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Micropropagation of Al-Taif Rose: Effects of Medium Constituents and Light on In Vitro Rooting and Acclimatization

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Abstract: Micropropagation facilitates the rapid production of roses. Nevertheless, in vitro rhizogenesis of essential oil roses has presented significant challenges, primarily attributed to low rates of rooting and poorer acclimatization compared to ornamental rose varieties. This study reports the optimization of in vitro rooting of Al-Taif rose (*Rosa damascena* f. *trigintipetala* (Diek) R. Keller) microshoots with the aim of increasing survival rate during acclimatization. We also investigated the effects of various parameters, including type and concentration of auxin (i.e., 2,4-Dichlorophenoxyacetic acid (2,4-D), indole acetic acid (IAA), indole butyric acid (IBA), and naphthaleneacetic acid (NAA) at concentrations of 0, 0.05, 0.1, 0.2, and 0.4 mg/L), salt strength (i.e., full- and half-strength Murashige and Skoog (MS) medium), sucrose concentration (i.e., 20, 30, 40, 60, and 80 g/L), light spectra (a 2:1 or 1:2 blue/red spectral ratio, cool or warm white light at a 1:1 ratio, and fluorescent light), light intensity (photosynthetic photon flux density (PPFD) values of 25, 50, and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and activated charcoal (i.e., 0 and 0.5 g/L) on the rooting and growth of in vitro regenerated Al-Taif rose axillary shoots. We found that half-strength MS medium supplemented with 0.2 mg/L NAA, 80 g/L sucrose, 0.5 g/L activated charcoal, and 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD were the optimal conditions for 100% induction of adventitious roots. Next, micropropagated Al-Taif rose plantlets were successfully transferred to a potting medium containing perlite/peatmoss (in a 1:1 ratio). We found that 98% of plants survived ex vitro conditions. The genetic fidelity of micropropagated Al-Taif rose clones along with their mother plant was tested using the inter-simple sequence repeats (ISSR) molecular marker. The genetic similarity between the micropropagated plantlets and the mother plant of Al-Taif rose plants was 98.8%, revealing high uniformity and true-to-type regenerated plants. These findings may therefore contribute toward the commercial micropropagation of Al-Taif roses.

Keywords: auxins; hardening; light irradiance; LEDs; sucrose; root induction

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1. Introduction

The genus *Rosa* consists of various economically valuable plant species. One variety, the Damask rose (*Rosa damascena* Herrm.), has numerous applications in the food industry, including the production of rosewater and desiccated blossoms, and its petals are also used in the creation of marmalades, preserves, and jellies. Moreover, it is known to possess notable medicinal properties [1–3], and has been used to produce a wide range of personal care products, including aromatic, therapeutic, astringent, sedative, and blood cholesterol-altering commodities. In addition, the remnants from the distillation process can be used to produce livestock feed and compost [4]. Furthermore, the Damask rose is also widely used as a decorative plant in parks and gardens.

The rose of Al-Taif (*Rosa damascena* f. *trigintipetala* (Diek) R. Keller) is a specific infraspecific taxon of *Rosa damascena* (https://www.worldfloraonline.org/taxon/wfo-0001001564, accessed on 19 February 2024). In the Arabian region, this variety of Damask rose is highly regarded for rose oil production [5,6] and for its deep and intense fragrance. The oil-rich Al-Taif rose is named as such because it thrives in the favorable temperatures, plentiful groundwater,

well-established irrigation systems, and fertile topsoil of Al-Taif city, although it is also called "Arabia's Rose". This ancient variety is among the most valuable rose varieties, and its export to Arab countries, where it is used for decorative purposes, medicinal applications, and in perfume manufacturing, is a significant economic activity [7].

In micropropagation systems, in vitro rooting is a crucial step that relies on both genotype and physiological conditions [8]. Improvements in in vitro rooting techniques and conditions may enable the production of more roots and healthier plantlets, thus leading to improved survival following transfer to ex vitro conditions [8]. Successful rooting of in vitro shoots can be affected by factors such as the salt strength of the nutrient medium, the type and concentration of growth regulators, sucrose concentration, and physical factors such as light intensity [9,10]. Acclimatization also plays a vital role in the overall success of micropropagation, since it is during this period that most plant losses occur [11]. Therefore, obtaining high-quality plantlets under in vitro conditions is necessary for ensuring a high survival percentage and appropriate growth in greenhouse conditions. However, for a plantlet to survive ex vitro, it must develop to a stage where it has sufficient new growth, foliage, and a well-developed root system, characterized by an appropriate number and length of roots [12].

Plant tissue culture techniques facilitate the rapid production of rose cultivars possessing desirable traits and aid in the production of healthy and pathogen-free plants [13]. However, in vitro rooting processes of essential oil roses have proven to be challenging, chiefly due to low rooting success rates and poorer acclimatization relative to ornamental rose varieties [14]. The acclimatization of rose plantlets depends on the successful rooting of microshoots as well as the overall number of roots per shoot. Kirichenko et al. [14] demonstrated that the ability to root using different auxins varies among plant species, and that inducing root formation in different varieties of rose, particularly those used for pharmaceutical purposes, often presents unique difficulties. In addition, Horan et al. [15] noted that successful establishment of rooted plantlets can be influenced by the age of specific microshoots. It is well-established that in vitro culture plays a crucial role in enhancing the rejuvenation process, which significantly influences the rooting efficiency of microshoots. However, the difficulty in rooting microshoots can be attributed to the carry-over effect of cytokinin [10,16]. Podwyszynska [17] postulated that inadequate levels of endogenous auxin, other growth regulators, phenolics, or enzymes may contribute to the root formation problems in certain rose genotypes. They further suggested that the presence of inhibitory materials in mature explants may be responsible for low observed rooting rates [17]. Moreover, other studies have reported notable differences in the rooting requirements, responses to culture conditions, and basic rooting capacities of various rose cultivars [18]. Specific studies have also reported variation in the rooting responses of different *Rosa* species, including a lower rooting ability for *Rosa damascena* and *Rosa canina* relative to *Rosa hybrida* [13].

The primary issue encountered during the in vitro propagation of Damask rose is related to its rooting phase. In our previous study on micropropagation of Al-Taif rose, we investigated axillary shoot proliferation and growth in response to rose type and the concentrations of cytokinins, light and dark incubation, and air-lift bioreactor culturing [19]. Continuing this line of experimentation, the present study investigated other factors, including medium composition (i.e., auxin, sucrose, and the salt strength of the medium), light spectra, and light intensity. Overall, our aim was to identify optimal in vitro rooting and ex vitro survival of micropropagated plantlets of Al-Taif roses. The genetic fidelity of the regenerated Al-Taif rose plants with their mother plant was assessed using the inter-simple sequence repeats (ISSR) molecular marker.

2. Materials and Methods

2.1. Plant Material

This study was conducted at the plant tissue culture laboratory of the College of Food and Agricultural Sciences at King Saud University (Riyadh, Saudi Arabia). In a previous

study, we performed in vitro multiplication of the axillary shoots of Al-Taif rose shrub (Figure 1a) using MS medium [20] supplemented with 6-benzylaminopurine (0.5 mg/L) and sucrose (30 g/L) [19]. This medium was solidified using 8.0 g·L^{-1} agar (Dephyte, Hanover, Germany) and its pH was adjusted to 5.8 before being autoclaved at 121 °C and 118 kPa pressure for 15 min. Shoot cultures were incubated under dark conditions for six weeks at 25 ± 2 °C air temperature. In this study, regenerated axillary shoots were used as initial explants for in vitro rooting experiments (Figure 1b,c).

