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ORIGINAL ARTICLE

Effect of explant type and growth regulators on in vitro regeneration of apricot (*Prunus armeniaca* L.) Al-Amar rootstock

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Abstract

Apricot is a highly recalcitrant species for shoot regeneration in addition the plant regeneration capacity is strongly genotype – dependent. Thus, this study aimed to establish in vitro regeneration of the Egyptian Al-Amar apricot rootstock. Two explant types (Cotyledon and hypocotyl) were cultured on woody plant medium (WPM) supplemented with three concentrations of thidiazuron (TDZ) in combination with eight concentration of indole-3-butyric acid (IBA) or α-naphthalene acetic acid (NAA). The cotyledons showed direct somatic organogenesis as the explants formed buds directly on regeneration media. The highest percentage of cotyledons producing buds was 78.00% when cultured on WPM containing 13.62 µM TDZ and 2.46 µM IBA. In contrast hypocotyls expressed indirect somatic organogenesis, as the explants produced callus first before bud formation. The highest percentage was 96.87% in hypocotyls cultured on WPM fortified with 15.89 µM TDZ and 1.61 µM NAA. The shoot proliferation was achieved when buds from cotyledons and hypocotyls were cultured on Soot Regeneration Medium (SRM) supplemented with 8.87 µM 6-Benzylaminopurine (BAP) and 0.54 µM NAA. The highest shoot formation with an average of 6.4 shoots per explant was obtained from hypocotyls, while an average of 2.0 shoots per explants was achieved from cotyledons. The regenerated shoots were rooted on WPM including 9.80 µM IBA. The results revealed that the regeneration of Al-Amar rootstock was more successful through hypocotyls and could therefore facilitate its genetic manipulation.

Key message

We were optimized a reproducible in vitro regeneration protocol for the Egyptian apricot rootstock (Al-Amar), the important step for improving traits of this rootstock using new techniques such as genetic transformation.

Keywords *Prunus armeniaca* L · *In-vitro* regeneration · Apricot rootstock · Explant type · Cotyledon · Hypocotyl · Growth regulators

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Abbreviations

MS	Murashige and Skoog medium
WPM	Woody plant medium
TDZ	Thidiazuron
IBA	Indole-3-butyric acid
NAA	α-naphtalene acetic acid
SRMs	Shoot Regeneration Media
BAP	6-Benzylaminopurine
mT	Meta-Topolin

Introduction

Apricot (*Prunus armeniaca* L.) is an important member of the family Rosaceae and one of the most global economic deciduous stone fruit crops. It is cultivated for its high-quality, attractive, delicious drupes, that is enriched with vitamin and mineral contents (Yehia et al. 2019; Basile et al. 2023). It is a diploid species (2n = 16) and has a small genome size of 251.3 Mbp (NCBI, 2021). Apricot is grown in diverse regions of the world, but predominantly cultivated in temperate and sub-tropical regions, especially in the Mediterranean basin and the Middle East (Zhebentyayeva et al. 2012; Siddiqui et al. 2023).

In Egypt, apricot is produced in commercial orchards, and the cultivated area recorded in 2003 was almost 8.8197 hectare, but decreased to 4.19982 hectare in 2021 (Abd El-Wahab and Maged 2024). One of the main pillars of Egypt's Sustainable Agricultural Strategy is increasing the cultivated area by expanding into new reclaimed areas. However, apricot cultivation and production could be affected by many genetic and environmental factors (Moustafa and Cross 2019). Apricot is sensitive to abiotic stress, such as salinity (Kemeç Hürkan and Akı 2024), drought (Ruiz-Sa´nchez et al. 2000) and severe low temperature (Gao et al. 2024). Moreover, in recent years, the ecological challenges are threatening most of fruit trees (Mendelné Pászti and Mendel 2022). Climate change causes environmental problems such as sudden temperature fluctuations, irregular precipitation and increasing soil issues, such as salinity, alkalinity, and acidity (Dhurve et al. 2023). Therefore, agriculture sustainability requires facing these climatic changes through adoption of new technologies and farming systems (Gainza et al. 2015; Kumar et al. 2024).

In apricot, the plant productivity and quality of the fruits are the result of the compatibility between the scion and the rootstock (Hernández et al. 2010; Mendelné Pászti et al. 2023). Apricot yield is not only controlled by the scion, but also by the rootstock's tolerance to biotic and environmental stresses (Rom 1991).

