.) 'Pyrodwarf®(S)' rootstock grown in vitro.

Treatment (mg·L^{-1})	Rooting (%)	Root Number	Root Length (cm)	Acclimatization (%)
0 IAA	0 ^{d}	0 ^{d}	0 ^{d}	0 ^{d}
0.5 IAA	50 ^{b} ± 2.87	3.50 ^{b} ± 0.14	1.18 ^{c} ± 0.12	40 ^{b} ± 3.02
1 IAA	95 ^{a} ± 5.13	5.50 ^{a} ± 0.31	2.39 ^{a} ± 0.08	90 ^{a} ± 5.35
0 IBA	0 ^{d}	0 ^{d}	0 ^{d}	0 ^{d}
0.5 IBA	20 ^{c} ± 2.22	2.00 ^{c} ± 0.082	1.75 ^{b} ± 0.046	15 ^{cd} ± 1.22
1 IBA	45 ^{b} ± 3.65	1.50 ^{cd} ± 0.036	2.68 ^{a} ± 0.20	20 ^{c} ± 0.98

Means with different letters in the same column are significantly different ($p < 0.05$) based on the LSD test.

The effect of auxin concentration on the root length was also significant (Table 2). The longest roots (2.68 and 2.39 cm per microshoot) were produced in the MS medium enriched with 1 mg·L^{-1} IBA or 1 mg·L^{-1} IAA (Figure 3B,D), respectively.

No survival of the microshoots from the control blocks was observed (Table 2). Low (15–20%) acclimatization success was found if the plantlets were rooted on the IBA-supplemented medium, regardless of its concentration. On the other hand, 90% of the plantlets from the treatment with 1 mg·L^{-1} IAA survived transplantation and acclimation to the greenhouse conditions (Figure 3).

4. Discussion

Multiplication rate, length, and the number of shoots in plant micropropagation depend, among other factors, on the type of culture medium, the concentration of PGRs, and the cultivar [31]. In the present experiment, a combination of 2 mg·L^{-1} BA and 1 mg·L^{-1} Kin in the MS medium was the most effective for shoot multiplication of *P. communis* 'Pyrodwarf®(S)' rootstock. An improvement in shoot proliferation as compared to BA or Kin alone may be due to the complementary effect of these two cytokinins. During axillary shoot proliferation, cytokinins are mostly utilized to overcome the apical dominance of

shoots. Nonetheless, studies on the simultaneous effect of these PGRs on in vitro organogenesis are scarce. Generally, BA is the cytokinin of choice for the in vitro propagation of many species and cultivars in the genus *Pyrus* [15]. Aygun and Dumanoglu [4] reported that the in vitro shoot proliferation of *P. elaeagrifolia* was affected by BA concentration. Similar findings were reported by several other researchers, who used BA at concentrations ranging from 1 to 5 mg·L^{-1} [19,20,22,24,26,32–34]. In *P. syrica*, increasing BA concentration to 1.5 and 2 mg·L^{-1} enhanced shoot proliferation significantly, while the shoot height decreased [26]. In *P. pashia*, the number of shoots increased with the increase in BA level up to 2 mg·L^{-1}; however, further elevation of BA levels reduced the number of shoots produced [35]. In *P. communis* 'Bartlett', the highest number of shoots (3.87) was obtained with 2.40 mg·L^{-1} BA [1]. On the other hand, the pear 'Conference' had the highest shoot proliferation rate with 1 or 2 mg·L^{-1} BA [36]. The optimum level of PGRs for maximum shoot proliferation is different in each species and explant type [37–39]. For example, successful shoot growth, proliferation, and establishment using 1.5 mg·L^{-1} BA was reported in *Pyracantha coccinea* [38]. This might be due to the different content of endogenous phytohormones in plant cells and tissues [37].

The use of other PGRs, such as auxins, gibberellins, or putrescine, along with cytokinins could further increase the rate of shoot multiplication in *P. communis* 'Pyrodwarf®(S)' rootstock. For the shoot production of the pear 'Beurre Bosc', an MS culture medium enriched with 1 mg·L BA, 0.2 mg·L GA_3 and 0.1 mg·L IBA has been suggested [40].

Although for pears most micropropagation methods were based on the MS medium, some other media, especially LP, DKW, and WPM, have also been used [1]. In the present study, the MS medium was better than WPM in terms of shoot proliferation. The main reasons for these differences lie within the genetic factor and media composition, including the content of inorganic ions, especially the source and concentration of nitrogen.