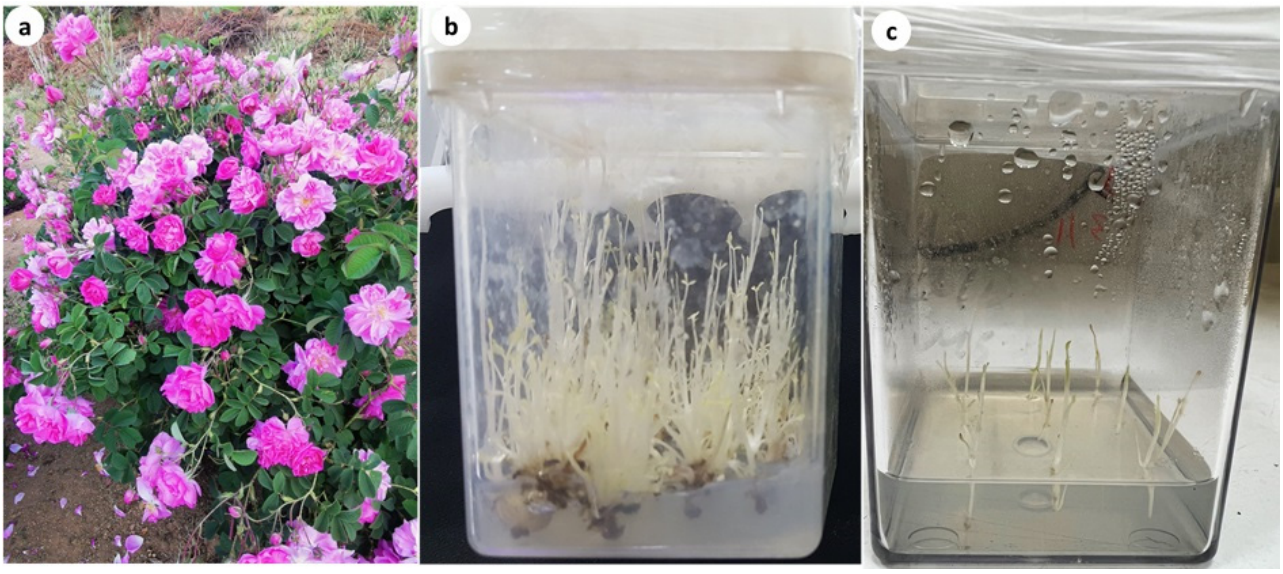

Figure 1. Al-Taif rose plant material used for in vitro rooting experiments. (**a**) Al-Taif rose shrub, (**b**) axillary shoots multiplication following six weeks of dark incubation, and (**c**) individual axillary shoots cultured for their rooting in vitro.

2.2. Effect of Auxin Type and Concentration on In Vitro Rooting of Al-Taif Rose

First, we examined the effect of auxin on rooting. Groups of nine axillary shoots of Al-Taif rose plants were cultured in Magenta GA-7 culture vessels (77 mm × 77 mm × 97 mm; Sigma Chemical Co., St. Louis, MO, USA) containing 60 mL MS medium supplemented with 30 g/L sucrose and auxins such as 2,4-Dichlorophenoxyacetic acid (2,4-D), indole acetic acid (IAA), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) at different concentrations (i.e., 0, 0.05, 0.1, 0.2, and 0.4 mg/L). Next, MS medium was gelled using 0.8% (*w*/*v*) agar and its pH was adjusted to 5.8 before being autoclaved at 121 °C and 1.2 kg cm^{-2} for 15 min. The cultures were then kept at 25 ± 2 °C air temperature under dark conditions for six weeks.

2.3. Effect of Medium Salt Strength and Sucrose Concentration on In Vitro Rooting of Al-Taif Rose

We then tested the importance of medium salt strength on rooting. MS salt (at full or half strength) and different sucrose concentrations (i.e., 20, 30, 40, 60, and 80 g/L) were tested as in vitro cultivation conditions for the rooting of Al-Taif roses. The axillary shoots were cultured onto MS medium supplemented with 0.2 mg/L NAA as an optimal auxin concentration. The medium was gelled using 0.8% (*w*/*v*) agar–agar, and the pH of the medium was adjusted to 5.8 before autoclaving at 121 °C and 1.2 kg cm^{-2} for 15 min. The cultures were kept at 25 ± 2 °C air temperature under dark conditions for six weeks.

2.4. Effect of Sucrose Concentration and Light Spectra on In Vitro Rooting of Al-Taif Rose

We then tested the interactive effect of sucrose and light spectra on rooting behavior. Al-Taif rose axillary shoots were cultured for six weeks on half strength MS medium containing 0.2 mg/L NAA and either 60 or 80 g/L sucrose. Next, four different light treatments were applied as per the protocol specified by Dewir et al. [21]. Briefly, three LED light (Shenzhen Lumini Technology Co., Ltd., Shenzhen, China) treatments were

tested alongside a standard fluorescent tube white spectrum. The LED treatments were as follows: a mixture of blue and red light at a 2:1 spectral ratio, a mixture of blue and red light at a 1:2 spectral ratio, and white light (i.e., cool + warm white; 1:1 ratio). Each treatment was performed using four Magenta vessels. LED light was provided under a 16:8 h (light/dark) photoperiod at a fixed light intensity of 50 µmol·m^{-2}·s^{-1} photosynthetic photon flux density (PPFD). Light emitted from the white fluorescent light was used as a control. The spectral energy distribution of the light treatments is shown in Figure 2.

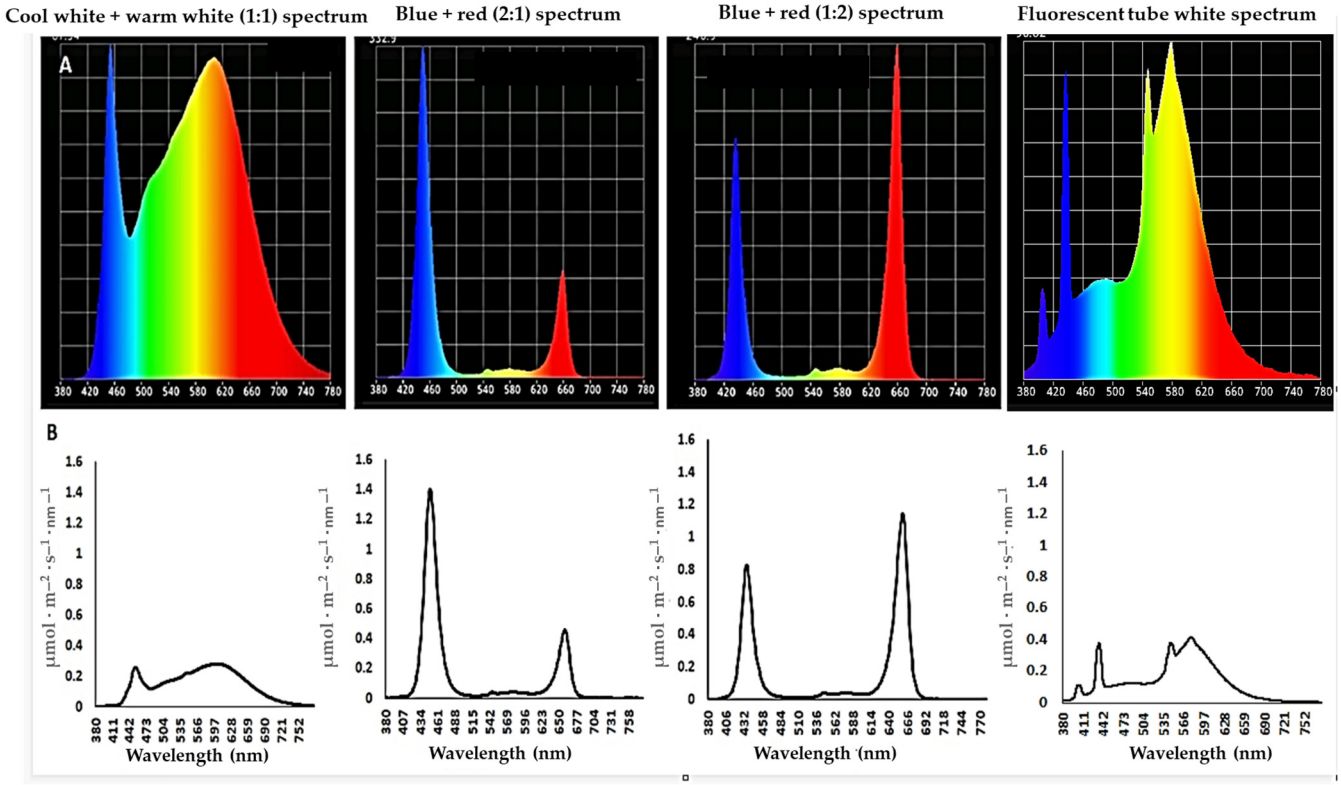

Figure 2. Measured spectra of the light treatments using a UPRtek spectrophotometer. (**A**) Relative light intensity. (**B**) Radiant density of the light spectrum intensity (Dewir et al. [21]).

2.5. Effect of Light Intensity and AC on In Vitro Rooting of Al-Taif Rose

We next examined the role played by light intensity and the presence of active charcoal on rose rooting. Al-Taif rose axillary shoots were cultured for six weeks on half-strength MS medium containing 0.2 mg/L NAA, 80 g/L sucrose that was supplemented with AC at concentrations of 0 or 0.5 g/L. The medium was gelled using 0.8% (*w*/*v*) agar, and the pH of the medium was adjusted to 5.8 before being autoclaved at 121 °C and 1.2 kg cm^{-2} for 15 min. The cultures were then kept at 25 ± 2 °C air temperature under fixed light intensities of 25, 50, and 100 PPFD provided by cool white fluorescent tubes under a 16:8 h (light/dark) photoperiod.