Rootstocks are playing an important role in agriculture productivity, especially for fruit cultivation, as they determine the orchard efficiency and sustainability (Santhi et al. 2020; Kumar et al. 2024). Rootstocks provide a root system which is responsible for water and mineral nutrient uptake and have a strong effect on the vegetative and generative characteristics of the scion such as productivity, blossom initiation, fruit set, flower set, fruit size and quality (Cinelli and Loreti 2004; Zarrouk et al. 2005; Jiménez et al. 2007). Also, rootstocks can provide other traits that are absent in the scion' cultivars, such as tolerance to some biotic stress (pests and diseases) and abiotic stresses (salinity and drought) (Gainza et al. 2015). In Egypt, there are three local apricot rootstocks in Egypt, Al-Amar, Hamawy, and Balady. These rootstocks are seed-planted trees, that exhibit variation in size, maturity date, production, and quality of fruits (Awad et al. 2019; Abd El-Wahab and Maged 2024). However, Al-Amar rootstock is distinguished from other varieties by its reddish color (Sharoba et al. 2007), notable fruit weight and flesh thickness (Mohamed et al. 2015), high total soluble solids (TSS) content (Mohamed et al. 2015; Abd El-Wahab and Maged 2024), and remarkable quality of taste and eating (Abd El-Wahab and Maged 2024). Additionally, some strains of Al-Amar apricot rootstock have demonstrated moderate tolerance to salinity up to 2000 ppm (Abd El-Aziz et al. 2019). These characteristics position Al-Amar apricot rootstock as a highly suitable candidate for further research to improve its traits for the purpose of modern breeding programs.

However, most of the research studies for improving apricot have been directed to scion cultivars, as breeding rootstocks for fruit plants is considerably slower and complex than scion breeding for the same fruit species (Cousins 2005; Kumar et al. 2024).There are few research studies to date dedicated to apricot specific rootstocks (Zhebentyayeva et al. 2012). Although, breeding of rootstocks is an essential component in modern agriculture systems, there are fewer number of rootstock breeding programs comparing to breeding programs of scion cultivars (Gainza et al. 2015). Yet, in the last few decades attention has been directed to apricot rootstocks and efforts have been directed to develop them (Kumar et al. 2024). In addition, the application of biotechnological techniques could accelerate the apricot breeding programs by introducing new important biotic and /or abiotic traits to circumvent the adverse climatic challenges. But, the availability of an efficient regeneration system is a prerequisite for a successful genetic manipulation approach (Hussein et al. 2004 and 2010; Assem et al. 2014; İpek et al. 2023).

In general, apricot is a highly recalcitrant species for shoot regeneration (Wang et al. 2013; Gao-Takai and Tao 2014; Pérez-Caselles et al. 2021; İpek et al. 2023). Yet, successful regeneration has been reported from different explants for certain genotypes. Shoot regeneration has been achieved from immature embryos (Lane and Cossio 1986), from cotyledons (Pieterse 1989; Laimer da Camara Machado et al. 1992; Goffreda et al. 1995; Wang et al. 2013; İpek et al. 2023), from hypocotyls (Wang et al. 2011; Pérez-Caselles et al. 2021), and from young in-vitro-derived leaves (Pérez-Tornero et al. 2000; Burgos and Alburquerque 2003; Mitrofanova et al. 2019).

However, in most plant species the plant regeneration capacity is strongly genotype - dependent (Hussein et al. 2010; Assem et al. 2014; Kodad et al. 2021; Lee and Wang 2023; Nowakowska et al. 2024). Therefore, the present

investigation has been designed to optimize a reproducible in vitro regeneration protocol for the Egyptian rootstock Al-Amar. This was conducted by evaluating the morphogenic potential of two different explants (cotyledons and hypocotyls) and determining the best medium by analyzing the effect of different growth regulators on the regeneration efficiency. This might facilitate the practical application of in vitro genetic manipulation of this rootstock.

Materials and methods

Plant materials and sterilization

The seeds from mature fruits of the Al-Amar apricot rootstock were kindly obtained from the Horticulture Research Institute (HRI), Agriculture Research Center (ARC), Giza, Egypt. After removing the endocarp, apricot seeds were washed under tap water for one hour. Then, seeds were subjected to surface sterilization in a laminar flow cabinet using a 3% (w/v) commercial bleach solution, containing approximately 20 µl Tween-20 per 100 mL solution, and shaking for 15 min. Afterward the sterilized seeds were thoroughly rinsed 3–4 times with sterile double distilled water and the disinfected seeds were kept overnight at room temperature (23 ± 2ºC).

Table 1 Formulation of in vitro regeneration induction media

Media	Growth regulators (µM)		Media	Growth regulators (µM)	
	TDZ	IBA		TDZ	NAA
control	00.00	0.00	control	00.00	0.00
WP1	11.35	0.25	WP25	11.35	0.27
WP2	11.35	0.49	WP26	11.35	0.54
WP3	11.35	0.98	WP27	11.35	1.07
WP4	11.35	1.48	WP28	11.35	1.61
WP5	11.35	1.97	WP29	11.35	2.15
WP6	11.35	2.46	WP30	11.35	2.69
WP7	11.35	2.95	WP31	11.35	3.22
WP8	11.35	3.44	WP32	11.35	3.76
WP9	13.62	0.25	WP33	13.62	0.27
WP10	13.62	0.49	WP34	13.62	0.54
WP11	13.62	0.98	WP35	13.62	1.07
WP12	13.62	1.48	WP36	13.62	1.61
WP13	13.62	1.97	WP37	13.62	2.15
WP14	13.62	2.46	WP38	13.62	2.69
WP15	13.62	2.95	WP39	13.62	3.22
WP16	13.62	3.44	WP40	13.62	3.76
WP17	15.89	0.25	WP41	15.89	0.27
WP18	15.89	0.49	WP42	15.89	0.54
WP19	15.89	0.98	WP43	15.89	1.07
WP20	15.89	1.48	WP44	15.89	1.61
WP21	15.89	1.97	WP45	15.89	2.15
WP22	15.89	2.46	WP46	15.89	2.69
WP23	15.89	2.95	WP47	15.89	3.22
WP24	15.89	3.44	WP48	15.89	3.76

