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In vitro Propagation of Two Prunus Rootstocks

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Stone fruit trees occupy about 17% of the total area of permanent crops. Myrobalan 29/C (*Prunus cerasifera*.) and GF677 (a natural hybrid of peach and almond) rootstocks, are attracting attention in Lebanese agriculture due to their diverse agricultural characteristics. Traditional vegetative propagation of these rootstocks results in a low rate of multiplication, and makes it difficult to achieve a high phytosanitary status for the final planting material. This study aims to develop an *in vitro* multiplication protocol for GF677 and Myrobolan 29/C using the meristem *in vitro* culture method. For the establishment phase, four culture media were tested; two based on the macro-

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elements of Schenk and Hildebrandt (SH), and two based on the macro-elements of Murashige and Skoog (MS). The four media contained the micro-elements of MS, supplemented with 6-Benzylaminopurine (BAP), Gibberellic acid (GA3) and 1-Naphthaleneacetic acid (NAA). The SH medium containing 0.5 mg.L-1 BAP, 0.5 mg.L-1 GA3 and 0.01 mg.L-1 NAA showed the best results with GF677 and Myrobolan 29/C rootstocks (63 and 63 shootlets regenerated from meristems). For the multiplication phase, the Quoirin and Lepoivre (QL) macro-elements with MS micro-elements, and 0.5 mg.L-1 of BAP presented the highest average number of shootlets/ explant (9.74) for GF677, while Driver and Kuniyuki Walnut (DKW) supplemented with 0.5 mg.L-1 of BAP showed the highest multiplication rate (12.8) after 8 subcultures (30 days apart between subcultures). The QL macro-elements and the DKW supplemented with 1 mg.L-1 of Indole-3-butyric acid (IBA) induced the highest percentage of rooting in GF677(85%) and Myrobolan 29/C (100%) respectively. This study underscores the significance of using SH during the establishment phase, and QL and DKW media during the multiplication and rooting phases for the two rootstocks.

Keywords: GF677; Myrobolan 29/C; Tissue culture; direct regeneration; meristem; growth regulators.

1. INTRODUCTION

Prunus or stone fruits represent an important component of fruit production in Lebanon, occupying up to 17% of the total area of permanent crops [1]. Cherry, almond, apricot, peach, and plum trees vary in their distribution among the Lebanese regions predominantly concentrated in Baalbeck-Hermel (55%) followed by the Bekaa with (16%), Mount Lebanon (10%), Aakkar (8%), North Lebanon (7%) with the lowest rates documented in Nabatiye (3%) and South Lebanon (1%) (Hajjar R, Ministry of Agriculture; personal communication).

The *Prunus* genus comprises around 98 species, representing considerable importance, and all the stone fruits are encompassed within this group [2].

Rootstocks are integral and play fundamental a role in the cultivation of stone fruits, such as almonds, peaches, plums, and cherries as they provide a strong foundation for the growth and development of the fruit-bearing scion, due to their resistance to diseases, adaptability to various soil conditions and improved tolerance to environmental stresses [3]. For instance, compatible rootstocks can aid in mitigating soil-borne diseases and enhancing the overall vigor of the tree [4,5].

Prunus rootstocks, have been widely propagated by seeds for thousands of years in Lebanon without a clear phytosanitary status of the produced plants. It usually leads to the obtaining of a low quantity of propagules and reduced quality of production; however, the high variability of these materials reduces their quality. Micropropagation, is a suitable method for propagation which offer multiple advantages when compared to the traditional propagation, it ensures a fast and large-scale system to produce true-to-type and virus free rootstocks [6].

Within the vegetative propagation, GF 677 (peach x almond hybrid) and Myrobolan 29/C rootstocks, are initially produced through French breeding programs such as INRA [7,8], and are becoming more prevalent in the Lebanese agriculture, where several rootstocks have been imported to Lebanon to produce certified propagation materials.

Hence, this research aims to establish an efficient protocol of *in vitro* propagation for two *Prunus* rootstocks. The propagation protocol includes initiating cultures using meristems, multiplication for several subcultures, rooting and acclimatization of shoots, thus obtaining certified and virus free plants. Along with this purpose the effects of plant growth regulators on shootlets proliferation and rooting are observed.

2. MATERIALS AND METHODS

2.1 Plant Material and Sterilization Process

Two rootstocks namely, GF677, “a peach-almond hybrid (*P. persica* × *P. amygdalus*) that is highly compatible with peach, nectarine and almond cultivars”, and Myrabolan 29/C (*P. Cerasifera*), “which is compatible with apricot and plum cultivars”, were used. The mother plants of these two rootstocks were obtained from the glasshouse of the Plant Biotechnology Department, Tissue Culture Unit at the Lebanese Agricultural Research Institute, Tal Amara (Fig. 1). Twenty-centimeter-long twigs were collected from both rootstocks on June 2022 and served as sources of explants for further isolation

of meristems. Twigs were first fragmented into small segments of 4 to 5 cm. These segments were placed under running tap water and then soaked in detergent for 5min. Following that, explants were rinsed with 70 % ethanol for 30 sec. Under laminar airflow, explants were immersed for 10 min in 20% solution of a commercial disinfectant with a concentration of 5% sodium hypochlorite (Na0Cl), and then were rinsed with sterile distilled water 5 more times.