2.6. Acclimatization of Al-Taif Rose Plantlets

Next, we examined acclimatization effects on freshly rooted plants. Al-Taif rose plantlets (7–9 cm) at the 4–5 leaf stage were first harvested from culture vessels and washed thoroughly to remove residual medium. The plantlets were then transplanted into plastic plug trays (38 cm × 28 cm; 54 cells per tray) filled with sterile peatmoss and perlite (1:1; *v*/*v*). Plantlets were covered with a transparent polyethylene sheet for the first ten days of growth, after which it was removed. Plants were grown under a 50% shade net and fertigated with 1 g/L NPK fertilizer (19:19:19; N:P_2O_5:K_2O) once per month. The environment in the conditioned plastic greenhouse was adjusted to a 25 ± 2 °C, 70–80% relative humidity

during the first week but the humidity was gradually decreased to 40–50% by the end of the fourth week.

2.7. DNA Extraction and ISSR-PCR Amplification

Total genomic DNA was extracted from the mother plant and nine regenerated rose plants. The leaves (150 mg) were ground using a mortar and pestle in the presence of liquid nitrogen. DNA was extracted by Cetyltrimethyl Ammonium Bromide (CTAB) according to Doyle and Doyle [22]. DNA concentration and purity were measured using an Implen NanoPhotometer p330 (Implen GmbH, München, Germany). Genetic fidelity was tested using five ISSR primers (Table 1). ISSR-PCR amplification was carried out in a 20 µL reaction mixture containing 2 µL (50 ng/µL) of genomic DNA, 2 µL (10 pm) primer, 2 µL (10×) PCR buffer, 0.6 µL (25 mM) $MgCl_2$, 2 µL (2.5 mM) dNTPs, 0.25 µL (1 unit) Taq DNA polymerase (Promega, Madison, WI, USA) and 11.15 µL ddH_2O. PCR amplification was performed in a Veriti™ 96-Well Thermal Cyclera thermal cycler (Applied Biosystems, Waltham, MA, USA). ISSR–PCR amplification conditions were as follows: 94 °C for 5 min followed by 35 cycles of 94 °C, 30 s for denaturation at 42 °C, 45 s for annealing at 72 °C, 45 s for polymerization and a final extension at 72 °C for 10 min. Separation of amplification product was carried out in 1.5% agarose gel using 0.5 × TBE buffer (44.5 mMTris/Borate, 0.5 mM EDTA, pH 8) against 100 bp DNA Ladder (Invitrogen, Waltham, MA, USA). The gels were stained with ethidium bromide and photographed by gel documentation system (UVITEC, Rugby, Warwickshire, UK).

Table 1. List of ISSR primers with their sequences.

No	Primer Code	Sequence 5′ → 3′
1	ISSR-UBC 818	CACACACACACACACAG
2	ISSR-UBC 840	GAGAGAGAGAGAGAGAYT
3	ISSR-UBC 836	AGAGAGAGAGAGAGAGYA
4	ISSR-UBC 814	CTCTCTCTCTCTCTCTCTA
5	ISSR-UBC 842	GAGAGAGAGAGAGAGAYG

2.8. Experimental Design, Data Recording, and Statistical Analysis

All experiments were performed using complete randomized designs with four replicates per treatment. Each replicate was represented by a culture vessel containing nine individual microshoots, thereby rendering a total of 36 microshoots per treatment. All cultures were maintained for six weeks and were followed throughout the culture period. The rooting response of Al-Taif microshoots was scored by measuring rooting percentage, the number of roots per plantlet, the length of the main root per plantlet, shoot length, and plantlet fresh weight. All percentage data were arcsine transformed before analysis. All data were subjected to analysis of variance (ANOVA) and Tukey's multiple range tests as implemented by SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Effect of Auxin Type and Concentration on the In Vitro Rooting Behavior of Al-Taif Rose Microshoots

Our experiments showed that auxin type, concentration, and their interaction all significantly ($p \leq 0.001$) predicted rose rooting percentage, the number of rose roots, and the length of the main root per explant. However, we also found that fresh weight was significantly influenced only by auxin type ($p \leq 0.05$) while auxin concentration and their interaction showed no significant effect (Table 2). Among the different auxins tested, NAA proved more effective than 2,4-D, IAA, or IBA for root induction of Al-Taif rose microshoots. Furthermore, NAA at concentrations of 0.2 and 0.4 mg/L produced the highest rooting percentages (56% for both), while the highest number of roots (2.0) and main root length (2.5 cm) were obtained from medium supplemented with 0.1 mg/L NAA. Interestingly, the highest fresh weight was obtained from plants grown in medium supplemented with

0.2 mg/L IBA, but this treatment also showed a relatively low rooting percentage of 44%. Low rooting percentages (≤22%) were observed on media supplemented with IAA or 2,4-D. Furthermore, microshoots cultured on media containing 0.4 mg/L IAA or 2,4-D—as well as medium devoid of auxin—showed no rooting whatsoever.

Table 2. Effect of auxin type and concentrations on in vitro rooting of Al-Taif rose microshoots after six weeks in culture under dark conditions.

Auxin Type	Concentration (mg L^{-1})	Rooting (%)	Number of Roots/Explant	Length of the Main Root/Explant (cm)	Fresh Weight/Explant (g)
Control	0.0	0 f	0.0 e	0.0 f	0.119 bcd
2,4-D	0.05	0 f	0.0 e	0.0 f	0.091 cd
	0.1	22 d	2.0 a	0.2 f	0.151 abc
	0.2	11 e	1.0 d	0.2 f	0.045 d
	0.4	0 f	0.0 e	0.0 f	0.075 cd
IAA	0.05	22 d	1.5 bc	1.6 bc	0.121 bcd
	0.1	11 e	1.0 d	1.0 d	0.105 cd
	0.2	22 d	1.0 d	0.3 ef	0.154 abc
	0.4	0 f	0.0 e	0.0 f	0.104 cd
IBA	0.05	22 d	2.0 a	1.1 cd	0.130 abcd
	0.1	33 c	1.3 bcd	0.4 ef	0.121 bcd
	0.2	44 b	1.3 cd	0.5 ef	0.215 a
	0.4	11 e	1.0 d	1.7 b	0.150 abc
NAA	0.05	11 e	1.0 d	0.4 ef	0.200 ab
	0.1	11 e	2.0 a	2.5 a	0.121 bcd
	0.2	56 a	1.4 bcd	0.7 de	0.092 cd
	0.4	56 a	1.8 ab	0.4 ef	0.156 abc
p-values					
Auxin type		<0.0001 ***	<0.0001 ***	<0.0001 ***	0.0272 *
Auxin concentration		<0.0001 ***	<0.0001 ***	<0.0001 ***	0.1164 ^{NS}
Auxin type × auxin concentration		<0.0001 ***	<0.0001 ***	<0.0001 ***	0.1353 ^{NS}

Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level, according to Tukey's multiple range test. NS, * and *** = nonsignificant, significant at $p \leq 0.05$, and $p \leq 0.001$, respectively.

Previous reports have indicated that auxin is indispensable for in vitro rooting of *R. damascena* microshoots [23,24]. In addition, the effective rooting response of *R. damascena* microshoots is known to vary depending on auxin type (i.e., IAA, IBA, or NAA) as well as their concentration (i.e., 0.1–3 mg/L) [25–28]. IBA is generally highly successful for facilitating rooting in plant tissue cultures. For example, a short treatment with IBA (100 mM; 12 h) was found to be effective in inducing rooting in *R. damascena* Mill. 'Jawala' [27]. In addition, Alsemaan [23] achieved 83% rooting using a medium supplemented with 2 mg/L IBA, although higher IBA concentrations produced lower root quality and a reduced number of roots. Interestingly, Jabbarzadeh and Khosh-Khui [26] reported that culturing *R. damascena* shoots on Murashige and Skoog's (MS) medium supplemented with 2.5 mg/L 2,4-D for two weeks followed by subculturing on a medium without auxin was the most efficacious treatment to facilitate rooting. Bhoomsiri and Masomboon [28] reported that rooting of *R. damascena* could be obtained only on MS medium supplemented with 0.5 mg/L NAA; in their study, 87.5% of all explants exhibited root development with 2.71 roots per explant. Similarly, Kornova et al. [29] reported that modified MS medium supplemented with 0.1 mg/L NAA also created good conditions for rooting.