Explants preparation

Cotyledons and hypocotyls were used as the explants source to determine the highest shoot regeneration percentage of Al-Amar apricot rootstock. Cotyledons were obtained from mature, sterilized seeds that had been soaked overnight (Fig. 4, A1), with the entire embryo section carefully discarded using a scalpel (Fig. 4. A2 & A3). Cotyledon explants were prepared by dividing each cotyledon by 3 cuts of adaxial side, and approximately 4–5 mm of the upper-middle part was used as the explant (as shown in Fig. 4, B1), which was then placed on regeneration media (Wang et al. 2013). The hypocotyl was obtained from mature embryos cultured under aseptic conditions on full strength Murashige and Skoog medium (MS) (Murashige and Skoog 1962), and incubated in the dark at 25 ± 2 °C for 2–3 weeks (Fig. 4, A4). After development, the hypocotyl region was evident by the naked eye and was isolated by carefully removing the epicotyl and radicle regions. A segment of approximately 2–3 mm from the middle part of the hypocotyl was then used (Fig. 4, C1).

Regeneration induction

Explants were cultured on forty-eight different induction media (WP1–WP48) in addition to control media (Table 1). All media were consisted of full-strength woody plant medium (WPM) (Lloyd and McCown 1980), supplemented with combinations of various concentrations of thidiazuron (TDZ, 0.0, 11.35, 13.62, and 15.89 µM) and, indole-3-butyric acid (IBA, 0.0, 0.25, 0.49, 0.98, 1.48, 1.97, 2.46, 2.95, 3.44 µM), or α-naphtalene acetic acid (NAA, 0.0, 0.27, 0.54, 1.07, 1.61, 2.15, 2.69, 3.22, 3.76 µM). The explants were incubated in growth chamber at 25 ± 2ºC in dark for one week before transferring to a photoperiod of 16 h under cool white fluorescent tubes, with light intensity of 20 µ Mol/m2/s, and relative humidity of 60–65%.

Shoot proliferation and elongation

The adventitious buds from cotyledons and the induced callus from hypocotyls were transferred to Shoot Regeneration Media (SRMs), composed of WPM supplemented with 6-Benzylaminopurine (BAP) only at concentrations of 0.0, 4.44, and 8.87 µM, or combined with both NAA at concentration of 0.54 µM and TDZ at concentrations of 4.54 and 9.08 µM.