One of the most important steps in the production of fruit tree rootstocks through tissue culture is the successful induction of rooting in the explants obtained from the multiplication stage. In vitro rooting of microcuttings is the most difficult stage of micropropagation to accomplish in woody species [1,34]. The initial differentiation of the root and its growth from parenchyma cells depends on the appropriate type and concentration of auxin. IBA is the most used auxin for inducing rooting followed by NAA, IAA, and 2,4-D [41]. Moreover, determining the most suitable culture medium for processing and rooting of different plants depends on the genotype, light, type of salts, and sugar compounds used [31]. Some researchers reported successful rooting in European pears, but the results were poorer with Asian cultivars [1,16]. Probably, Asian pear cultivars have harder wood than European cultivars and it is more difficult to induce rooting in them. In most studies, the MS medium supplemented with auxins was utilized for rooting of *Pyrus* [34]. The use of IBA has been reported to be effective in stimulating the rhizogenesis of the regenerated shoots of the Pyrodwarf rootstock [15]. It has also been effective in inducing rooting of *P. betulaefolia* explants, and the highest percentage of rooting was reported in explants grown in $\frac{1}{2}$MS medium containing 2 mg·L^{-1} IBA [42]. The high efficiency of IBA in inducing rooting is due to the slow rate of oxidation of this PGR in plant tissues [43]. Contrary to these reports, our findings revealed that IAA is better than IBA for rhizogenesis induction in *P. communis* 'Pyrodwarf®(S)' rootstock. Similar results were reported in some other pear species and cultivars [44]. The difference in the indigenous concentration of auxins in various cultivars is the main reason for the varied results. Shibli et al. [26] found that IBA, IAA, and NAA induced in vitro rooting of *P. syrica*, and a maximum of 72% rooting was obtained at 3 mg·L^{-1} IAA. The concentrations of 1 mg·L^{-1} NAA and 0.1 mg·L^{-1} IBA in $\frac{1}{2}$MS medium induced 27.46 and 14.08% rooting in *P. pashia* explants, respectively [45]. Different results were reported by some other researchers [21,46]. Anirudh and Kanwar [13] revealed that the rooting response was better with lower concentrations (0.125 and 0.25 mg·L^{-1}) of auxins. At higher concentrations (0.5–2 mg·L^{-1}), the rooting response was poor. These reports contradict our finding, as 1 mg·L^{-1} IAA was the best for *P. communis* 'Pyrodwarf®(S)' rootstock. At higher auxin concentrations, a callus is

formed at the shoot base that inhibits normal root development [1,13]. Moreover, the root elongation phase is very sensitive to auxin concentration and it is hampered by a high concentration of these PGRs [47]. In our study, the average root length increased with increasing auxin concentration from 0.5 to 1 mg·L^{-1}, both with IAA and IBA. On the other hand, the average root elongation decreased with increasing auxin concentration in the root induction medium in *P. pyrifolia* [13].

The success of any micropropagation system is related to the effective transfer of plantlets from tissue culture vessels to the ambient conditions found during acclimatization [48]. Ex vitro conditions, such as those in the greenhouse or field, are characterized by lower relative humidity and higher light level when compared to in vitro conditions. A good potting substrate with high water holding capacity, porosity, and drainage is necessary for the proper growth and development of in vitro-produced plantlets [37,39,49,50]. Moreover, acclimatization efficiency is affected by ambient conditions, i.e., light intensity, humidity, and air temperature [51]. The high survival of plantlets obtained (reaching up to 90%) highlights the utility of the protocol developed here. Interestingly, in the present study, microshoots rooted in the presence of IAA showed a much higher survival rate during acclimatization (40–90%) when compared to those from the IBA-supplemented medium. This suggests that the tatter auxin stimulates the development of a less-functional rooting system in *P. communis* 'Pyrodwarf®(S)' rootstock.

It is also worth mentioning that in the present study, due to the use of meristematic explants and relatively low concentration of PGRs, no callus formation was observed, indicating the direct origin of the shoots and roots. This is of significant importance from the horticultural point of view as callus is known to be a genetically unstable tissue that leads to somaclonal variation occurrence [52,53]. The direct development of organs from the meristematic cells suggests the maintenance of genetic stability of the plant material produced.

5. Conclusions

Owing to the high economic value of pears, developing a successful in vitro regeneration program is essential for its genetic improvement and delivering elite clones for commercial production. In this study, by analyzing the effect of various basal media types and combinations of PGRs, we have optimized a complete micropropagation protocol of *Pyrus communis* 'Pyrodwarf®(S)' rootstock through direct organogenesis. The highest microshoot proliferation rate (4.3 new shoots) was obtained with a combination of 2 mg·L^{-1} BA and 1 mg·L^{-1} Kin in the MS medium. On the other hand, the largest number of roots was regenerated in the MS medium augmented with IAA at 1 mg·L^{-1}. The suitability of the protocol developed here was confirmed by the high acclimatization success of the micropropagated plantlets (90%). In vitro propagation of this economically valuable fruit crop can be implemented for mass production, particularly since the use of a meristematic explant and direct organogenesis should guarantee the genetic fidelity of plants.

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