3.2. Effect of Medium Salt Strength, Sucrose Concentration, and Light Spectrum on the In Vitro Rooting of Al-Taif Rose Microshoots

Our data show that medium salt strength and sucrose concentration significantly influenced the in vitro rooting behavior of Al-Taif rose microshoots (Table 3). Specifically, we found that medium salt strength had no significant effect on the number of roots per plantlet but significantly improved rooting percentage, the length of the main root, and the

fresh weight per plantlet. Moreover, sucrose concentration significantly affected all rooting parameters of the Al-Taif rose. For example, at full strength MS medium, we observed no rooting when the sucrose concentration was reduced to 20 g/L, and the highest rooting percentage (89%) was obtained when half-strength MS medium was supplemented with 80 g/L sucrose. Interestingly, this treatment also resulted in a high number of roots (i.e., three per plantlet) and relatively long mean root length (i.e., 1.87 cm) per plantlet (Table 3 and Figure 3a,b). Reducing the salt concentration of the MS medium to half, one-third, or one-quarter strength [18,25,30,31] or modification of available MS macronutrients [32] have also been found to promote rooting in roses. For example, Kornova and Michailova [33] reported that optimum in vitro rooting behavior (90.4%) of the Kazanlak oil-bearing rose was obtained using a nutrient medium with 25% of the mineral salts normally present in the medium plus 0.1 mg/L NAA and 126 mg/L phloroglycinol in the presence of light (2500 lx). In comparison, the most favorable conditions for rhizogenesis induction of the Kazanlak oil-bearing rose 'Iskra' were obtained using 1/4 salt strength MS medium with 0.1 mg/L NAA [30]. Moreover, the in vitro rooting of *R. damascena* microshoots (80%) was obtained using half-strength MS liquid medium supplemented with 10.0 µM IBA and 3% sucrose for one week in the dark; these plants were later transferred to plant growth regulator (PGR)-free medium and kept in the light. All plantlets remained in the latter medium for 5–6 weeks and showed 90% survival after being transferred to the soil [34]. Here, low IBA concentrations and reduced nutrient concentrations favored in vitro root induction in Damask rose shoots. In another study, reducing the strength of NH_4NO_3 and $CaCl_2$ present in MS medium supplemented with 0.1 mg/L IBA resulted in the highest rates of in vitro rooting (71%) for *R. damascena* [32]. In addition, Mamaghani et al. [31] investigated the impact of MS media containing reduced macronutrient concentrations (i.e., 1/2, 1/3, and 1/4) as well as different concentrations of NAA (i.e., 0.1, 0.2, 0.5, and 1 mg/L) on the in vitro rooting of three elite Iranian *R. damascena* accessions, M6 (Kashan), G1 (East Azerbaijan), and G2 (West Azerbaijan). Their results indicated that root regeneration of shoots under in vitro conditions was relatively difficult. Shoots in the G1 (East Azerbaijan) accession rooted only moderately well (25%) on modified 1/3 MS medium supplemented with 0.1 mg/L NAA. Conversely, root regeneration of the G2 (West Azerbaijan) accession was obtained on modified 1/2 MS medium in the presence of 0.2 mg/L NAA. For *R. damascena* and *R. bourboniana*, rooted microshoots were obtained via a two-step procedure in which explants were first cultured on an MS medium containing 2 mg/L IBA, then shoots were cultured on half-strength MS medium without PGRs [35]. Lowering the salt concentration of the rooting medium has also proved to effectively increase the in vitro rooting of other *Rosa* species, including *Rosa hybrida* [36,37] as well as many woody and recalcitrant species [10].

Table 3. Effect of medium salt strength and sucrose concentrations on in vitro rooting of Al-Taif rose microshoots after six weeks in culture under dark conditions.

Medium Salt Strength	Sucrose Concentration (g L^{-1})	Rooting (%)	Number of Roots/Explant	Length of the Main Root/Explant (cm)	Fresh Weight/Explant (g)
Full strength	20	0 e	0.0 d	0.00 b	0.117 d
	Control (30)	56 cd	1.8 c	0.70 b	0.132 cd
	40	44 d	3.3 a	0.75 b	0.129 cd
	60	67 c	3.2 a	1.00 b	0.165 bcd
	80	67 c	3.2 a	0.95 b	0.141 bcd
Half strength	20	33 d	1.7 c	0.65 b	0.195 b
	30	33 d	1.3 c	0.50 b	0.144 bcd
	40	44 d	2.0 b	1.43 a	0.183 b
	60	78 b	3.5 a	1.65 a	0.279 a
	80	89 a	3.0 ab	1.87 a	0.174 b
p-values					
Medium salt strength		0.0051 **	0.3479 ^{NS}	0.0001 ***	<0.0001 ***
Sucrose concentration		<0.0001 ***	<0.0001 ***	0.0020 **	0.0313 *
Medium salt strength × sucrose concentration		0.0142 *	0.0008 ***	0.0735 ^{NS}	0.0070 **

Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level, according to Tukey's multiple range test. NS, *, **, and *** = nonsignificant, significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Figure 3. In vitro rooting of Al-Taif microshoots. (**a**,**b**) In vitro rooting of microshoots following six weeks culture onto half strength MS medium supplemented with 0.2 mg/L NAA and 80 g/L sucrose under dark conditions, and (**c**) in vitro rooting of microshoots following six weeks culture onto half strength MS medium supplemented with 0.2 mg/L NAA and 60 or 80 g/L sucrose under light spectra at 50 PPFD.

Carbohydrates have been shown to promote adventitious root formation in many species, mainly by acting as an energy source [10]. Sugars regulate root initiation by a coordinated modulation of gene expression and enzyme activity in meristematic cells [38].

Varying the concentration of sucrose present in the rooting medium can therefore positively affect root induction and development. For example, in vitro shoot cultures of *Astragalus chrysochlorus* failed to generate roots in MS medium containing 30 g L^{-1} of sucrose, but rooting was strongly stimulated (i.e., in 93% of shoots) when the sucrose concentration was reduced to 20 g/L [39]. Further tests of different sucrose concentrations showed that adding 45 g/L of sucrose along with IBA facilitated optimal root induction in chickpea [40]. Here, the effect of sucrose concentration under different light spectra varied significantly, affecting rooting percentage, the length of the main root, shoot length, and fresh weight. However, it had no effect on the number of roots per plantlet (Table 4). In general, a sucrose concentration of 80 g/L resulted in higher rooting and growth values relative to 60 g/L treatments. Light spectra also had a significant effect on rooting percentage, the length of the main root and fresh weight per plantlet but no significant effect on the number of roots or shoot length. Furthermore, a cool white + warm white (1:1) treatment resulted in 100% rooting when Al-Taif rose microshoots were cultured on medium supplemented with 80 g/L sucrose while a blue + red (1:2) treatment resulted in the greatest root length and number of roots values per plantlet. Thus, our results show that all rooting and growth parameters were significantly influenced by the interaction of sucrose and light spectrum (Table 4 and Figure 3c).

Table 4. Effect of sucrose concentration and light spectra on in vitro rooting of Al-Taif rose microshoots after six weeks in culture.

Sucrose Concentration (g L^{-1})	Light Spectra	Rooting (%)	Number of Roots/Plantlet	Length of the Main Root/Plantlet	Shoot Length (cm)	Fresh Weight/ Plantlet (g)
60	Fluorescent	73 d	3.56 bc	1.29 d	4.41 d	0.292 d
	Cool white + warm white (1:1)	73 d	4.56 abc	2.92 ab	4.81 cd	0.324 bc
	Blue + red (2:1)	83 cd	4.77 ab	1.38 cd	5.13 bc	0.332 bc
	Blue + red (1:2)	88 bc	4.56 abc	1.03 d	4.94 bcd	0.297 cd
80	Fluorescent	94 ab	5.44 ab	1.60 bcd	5.78 a	0.420 a
	Cool white + warm white (1:1)	100 a	3.11 c	2.82 ab	5.46 ab	0.290 d
	Blue + red (2:1)	83 cd	3.78 bc	2.62 abc	4.54 cd	0.305 bcd
	Blue + red (1:2)	90 abc	5.78 a	3.69 a	5.78 a	0.408 a
p-values						
Sucrose concentrations		<0.0001 ***	0.4272 ^{NS}	0.0467 *	<0.0007 ***	<0.0001 ***
Light spectra		0.0206 *	0.3859 ^{NS}	<0.0014 **	0.1520 ^{NS}	0.0001 ***
Sucrose concentrations × LEDs		<0.0001 ***	0.0068 **	<0.0192 *	<0.0005 ***	<0.0001 ***

Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$ level, according to Tukey's multiple range test. NS, *, **, and *** = nonsignificant, significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Light is known to influence root elongation via photomorphogenic action; for example, root elongation may be controlled by phytochromes [41]. Light emitting diodes (LEDs) can be used to accurately and flexibly control light spectra to provide optimal light wavelengths that match plant photoreceptors and their photosynthetic pigments. Hence, LED light is often used to optimize plant growth and metabolism [42]. Moreover, light spectra can affect the ability of roses to induce rooting. Unfortunately, to date few studies have assessed this effect in detail. In one study, Skirvin and Chu [43] reported that the microshoots of the miniature rose cultivars 'Ginney' and 'Fairlane' rooted more quickly and produced more roots under warm white light than under cool white fluorescent light. In addition, it has been reported that red light can positively affect the rooting of miniature roses (*R. chinensis*) [44]. In another study, Kumar et al. [45] reported that *Rosa damascena* plantlets raised under photosynthetically active radiation including only emissions in the red range (660 nm) showed maximum rooting rates (78.3%) as well as higher survival (i.e., 70% in agar and 80% in media gelled with phytagel) and better ex vitro growth relative to control plants cultured under cool white fluorescent lights. Pawlowczyk et al. [46] also conducted a study exploring the effectiveness of single-color LEDs for facilitating the rooting of *Rosa canina* buds by subjecting them to different LED light treatments. They found that an RB (i.e., 50% red and 50% blue) treatment significantly increased the number of roots per plant