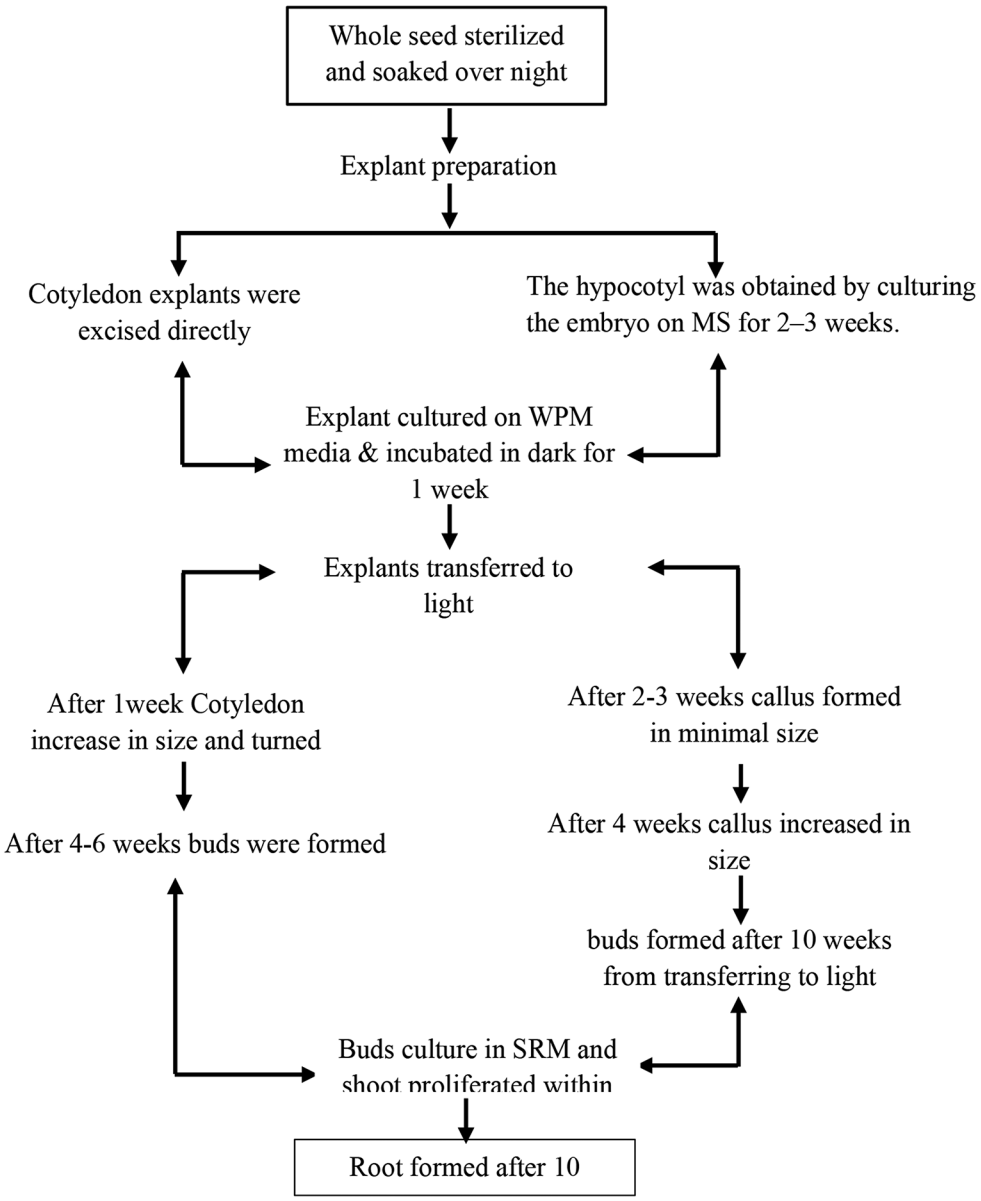


Fig. 1 The Processes and Time-line for In Vitro Regeneration of apricot Al-Amar Rootstock

Rooting

The newly developed shoots (4–6 cm long) were then

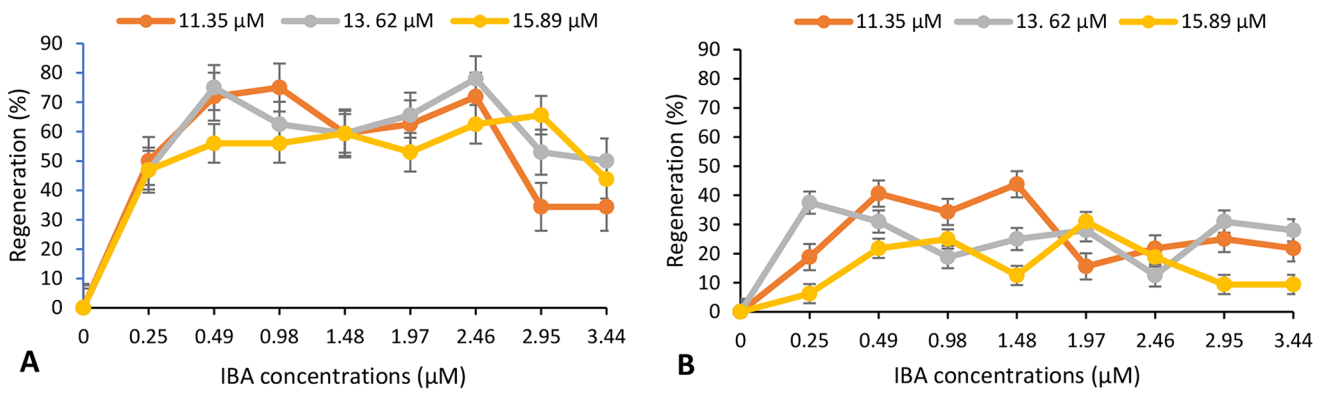


Fig. 2 The effect of TDZ and IBA on: (**A**) adventitious bud regeneration from cotyledons, and (**B**) callus formation from hypocotyls. Vertical bars represent standard errors

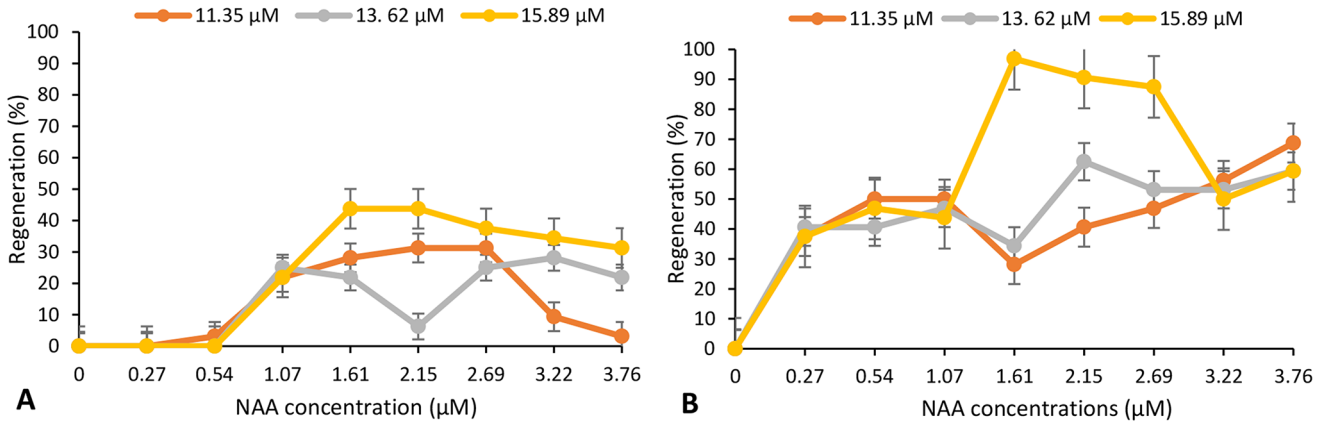


Fig. 3 The effect of TDZ and NAA on: (**A**) adventitious bud regeneration from cotyledons, and (**B**) callus formation from hypocotyls. Vertical bars represent standard errors

separated and utilized for in vitro induction of roots where two types of media were tested, i.e. WPM or half strength MS medium, supplemented with IBA at concentrations of 4.90 and 9.80 µM.

Sucrose (2%, w/v) and agar (6%, w/v) were added to all media, and pH was adjusted to 5.7 ± 1 with 1 N NaOH before adding agar. A 20 ml of prepared media was dispensed in jars (350 ml), then autoclaved at 121ºC at a pressure of 1.1 kg/cm2 for 20 min.

Experimental design and data analysis

The regeneration experiments were conducted in a completely randomized design with eight replicates (jars) for each treatment. Each jar contained 4 explants, and the experiments were repeated at least twice. The collected data were subjected to statistical analysis of Variance (ANOVA) using Tukey's Honest Significant Difference test (Tukey 1949) to evaluate significant differences in mean values across the different treatments, Differences were considered significant when $p < 0.05$.

Results

Regeneration of both explants was conducted using woody plant basal medium. Forty-eight media combinations in addition to control were tested for shoot induction, while, for shoot proliferation and rooting, seven and five media were tested, respectively.

Effect of explants type

Our finding revealed two pathways for shoot regeneration depending on the explant type. Cotyledon explants showed direct organogenesis as the explants formed buds on regeneration media without intermediate callus stage (Fig. 4. B2 & B3). In contrast, the hypocotyl explants formed callus first when cultured on the same media (Fig. 4, C2 & C3). After one week of dark incubation, the cotyledon explants had grown to twice their initial size and their color changed between white-greenish yellow and radish purple. Then, the color of the cotyledon explants changed to light-dark green in the next week after transferring to light and their size increased to about four-fold, forming green buds as direct organogenesis. The buds became evident for naked eye as

Table 2 Mean numbers of shoot proliferation, shoot number, and shoot length from cotyledons and hypocotyls on WPM supplemented with BAP, TDZ and NAA

Growth regulators (µM)			Cotyledon			Hypocotyl		
BAP	TDZ	NAA	Proliferation rate	Shoots number/ explant	Shoot length	Proliferation rate	Shoots number/ explant	Shoot length
0.00	0.00	0.00	0.0 ± 0.00 ^{c}	0.0 ± 0.00^{c}	0.0 ± 0.00 ^{c}	0.0 ± 0.00 ^{c}	0.0 ± 0.00 ^{c}	0.0 ± 0.00 ^{c}
8.87	0.0	0.00	3.0 ± 0.00 ^{a}	1.3 ± 0.14 ^{bc}	1.3 ± 0.23 ^{c}	3.0 ± 0.00 ^{a}	3.2 ± 0.27 ^{b}	2.0 ± 0.40 ^{c}
8.87	0.0	0.54	3.0 ± 0.00 ^{a}	2.0 ± 0.21 ^{a}	5.5 ± 0.28 ^{a}	3.0 ± 0.00 ^{a}	6.4 ± 0.41 ^{a}	6.5 ± 0.50 ^{a}
8.87	9.08	0.54	2.5 ± 0.28 ^{ab}	1.5 ± 0.31 ^{b}	5.8 ± 0.46 ^{a}	2.5 ± 0.28 ^{ab}	3.2 ± 0.51 ^{b}	5.6 ± 0.55 ^{ab}
8.87	4.54	0.54	2.8 ± 0.25 ^{a}	1.2 ± 0.16 ^{bc}	5.9 ± 0.15 ^{a}	2.3 ± 0.25 ^{ab}	3.1 ± 0.57 ^{b}	5.9 ± 0.42 ^{ab}
4.44	9.08	0.54	2.0 ± 0.00 ^{bc}	1.1 ± 0.28 ^{bc}	5.0 ± 0.40 ^{ab}	1.8 ± 0.47 ^{b}	1.5 ± 0.45 ^{c}	4.8 ± 0. 47 ^{b}
4.44	4.54	0.54	1.5 ± 0.28 ^{c}	0.7 ± 0.22 ^{c}	4.3 ± 0.25 ^{b}	1.8 ± 0.25 ^{b}	0.8 ± 0.27 ^{c}	4.5 ± 0.64 ^{b}

means followed by the same letter within each column are not significantly different at $P < 0.05$

Table 3 Mean number of shoots forming roots on WPM and 1/2 MS supplemented with two concentrations of IBA after 10 weeks of culture