(i.e., 3.9). More recently, Al-Rekaby [47] pointed out that the highest values for several rooting parameters (i.e., root length, the number of roots, and fresh and dry root weight) for *Rosa damascena* and *Rosa hybrida* occurred when cultured under red light. Blue and red light are the most efficiently absorbed by chlorophyll during photosynthesis [48]. Therefore, red and blue light have been studied extensively in plant photobiology studies. However, to date relatively few studies on the root growth and physiology of plants under varying combinations of LED light have been conducted. In one study, Wu and Lin [49] found that the rooting percentage was higher in in vitro *Protea cynaroides* plantlets cultured under red LED light (67%) than under cool white fluorescent light (7%). Red LED light has also been found to stimulate root formation in *Anthurium andreanum* [50] and *Chrysanthemum morifolium* [51]. Moreover, a combination of blue + red light (1:2) recorded the highest rooting values in *Rubus fruticosus*, thereby indicating that red light favors root growth and development to a greater degree than fluorescent light in that species [24]. Thus, in view of previous studies, it seems clear that the light spectrum significantly affects the growth and morphology of the rooting system in a species-dependent manner. For Al-Taif rose, a combination of blue + red light (1:2) was found to increase the root length and the number of roots while cool white + warm white (1:1) resulted in 100% rooting. Taken together, these results confirm previous findings in other rose species, including *Rosa chinensis* [43], *Rosa canina* [46], *Rosa damascena*, and *Rosa hybrida* [47].

3.3. Effect of Light Intensity and Activated Charcoal on In Vitro Rooting of Al-Taif Rose Microshoots

Next, we examined the effect of light intensity and activated charcoal (AC) on in vitro rooting and growth of Al-Taif rose microshoots. We found that light intensity at 50 PPFD resulted in the highest number of roots, greatest length of the main root, greatest shoot length, and the highest fresh weight per plantlet (Figure 4). However, at this level of light intensity, only shoot length was affected by AC supplementation. Moreover, varied light intensity (i.e., at 25 or 100 PPFD) was found to significantly reduce rooting parameters. At 100 PPFD, AC supplementation enhanced the number of roots, the length of the main root, and shoot length, but showed only a non-significant overall effect on fresh weight per plantlet. We also note that AC supplementation prevented browning of adventitious roots and resulted in 100% rooting of microshoots as compared with 95% in cultures without AC. Different light intensities did not affect percentage of rooting. In general, a combined treatment of 50 PPFD treatment and 0.5 g/L AC was optimal for in vitro rooting and growth of Al-Taif rose microshoots.

According to a study by Khosh-Khui and Sink [25], rose shoots grown at low light intensity (i.e., 1.0 Klx) yielded a higher rooting percentage (84%) than those grown under higher light intensity (i.e., 3.0 Klx). However, in our study, light intensity did not influence rooting percentage and a treatment of 50 PPFD was found to be optimal for plant growth. This result is consistent with a study of *Rose hybrida*, in which a medium light intensity of 66 PPFD for 12–14 h proved to be optimal for in vitro rooting [52]. Moreover, in our study the addition of AC to the rooting medium resulted in 100% rooting of Al-Taif rose. The effect of AC in promoting morphogenesis may be due to its ability to adsorb inhibitory composites present in the culture medium and significantly decrease the concentrations of toxic metabolites, phenolic exudates, and accumulated brown exudates [53]. In other studies, the use of AC had an outstandingly positive impact on the efficiency of root establishment [54]. Moreover, a combined treatment in which AC was applied to the rooting medium followed by culturing in the dark may influence the different phases of auxin metabolism and consequently the formation of roots by altering the activities of endogenous phenolic compounds and peroxidases [55]. For example, Alsemaan [23] reported that the incorporation of 3 g/L AC in the rooting medium of Syrian Damask rose microshoots significantly reduced the production of phenolic compounds.

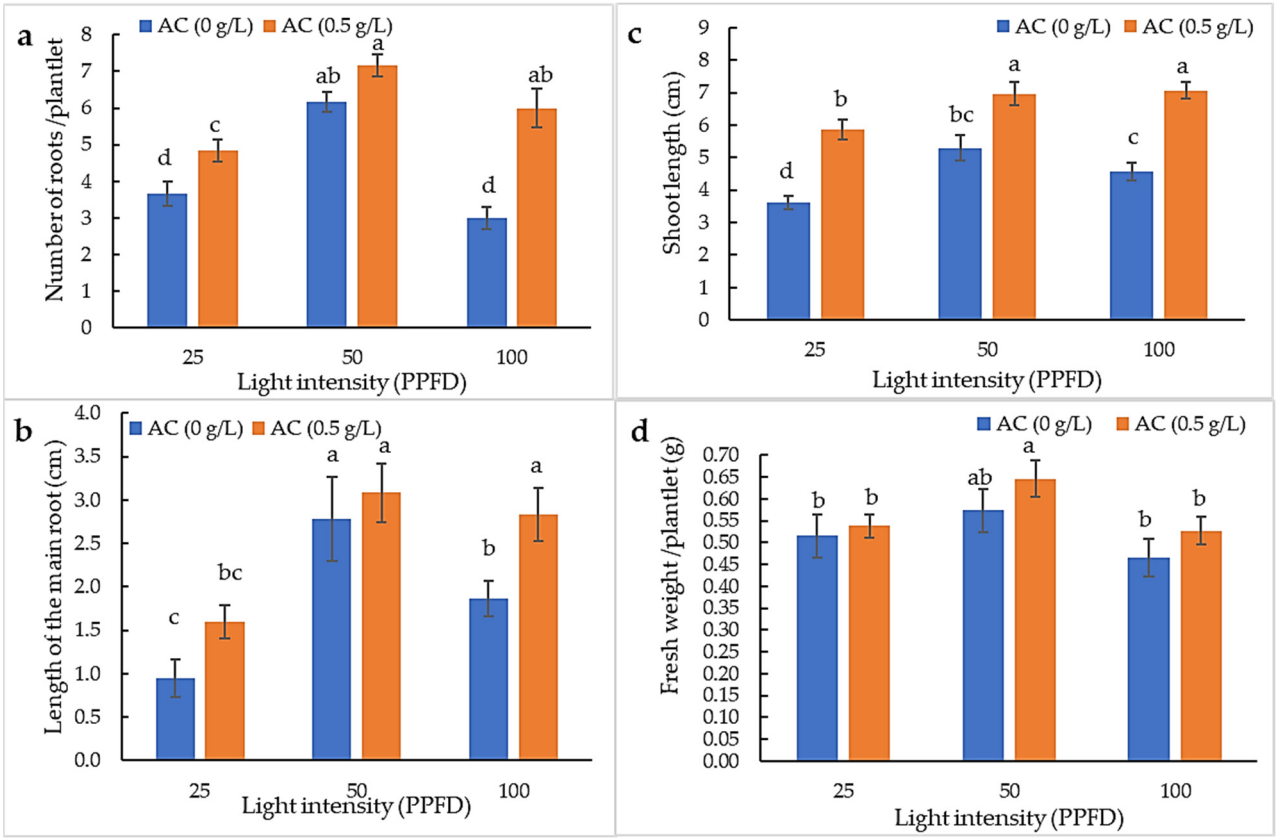

Figure 4. In vitro rooting of Al-Taif microshoots as influenced by fluorescent light intensity and activated charcoal. (**a**) Number of roots per plantlet, (**b**) length of the main root, (**c**) shoot length, and (**d**) fresh weight per plantlet. Values followed by the same letter in each graph are not significantly different at $p \leq 0.05$ level, according to Tukey's multiple range test.

3.4. Acclimatization of Micropropagated Al-Taif Rose Plantlets

Finally, we examined the acclimatization of micropropagated plantlets. Al-Taif rose plantlets were grown in trays filled with sterile peatmoss and perlite (Figure 5a) and the root system was developed over four weeks of acclimatization (Figure 5b). The survival percentage was 98% for a sample of 1080 plantlets (i.e., 20 trays) after 12 weeks of acclimatization (Figure 5c,d). The last and most important step in micropropagation is the establishment of plantlets to ex vitro conditions [56]. That is, once plantlets are well rooted, they must be acclimatized to a normal greenhouse environment. Roses can be successfully grown on a wide range of soils, but they do best on well-drained soils with a soil pH of 6.0 to 6.5 [26]. In our study, potted plants were first covered with a perforated plastic bag that can provide a favorable environment for rose growth. Pati et al. [35] found that the period during which microshoots are incubated in a rooting vessel influences the survival percentage of *R. damascena* plantlets under greenhouse conditions. Here, a significantly higher survival percentage (96.66%) was observed after six weeks of incubation relative to the low value (3.3%) observed after only one week. This suggests that there is a clear correlation between the incubation period during root induction and the survival of plantlets during hardening or acclimatization. Previous reports have also shown different survival rates of *R. damascena* in response to different ex vitro conditions. Kornova et al. [30] reported a procedure that resulted in 81.8% of plants being suitable for adaptation, while Kumar et al. [27] reported 70% survival of rooted plants that were successfully transferred to pots after 15 d of hardening in a mist chamber. In another study, Anil Kumar et al. [45] showed that survival rate of micropropagated *R. damascena* plants was 70% for those grown in media gelled with agar but reached 80% for those in media gelled with phytagel. Thus, it may be that the culture

conditions during the in vitro rooting stage and the hardening stage affect the survival rate of *R. damascena* when exposed to ex vitro conditions.

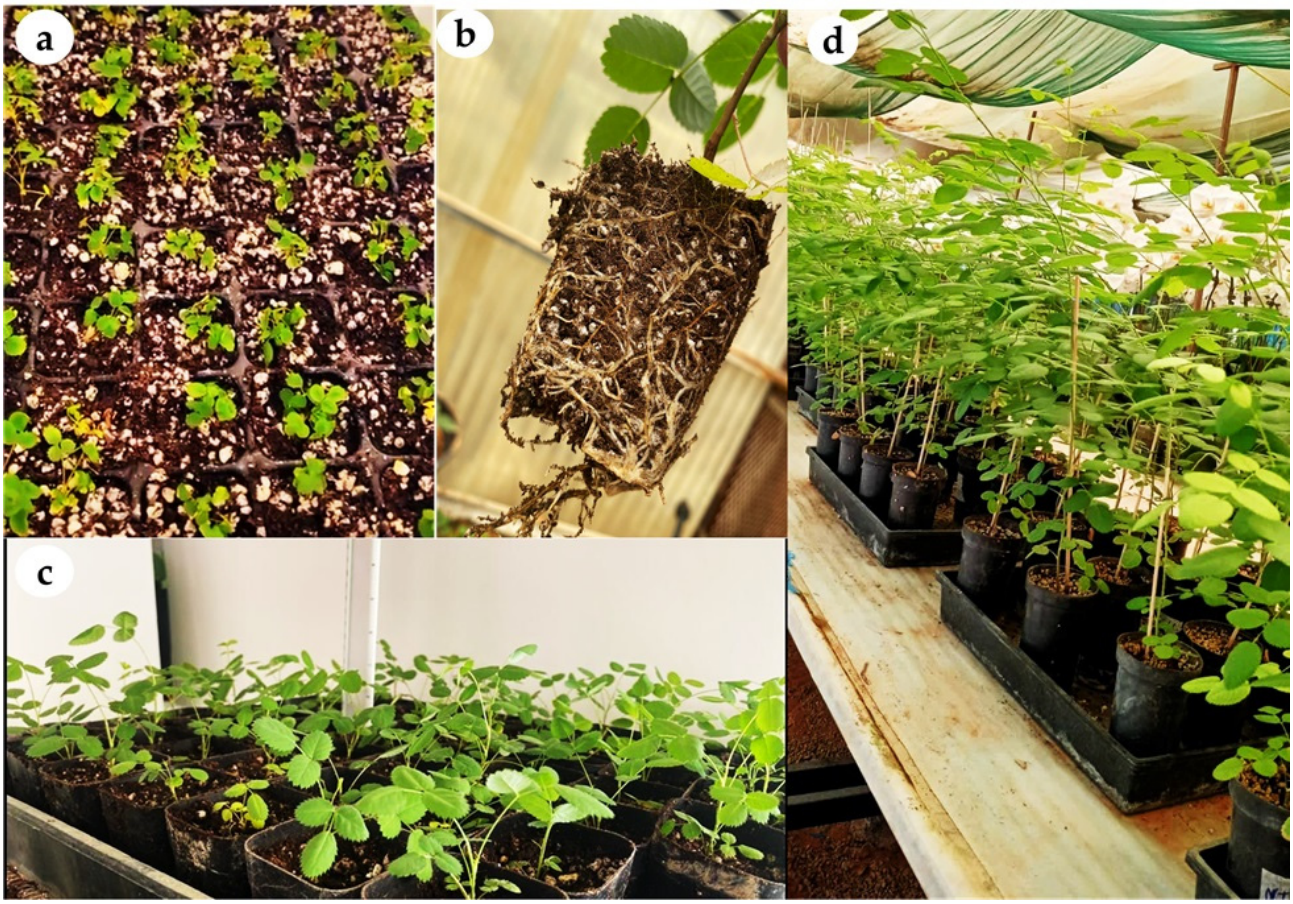

Figure 5. Acclimatization of Al-Taif rose to ex-vitro conditions. (**a**) Transplanted micropropagated plantlets in trays filled with sterile peatmoss and perlite (1:1; *v*/*v*), (**b**) root development of Al-Taif rose seedlings after four weeks acclimatization, (**c**) Al-Taif rose seedlings growing in greenhouse after six weeks acclimatization, and (**d**) micropropagated Al-Taif rose plants after 12 weeks following acclimatization.

3.5. Genetic Fidelity of Micropropagated Al-Taif Rose Plantlets

In ISSR analysis, five primers produced 28 bands which were clear, distinct, and scorable with 1.2% polymorphism (one band), while all the other bands produced were monomorphic. The number of bands produced by the five primers ranged between three and nine with an average of 5.4 bands/primer with band size (in bp) ranging from 100 to 3000 (Figure 6 and Table 5). PCR-based molecular markers, i.e., Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeats (ISSR), Random amplified polymorphic DNA (RAPD), Start codon targeted (SCoT), and Simple Sequence Repeats (SSR) are employed for confirming the true-to-type nature of the in vitro regenerated plants. The ISSR technique provides reliable and reproducible results and requires a small quantity of DNA [57,58], therefore, it is utilized to evaluate the genetic uniformity and resemblance of the micropropagated plants to the mother plant. Few studies assessed the genetic uniformity of the micropropagated rose plants with their mother plant. RAPD and ISSR were utilized to assess the genetic fidelity of in vitro grown plantlets of *Rosa hybrida* and a polymorphism rate of <2% was detected, indicating the uniformity of the regenerated plants [59]. For *Rosa damascena*, SCoT and CAAT-box derived polymorphism (CBDP) markers were utilized to detect the genetic variations in in vitro cultures. The results of cluster analysis showed that the use of 2,4-D in particular in higher concentrations (1 mg/L)

in medium can increase the rate of genetic changes [60]. In our study, high uniformity and true-to-type fidelity of Al-Taif rose (98.8%) was revealed by the ISSR molecular marker.

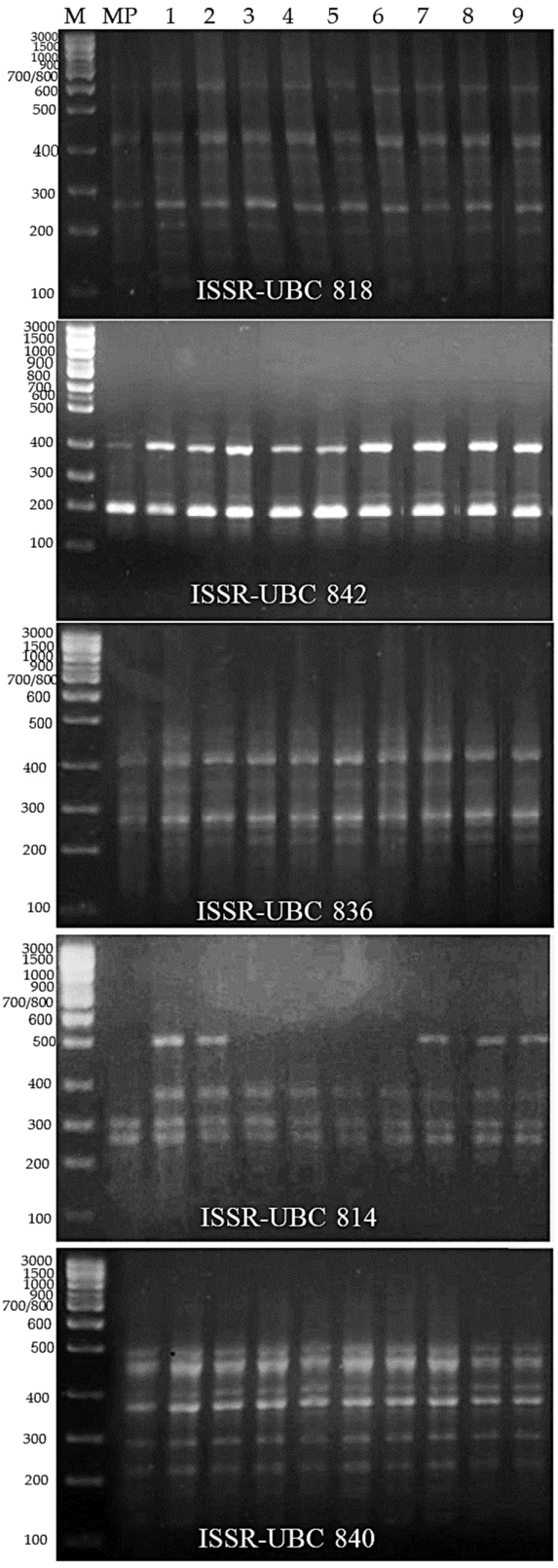

Figure 6. DNA amplification pattern obtained with the ISSR primer. Lane M = DNA ladder; Lane MP = DNA from mother plant; Lane 1–9 DNA from micropropagated Al-Taif rose plants.