Media	IBA concentrations (µM)	Rooting rate	Roots numbers/ shoot
control	0.00	0.00 ± 0.00 [b]	0.0 ± 0.00 [b]
WPM	4.90	0.25 ± 0.25 [b]	0.5 ± 0.50 [ab]
WPM	9.80	1.25 ± 0.25 [a]	3.0 ± 1.08 [a]
½ MS	4.90	0.00 ± 0.00 [b]	0.0 ± 0.00 [b]
½ MS	9.80	0.00 ± 0.00 [b]	0.0 ± 0.00 [b]

means followed by the same letter within each column are not significantly different at $P < 0.05$

a cluster within 4–6 weeks (Figs. 1 and 4. B2). While, for the hypocotyl, white-yellow callus was induced through the dark incubation. After transferring to light, the callus turned green, but showed minimal size increasing during the initial 2–3 weeks. Then, within the next four weeks, the callus doubled its size and produced buds after 10 weeks from transferring to light (Figs. 1 and 4. C2).

It is worth noting that all media containing IBA were successful in inducing buds from cotyledons and calli from hypocotyls. While, five media containing NAA (WP 25, WP 33, WP 34, WP 41 and WP 42), specifically consisting of 11:35 µM TDZ + 0.27 µM NAA, 13.62 µM TDZ + 0.27 µM NAA, 13.62 µM TDZ + 0.54 µM NAA, 15:89 µM TDZ + 0.27 µM NAA, 15.89 µM TDZ + 0.54 µM NAA, respectively, did not induce bud formation from cotyledons (Fig. 2. A).

Most of the buds obtained from both explants, cotyledons and hypocotyls, exhibited potential of shoot regeneration when cultured on the shoot regeneration media (WPM + BAP alone or combined with TDZ and NAA). As shown in Table 2 the results revealed significant differences between the number of shoots produced by the two types of