Table 5. Polymorphism percentage of micropropagated Al-Taif rose plantlets obtained with ISSR primers.

Primer Code	Total Bands	Monomorphic Bands	Polymorphic Bands	Polymorphism (%)
ISSR-UBC 818	9	9	0	0
ISSR-UBC 840	8	8	0	0
ISSR-UBC 836	4	4	0	0
ISSR-UBC 814	3	3	0	0
ISSR-UBC 842	4	3	1	25
Total	28	27	1	1.2

4. Conclusions

Our results show that the in vitro rooting of Al-Taif rose microshoots was influenced by auxin type and concentration, salt strength of the growth medium, sucrose concentration, light spectrum and intensity, and charcoal supplementation in the rooting medium. The optimal incubation conditions for adventitious root induction of Al-Taif rose microshoots were determined to be as follows: half strength MS medium supplemented with 0.2 mg/L NAA, 80 g/L sucrose, 0.5 g/L AC, and 50 PPFD. Moreover, well-rooted Al-Taif rose plantlets showed high levels of survival (98%) following ex vitro acclimatization. The ISSR molecular marker revealed a high degree of genetic fidelity (98.8%) of micropropagated Al-Taif rose clones.

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References

1. Ozkan, G.; Sagdic, O.; Baydar, N.G.; Baydar, H. Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. *Food Sci. Technol. Int.* **2004**, *10*, 277–281. [CrossRef]
2. Achuthan, C.R.; Babu, B.H.; Padikkala, J. Antioxidant and hepatoprotective effects of *Rosa damascena*. *Pharm. Biol.* **2003**, *41*, 357–361. [CrossRef]
3. Mahmood, N.; Piacente, S.; Pizza, C.; Burke, A.; Khan, A.; Hay, A. The anti-HIV activity and mechanisms of action of pure compounds isolated from *Rosa damascene*. *Biochem. Biophys. Res. Commun.* **1996**, *229*, 73–79. [CrossRef] [PubMed]
4. Ginova, A.; Tsvetkov, I.; Kondakova, V. *Rosa damascena* Mill.—An overview for evaluation of propagation methods. *Bulg. J. Agric. Sci.* **2012**, *18*, 545–556.
5. Bahaffi, S.O. Volatile oil composition of Taif rose. *J. Saudi Chem. Soc.* **2005**, *9*, 401–406.
6. Kürkçüoglu, M.; Abdel-Megeed, A.; Başer, K. The composition of Taif rose oil. *J. Essent. Oil Res.* **2013**, *25*, 364–367. [CrossRef]
7. Bazaid, S.A. Protein and DNA fragments variation in relation to low temperature in four *Rosa hybirida* cultivars in Taif, Saudi Arabia. *J. Egypt. Acad. Dev.* **2004**, *5*, 77–90.
8. George, E.F.; Debergh, P.C. Micropropagation: Uses and methods. In *Plant Propagation by Tissue Culture*, 3rd ed.; George, E.F., Hall, M.A., De Klerk, G.J., Eds.; Spinger: Dordrecht, The Netherlands, 2008; pp. 29–64.
9. Pospisilova, J.; Ticha, I.; Kadleck, P.; Haisel, D.; Plazakova, S. Acclimatization of micropropagated plants to ex vitro conditions. *Biol. Plant.* **1999**, *42*, 481–497. [CrossRef]
10. Dewir, Y.H.; Murthy, H.N.; Ammar, M.H.; Alghamdi, S.S.; Al-Suhaibani, N.A.; Alsadon, A.A.; Paek, K.Y. In vitro rooting of leguminous plants: Difficulties, alternatives, and strategies for improvement. *Hortic. Environ. Biotechnol.* **2016**, *57*, 311–322. [CrossRef]

11. Van Huylenbroeck, J.; Piqueras, A.; Debergh, P. Photosynthesis and carbon metabolism in leaves formed prior and during ex vitro acclimatization of micropropagated plants. *Plant Sci.* **1998**, *134*, 21–30. [CrossRef]
12. Diaz, L.P.; Namur, J.J.; Bollati, S.A.; Arce, O.E.A. Acclimatization of *Phalaenopsis* and *Cattleya* obtained by micropropagation. *Rev. Colomb. Biotecnol.* **2010**, *12*, 27–40.
13. Pati, P.K.; Rath, S.P.; Sharma, M.; Sood, A.; Ahuja, P.S. In vitro propagation of rose—A review. *Biotechnol. Adv.* **2006**, *24*, 94–114. [CrossRef] [PubMed]
14. Kirichenko, E.B.; Kuzmina, T.A.; Kataeva, N.V. Factors in optimizing the multiplication of ornamental and essential oil roses in vitro. *Byulleten Gl. Bot. Sada* **1991**, *159*, 61–67.
15. Horan, I.; Walker, S.; Roberts, A.V.; Mottley, J.; Simpkins, I. Micropropagation of roses: The benefits of pruned mother-plantlets at stage-II and a greenhouse environment at stage III. *J. Hort. Sci.* **1995**, *70*, 799–806.
16. Huettman, C.A.; Preece, J.E. Thidiazuron: A potent cytokinin for woody plant tissue culture. *Plant Cell Tissue Org. Cult.* **1993**, *33*, 105–119. [CrossRef]
17. Podwyszynska, M. Rooting of micropropagated shoot (Cell Tissue and Organ culture). In *Encyclopedia of Rose Science*; Roberts, A.V., Debener, T., Gudin, S., Eds.; Elsevier Press: Amsterdam, The Netherlands, 2003; pp. 66–76.
18. Hasegawa, P.M. Factors affecting shoot and root initiation from cultured rose shoot tips. *J. Am. Soc. Hortic. Sci.* **1980**, *105*, 216–220. [CrossRef]
19. Al-Ali, A.M.; Dewir, Y.H.; Al-Obeed, R.S. Influence of cytokinins, dark incubation and air-Lift bioreactor culture on axillary shoot proliferation of Al-Taif rose (*Rosa damascena trigintipetala* (Diek) R. Keller). *Horticulturae* **2023**, *9*, 1109. [CrossRef]
20. Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant* **1962**, *15*, 473–495. [CrossRef]
21. Dewir, Y.H.; Al-Ali, A.M.; Rihan, H.Z.; Alshahrani, T.; Alwahibi, M.S.; Almutairi, K.F.; Naidoo, Y.; Fuller, M.P. Effects of artificial light spectra and sucrose on the leaf pigments, growth, and rooting of blackberry (*Rubus fruticosus*) microshoots. *Agronomy* **2023**, *13*, 89. [CrossRef]
22. Doyle, J.J.; Doyle, J.L. Isolation of plant DNA from fresh tissue. *Focus* **1990**, *12*, 13–15.
23. Alsemaan, T. Micro-propagation of Damask rose (*Rosa damascena* Mill.) cv. Almarah. *Int. J. Agric. Res.* **2013**, *8*, 172–177. [CrossRef]
24. Mirshahi, H.; Mahdinezhad, N.; Soloki, M.; Samiei, L. Effect of plant growth adjuvants on direct regeneration of Mohammadi flower (*Rosa damascena* Mill.) using thin cell layering technique. *Acta Sci. Pol. Hortorum Cultus.* **2020**, *19*, 167–177. [CrossRef]
25. Khosh-Khui, M.; Sink, K.C. Micropropagation of new and old world species. *J. Hort. Sci.* **1982**, *57*, 315–319. [CrossRef]
26. Jabbarzadeh, Z.; Khosh-Khui, M. Factors affecting tissue culture of Damask rose (*Rosa damascene* Mill.). *Sci. Hortic.* **2005**, *105*, 475–482. [CrossRef]
27. Kumar, A.; Sood, A.; Palni, U.; Gupta, A.; Palni, L.M. Micropropagation of *Rosa damascena* Mill. from mature bushes using thidiazuron. *J. Hortic. Sci. Biotechnol.* **2001**, *76*, 30–34. [CrossRef]
28. Bhoomsiri, C.; Masomboon, N. Multiple shoot induction and plant regeneration of *Rosa damascena* Mill. *Silpakorn Univ. Int. J.* **2003**, *3*, 229–239.
29. Kornova, K.; Mihailova, J.; Stefanova, A. Propagation of *Rosa Kazanlika* Top. (*Rosa damascena* var. *Trigintipetala*) using the in vitro method. *Sci. Work.* **2001**, *46*, 61–66.
30. Kornova, K.; Michailova, J.; Astadjov, N. Application of in vitro techniques for propagation of Rosa kazanlika Top. (*Rosa damascena* var. trigintipetala). *Biotechnol. Biotechnol. Equip.* **2000**, *14*, 78–81. [CrossRef]
31. Mamaghani, B.A.; Ghorbanli, M.; Assareh, M.H.; Zare, A.G. In vitro propagation of three Damask roses accessions. *Iran. J. Plant Physiol.* **2010**, *1*, 85–94.
32. Noodezh, H.M.; Moieni, A.; Baghizadeh, A. In vitro propagation of the Damask rose (*Rosa damascena* Mill.). *Vitr. Cell. Dev. Biol.-Plant* **2012**, *48*, 530–538. [CrossRef]
33. Kornova, K.M.; Michailova, J. Study of the in vitro rooting of Kazanlak oil-bearing rose (*Rosa damascena* Mill.). *J. Essent. Oil Res.* **1994**, *6*, 485–492. [CrossRef]
34. Pati, P.K.; Sharma, M.; Sood, A.; Ahuja, P.S. Direct shoot regeneration from leaf explants of *Rosa damascena* Mill. *Vitr. Cell. Dev. Biol.-Plant* **2004**, *40*, 192–195. [CrossRef]
35. Pati, P.K.; Sharma, M.; Sood, A.; Ahuja, P.S. Micropropagation of *Rosa damascena* and *R. bourboniana* in liquid cultures. In *Liquid Systems for In Vitro Mass Propagation of Plants*; Hvoslef-Eide, A.K., Preil, W., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2005; Volume III, pp. 373–385.
36. Nak-Udom, N.; Kanchanapoom, K.; Kanchanapoom, K. Micropropagation from cultured nodal explants of rose (*Rosa hybrida* L. cv.'Perfume Delight'). *Songklanakarin J. Sci. Technol.* **2009**, *31*, 583–586.
37. Nizamani, F.; Nizamani, G.S.; Nizamani, M.R.; Ahmed, S.; Ahmed, N. Propagation of rose (*Rosa hybrida* L.) under tissue culture technique. *Int. J. Biol. Res.* **2016**, *1*, 23–27.
38. Pawlicki, N.; Welander, M. Influence of carbohydrate source, auxin concentration and time of exposure on adventitious rooting of the apple rootstock Jork 9. *Plant Sci.* **1995**, *106*, 167–176. [CrossRef]
39. Hasançebi, S.; Turgut Kara, N.; Çakir, Ö.; Ari, S. Micropropagation and root culture of Turkish endemic *Astragalus chrysochlorus* (Leguminosae). *Turk. J. Bot.* **2011**, *35*, 203–210. [CrossRef]
40. Aasim, M.; Day, S.; Rezai, F.; Hajyzadeh, M.; Mahmud, S.T.; Ozcan, S. In vitro shoot regeneration from pre-conditioned explants of chickpea (*Cicer arietinum* L.) cv. Gokce. *Afr. J. Biotechnol.* **2011**, *10*, 2020–2023.

41. Vinterhalter, D.; Grubisic, D.; Vinterhalter, B.; Konjevic, R. Light controlled root elongation in in vitro cultures of *Dracaena fragrans* Ker-Gawl. *Plant Cell Tissue Org. Cult.* **1990**, *22*, 1–6. [CrossRef]
42. Mohamed, S.J.; Rihan, H.Z.; Aljafer, N.; Fuller, M.P. The Impact of Light Spectrum and Intensity on the Growth, Physiology, and Antioxidant Activity of Lettuce (*Lactuca sativa* L.). *Plants* **2021**, *10*, 2162. [CrossRef]
43. Skirvin, R.M.; Chu, M.C. The effect of light quality on root development on in vitro grown miniature roses. *Hortic Sci.* **1984**, *19*, 575.
44. Skirvin, R.M.; Chu, M.C.; Young, H.J. Rose. In *Handbook of Plant Cell Culture*; Ammirato, P.V., Sharp, W.R., Evans, D.A., Eds.; McGraw Hill Publishing Co., Ltd.: New York, NY, USA, 1990; Volume 5, pp. 716–743.
45. Kumar, A.; Palni, L.M.S.; Nandi, S.K. The effect of light source and gelling agent on micropropagation of *Rosa damascena* Mill. and *Rhynchostylis retusa* (L.) Bl. *J. Hortic. Sci. Biotechnol.* **2003**, *78*, 786–792. [CrossRef]
46. Pawłowska, B.; Szewczyk-Taranek, B.; Dziedzic, E.; Żupnik, M. Rooting response under LED systems in *Rosa canina* in vitro cultures. *Acta Hortic.* **2017**, *1155*, 519–524. [CrossRef]
47. Al-Rekaby, L.S. Response of Two *Rosa* sp. to light quality in vitro. *Iraqi J. Sci.* **2023**, *64*, 5064–5072. [CrossRef]
48. Chen, M.; Blankenship, R.E. Expanding the solar spectrum used by photosynthesis. *Trends Plant Sci.* **2011**, *16*, 427–431. [CrossRef] [PubMed]
49. Wu, H.C.; Lin, C.C. Red light-emitting diode light irradiation improves root and leaf formation in difficult-to-propagate *Protea cynaroides* L. plantlets in vitro. *HortScience* **2012**, *47*, 1490–1494. [CrossRef]
50. Budiarto, K. Spectral quality affects morphogenesis on *Anthurium* plantlet during in vitro culture. *Agrivita* **2010**, *32*, 234–240.
51. Kurilčik, A.; Miklušytė-Čanova, R.; Dapkūnienė, S.; Žilinskaitė, S.; Kurilčik, G.; Tamulaitis, G.; Duchovskis, P.; Žukauskas, A. In vitro culture of Chrysanthemum plantlets using light-emitting diodes. *Cent. Eur. J. Biol.* **2008**, *3*, 161–167. [CrossRef]
52. Bressan, P.H.; Kim, Y.J.; Hyndman, S.E.; Hasegawa, P.M.; Bressan, R.A. Factors affecting in vitro propagation of rose. *J. Am. Soc. Hortic. Sci.* **1982**, *107*, 979–990. [CrossRef]
53. Thomas, T.D. The role of activated charcoal in plant tissue culture. *Biotechnol. Adv.* **2008**, *26*, 618–631. [CrossRef]
54. Siddique, I.; Anis, M. In vitro shoot multiplication and plantlet regeneration from nodal explants of *Cassia angustifolia* (Vahl.): A medicinal plant. *Acta Physiol. Plant* **2007**, *29*, 233–238. [CrossRef]
55. Druart, P.; Kevers, C.; Boxus, P.; Gaspar, T. In vitro promotion of root formation by apple shoots through darkness effect on endogenous phenols and peroxidases. *Z. Pflanzen. Physiol.* **1982**, *108*, 429–436. [CrossRef]
56. Rout, G.R.; Samantaray, S.; Mottley, J.; Das, P. Biotechnology of the rose: A review of recent progress. *Sci. Hortic.* **1999**, *81*, 201–228. [CrossRef]
57. Jabbarzadeh, Z.; Khosh-khui, M.; Salehi, H.; Saberivand, A. Inter simple sequence repeat (ISSR) markers as reproducible and specific tools for genetic diversity analysis of rose species. *Afr. J. Biotechnol.* **2010**, *9*, 6091–6095.
58. Wu, K.; Jones, R.; Dannaeberger, L.; Scolnik, P.A. Detection of microsatellite polymorphisms without cloning. *Nucleic Acids Res.* **1994**, *22*, 3257–3258. [CrossRef] [PubMed]
59. Senapati, S.K.; Aparajita, S.; Rout, G.R. An assessment of genetic fidelity of in vitro grown plantlets of rose (*Rosa hybrida*) through molecular markers. *Afr. J. Biotechnol.* **2012**, *11*, 16532–16538.
60. Asadi, A.; Shooshtari, L. Assessment of somaclonal variation in micropropagation of Damask Rose (*Rosa damascena* Mill.) using molecular markers. *MGj* **2021**, *15*, 327–335.