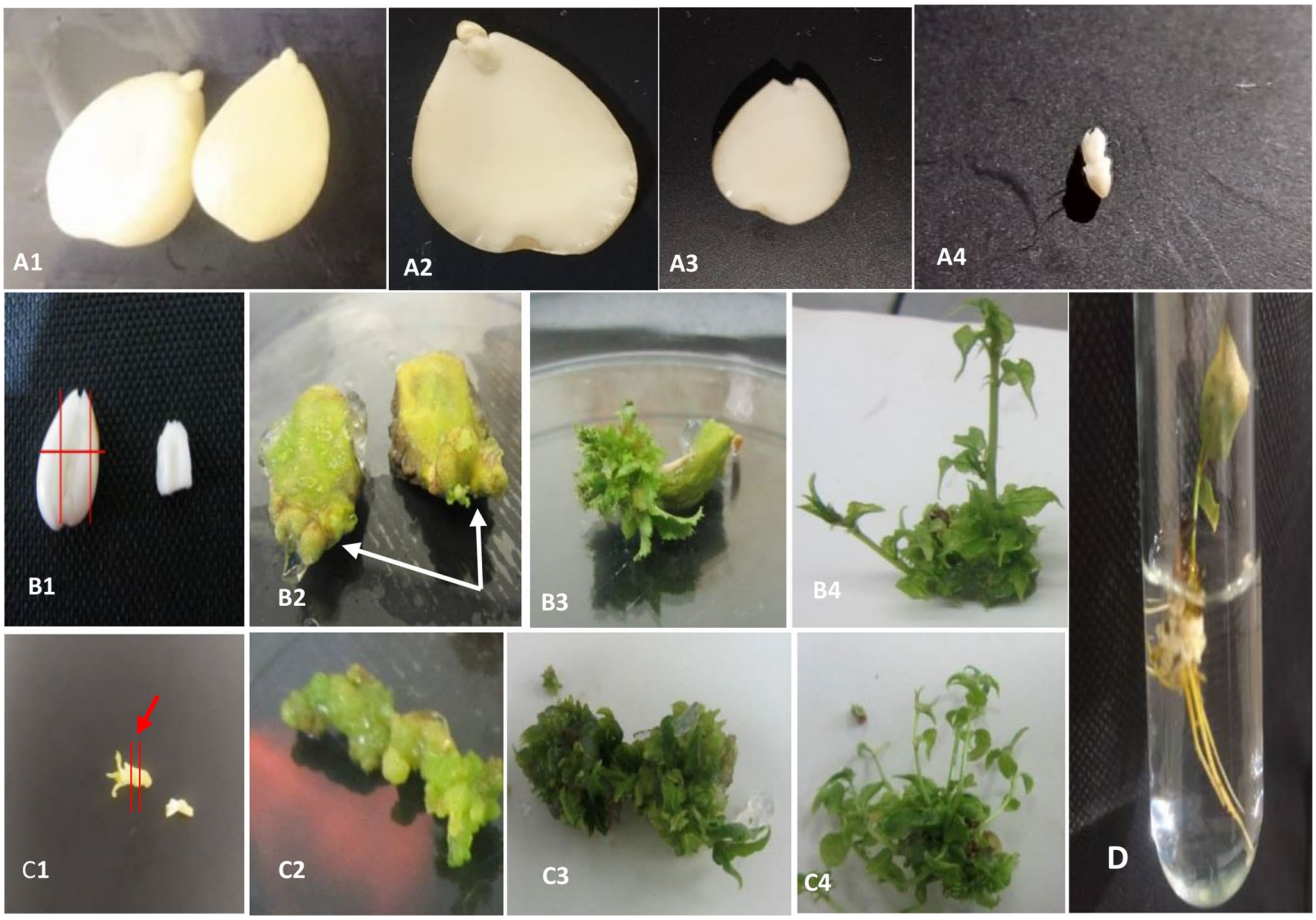


Fig. 4 Explant preparation and developmental stages of regeneration in Al-Amar apricot rootstock: (**A**) Explant types include A1: the whole apricot seed, A2: the cotyledon with the attached embryo, A3: the cotyledon after the removal of the embryonic axis, and A4: the whole embryo prior to culture on MS medium to identify the hypocotyl region. (**B**) direct somatic organogenesis from cotyledon explants, B1: cotyledon explants were separated by 3 axis cuts and after the embryos are removed, B2: the buds start forming in the explant; after 4–6 weeks on induction media, B3: proliferation of shoots on SRM, B4: elongated shoots before transferring to rooting media.(**C**) indirect somatic organogenesis from hypocotyl explants, C1: hypocotyl explants; the red lines and red arrow indicate the region between the apical and radicle sections that was used for regeneration, the middle part of the hypocotyl. C2: callus induction on induction media after 8 weeks from culture, C3: the shoot proliferation after transferring callus on SRM media, C4: elongated shoots. **D**: rooted shoots

explants on the different SRM media. In general, the hypocotyl explants produced more shoots compared to the cotyledon explants. The mean number of shoots from hypocotyls ranged from 0.8 to 6.4 shoots per explant. While, cotyledons produced a range of 0.7 to 2.0 shoots per explant.

Rooting was obtained from shoots derived from hypocotyls when cultured on WPM supplemented with IBA after 10 weeks (Table 3; Fig. 4. D).

Effect of plant growth regulators

Effect of TDZ and IBA on shoot induction

Results presented in Fig. 2 indicated that supplementing the WPM medium with the three different concentrations of TDZ (11.35, 13.62 or 15.89 µM) in combination with the eight concentrations of IBA enhanced the percentage of adventitious bud formation from cotyledons and callus induction from hypocotyls compared to the control. However, the positive effect was more pronounced on the bud formation from cotyledons. The highest percentage of cotyledons producing buds was 78.00% achieved by medium supplemented with 13.62 µM TDZ and 2.46 µM IBA. This was followed by 75.00% on WPM supplemented with two different concentrations of TDZ and IBA. i.e., 11.35 µM TDZ + 0.98 µM IBA and 13.62 µM TDZ + 0.49 µM IBA (Fig. 2, A). While, the highest percentage of hypocotyl producing calli was 43.80% obtained on medium supplemented with 11.35 µM TDZ + 1.48 µM IBA, followed by 40.60% on medium containing the same concentration of TDZ with 0.49 µM IBA (Fig. 2, A).

Effect of TDZ and NAA on shoot induction

In contrast to the effect of IBA with TDZ, the combination of NAA and TDZ was significantly effective in enhancing the number and percentage of hypocotyls initiating calli (Fig. 3, B) where the highest percentage reached 96.87% on medium WP44 (15.89 µM TDZ + 1.61 µM NAA). Similarly, the same medium (WP44), induced the highest percentage of cotyledons initiating buds (43.75%) (Fig. 3, A).

Effect of BAP, TDZ and NAA on shoot proliferation

Most of the buds obtained from either cotyledon or hypocotyl explants expressed potential of shoot regeneration when cultured on SRM supplemented with BAP alone or in combination with NAA or in combination with TDZ and NAA (Table 2; Fig. 4, B4 & C4). Application of BAP at a concentration of 8.87% µM had significant effect on soot proliferation ($p < 0.05$) compared to the control medium (0.0% BAP) or the media containing 4.44 µM BAP. Also, adding NAA (0.54 µM) with BAP (8.87 µM) to the medium further enhanced the number and elongation of the differentiated shoots. While, applying TDZ did not significantly affect the number of shoots per explant nor the shoot length. In addition, the results displayed in Table 2 indicate significant difference between the mean number of shoots produced by the two explant types.

Effect of media composition on in vitro rooting

WPM and 1/2 MS basal media supplemented with either 4.9 µM or 9.8 µM IBA, were tested for their potentiality in inducing root formation. Rooting was achieved from shoots cultured on WPM medium while, MS medium failed to induced root formation (Fig. 4. D and Table 3). Meanwhile, the mean number of rooted shoots on medium WPM supplemented with 9.8 µM IBA was higher (1.25) than on WPM + 4.9 µM IBA (0.25).

Discussion

To the best of our knowledge, this is the first experiment for in vitro regeneration of apricot rootstocks in Egypt. Al-Amar rootstock has been chosen on the basis of being the most successful rootstock variety currently grown in Egypt, and it has many characteristics that could be improved through genetic modification techniques. Therefore, the study focused on establishing a reproducible protocol as a key factor for a successful genetic transformation system for apricot improvement.

The regeneration potentiality of any plant is influenced by several factors. Selection of the right explant is crucial for establishing a successful plant regeneration protocol, it greatly affects the embryogenic or organogenic potential (Nas et al. 2010; Piqueras et al. 2010; Ruiz et al. 2011; Gitonga et al. 2018; Nurokhman et al. 2019; Martini et al. 2022). In the present study, two types of explant (mature cotyledons and hypocotyls), have been evaluated. The results suggested that the type of explant has a strong influence on the regeneration percentage of the Al-Amar apricot. Although both explants investigated were able to regenerate, the hypocotyl explant responded better than cotyledon in both adventitious bud induction and shoot regeneration. This result is in agreement with Nas et al. (2010) that found different regeneration responses between two explants of *Prunus microcarpa* (cotyledon and hypocotyl) when cultured on media containing different types of cytokinin (BA, or meta-Topolin (mT), or TDZ). Sefasi et al. (2013), pointed out that the stem explant was more effective than leaf explant in shoot regeneration of (*Ipomoea batatas* L), and indicated that the mean number of explants regenerating shoots and

the number of shoots per explant had been affected significantly according to the type of explants.

Interestingly, Pérez-Caselles et al. (2021) observed different regeneration responses when they sliced the explant (apricot hypocotyl) from Canino cultivar into three cross sections: (1) section closer to radical, (2) central section, (3) section closer to epicotyl, and placed them on media supplemented with TDZ and IBA. They found that the highest regeneration percentage was in the section close to epicotyl, followed by central section. These finding are in partial consistence with our results.

The type and concentration of the PGRs added to the regeneration medium are considered another important factor affecting shoot regeneration of plants (Ricci et al. 2020). Pérez-Tornero et al. (2000) confirmed that TDZ was required for efficient shoot regeneration of apricot. They found that the regeneration percentages were significantly affected by TDZ concentration, and the proportion of leaves that produced adventitious buds was significantly greater with TDZ compared to BAP. TDZ is considered to be the best synthetic cytokinin for the regeneration of many plant species (Guo et al. 2011). While, Vinoth and Ravindhran (2018) pointed out that the effect of TDZ in regeneration media, is greatly dependent on other factors such as type of explant, plant maturity, TDZ concentration, combination with other PGRs and organic additives. Deepa et al. (2018) stated that TDZ was found more effective in some regeneration systems if combined with other cytokinin or auxin, and this depends on the plant genotype, type of explant, and duration of exposure. Moreover, Wang et al. (2011), indicated that the combination of TDZ and IBA successfully induced shoot regeneration of three cultivars of apricot from mature seed hypocotyl. In a subsequent study the authors reported direct organogenesis from mature cotyledon of five cultivars of apricot, when cultured on media supplemented with TDZ and IBA (Wang et al. 2013). In this context, the results of the present investigation confirmed that TDZ plays an important role in callus induction and bud formation in in-vitro regeneration of apricot, but the percentage of regeneration depends on the type of explant and other auxins combined with TDZ. The results proved that IBA increased the percentage of bud formation in cotyledons, with optimal concentrations depending on the accompanying TDZ level. In contrast, adding NAA with TDZ proved more successful for hypocotyl explants. In conformity with these findings, Goffreda et al. (1995) reported that addition of IBA with TDZ in the media, had pronounced effect on regeneration capacity of cotyledon and the shoot initiated continued to develop.

Also, the results of the present study showed that adding TDZ in SRM did not significantly affect the shoot proliferation. Although, the TDZ enhanced the callus and bud formation for both explants, but the shoots differentiation was very low in SRM media supplemented with TDZ. However, the shoots were significantly affected by adding BAP to SRM media alone or in combination with NAA (Table 3). This is in accordance with Panda and Hazra (2012). They indicated that while numerous buds were regenerated on the surface of the hypocotyl of *Semecarpus anacardium*; only a few of them differentiated to shoots on nutrient medium containing TDZ. Also, Goffreda et al. (1995) stated that TDZ can have undesirable effects on shoot elongation and root formation. Furthermore, İpek et al. (2023) demonstrated that the number of shoots obtained from cotyledon explants of three cultivars of apricot, remained very low when cotyledons were cultured on different media, and the highest average was observed on media supplemented with combination of BAP and NAA.

Conclusion

The in vitro regeneration of Al-Amar apricot rootstock was attempted using cotyledons and hypocotyls. Two pathways were obtained when explants were cultured on regeneration media containing different concentrations of TDZ and of IBA or NAA. The cotyledons expressed direct organogenesis, while indirect organogenesis was achieved from hypocotyls. The results revealed that IBA was more suitable for bud induction from cotyledon explants, and NAA was more effective to hypocotyls. For shoots proliferation and elongation, BAP and NAA, positively affected the both explants, while, adding TDZ has not enhanced shoot proliferation. Also, the average number of shoots obtained from hypocotyl explants were significantly more than shoots produced from cotyledons. The WPM medium supplemented with IBA was the best medium for rooting. Therefore, the hypocotyls proved to be more promising in terms of the regeneration capacity of the Egyptian apricot Al-Amar rootstock.

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Data availability All data generated or analyzed in this study are involved in this manuscript.

Declarations

Competing interests The authors declared that they have no competing interests.

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