2.2 Establishment Phase

Following the sterilization of GF677 and 29/C explants, meristems (approx. 0.5 mm long), were isolated using a binocular magnifying glass (20 x 40 magnification) and sterilized instruments (forceps, scalpels, needles, etc.). Four media were tested for meristem culture, two Murashige and Skoog (MS) [9] media: P1 and P2, and two Schenk and Hildebrandt (SH) [10] media: P3 and P4. The four media contained MS micro-elements**,** sucrose (30 g.L-1), vitamin and ascorbic acid (25 mg.L-1) to prevent oxidation; P1 and P3 lacked any growth regulators, while P2 and P4 contained 0.5 mg.L-1 6-Benzylaminopurine (BAP), 0.5 mg.L-1 Gibberellic acid (GA3) and 0.01 mg.L-1 1-Naphthaleneacetic acid (NAA). The pH was adjusted to 5.7, and all media were solidified using 7.5 g.L-1agar, then autoclaved at 118°C for 20 min and dispensed into sterile petri dishes (10 ml/dish) (9 cm). A total of 260 meristems extracted from each of GF677 or Myrobolan 29/C rootstocks, were cultured on the four media P1, P2, P3 and P4 at a rate of 13 replicates/culture medium and five meristems/replicate. Cultures were then incubated in a culture room at a temperature of 22°C ± 2°C, a photoperiod of 16 h per day, with light intensity of 3000 lux using white fluorescent lamps. The number of reactive meristems, the survival percentage and the number of regenerated shootlets were calculated 4 weeks after culturing the meristems.

2.3 Multiplication Phase

During this phase, shoot tips generated at the end of the establishment phase were transferred into jars containing 40 ml of the nine-propagation media P1, P2, P5, P6, P7, P8, P9, P10 and P11 as presented in Table 1, constituting the first subculture. The subculture was repeated every 30 days to study the effect of mineral composition “MS [9], Driver and Kuniyuki Walnut (DKW) [11] or Quoirin and Lepoivre (QL) [12]” and the growth regulators BAP (0, 0.5 or 1 mg.L-1) and GA3 (0, 0.5 mg.L-1) on shootlets proliferation. At the end of each subculture, the multiplication coefficient was estimated as follows:

> Multiplication coefficient = Number of new shootlets / Initial number of shootlets

2.4 Rooting Phase

After eight successive subcultures, 2 to 3 cm length shootlets were sectioned at their base and transferred to rooting media. For this purpose, nine rooting media were tested as shown in Table 2: P12, P13, P14, P15, P16, P17, P18, P19 and P20, differing in their mineral composition (MS, DKW or QL) and in their Indole-3-butyric acid (IBA) concentration (0, 1 or 2 mg.L-1). The pH of the rooting media was adjusted to 5.9. After 30 days, the rate of rooted shootlets, the average number of roots and the average length of roots were recorded.

Fig. 1. *Prunus* rootstocks growing at LARI glasshouses; A: 29/C and B: GF677

Table 1. Composition of the different media (macronutrients, micronutrients, and growth regulators) used during multiplication phase

	P1	P2	P5	P6	P7	P8	P9	P10	P11
Macro elements	MS	MS	MS	DKW	DKW	DKW	QL	QL	QL
Micro elements	MS	MS	MS	DKW	DKW	DKW	MS	MS	MS
BAP (mg.L-1)	0	0.5	1	0	0.5	1	0	0.5	1
GA3 (mg.L-1)	0	0.5	0.5	0	0.5	0.5	0	0.5	0.5
NAA (mg.L-1)	0	0.01	0.01	0	0.01	0.01	0	0.01	0.01

** MS [9], DKW [11] or QL [12]*

Table 2. Composition of the different media (macronutrients, micronutrients, and growth regulators) used during rooting phase

	P12	P13	P14	P15	P16	P17	P18	P19	P20
Macro elements	MS	MS	MS	DKW	DKW	DKW	QL	QL	QL
Micro elements	MS	MS	MS	DKW	DKW	DKW	MS	MS	MS
IBA (mg.L-1)	0	1	2	0	1	2	0	1	2

** MS [9], DKW [11] or QL [12]*

2.5 Acclimatization

The rooted shootlets were transferred to the greenhouse in April 2023. The shootlets were firstly cleaned from the agar by washing under tap water and soaking in a fungicide solution (1 g.L-1). Then, they were transplanted into potting trays of 50 square holes (6 x 6 cm) containing a mixture of peat moss and perlite (1:1 v/v), and placed in the greenhouse in mini tunnels at a relative humidity of 95%. These mini-tunnels were completely closed for the first 15 days (only opened for watering), then were gradually opened to lower the relative humidity and allow the shootlets to adapt to the external conditions. The above fungicide treatments and foliar fertilizers (20.20.20) were regularly applied every 7 days.

2.6 Statistical Analysis

Data recorded during each step of the micropropagation protocol, were analyzed by using standard analysis of variance (ANOVA). Duncan's multiple range tests were used to show differences among the treatments means. All statistical analyses were performed using SAS for windows (S.A.S Institute Inc, 1995).

3. RESULTS AND DISCUSSION

3.1 Establishment Phase

Meristems cultures showed the absence of bacterial contamination while fungal contamination and oxidation of phenolic compounds were encountered. This reflects the effectiveness of the sterilization protocol used, which was therefore suitable for the rest of this study.

The number of shootlets regenerated from meristems that survived the sterilization protocol, indicated a total of 493 reactive meristems out of 520 cultured ones.

For the effect of media (Table 3), P3 and P4 containing SH macro-elements recorded the highest numbers of reactive meristems for both rootstocks (50 and 63 reactive meristems for GF677 rootstock and 60 and 63 reactive meristems for Myrobolan 29/C respectively) as compared to MS medium. This reflects the importance of the SH medium, as an optimal medium for the induction of meristem reactivity.

Regarding the effect of growth regulators, the highest significant number of reactive meristems (63) for GF677 was observed on P4 medium containing (0.5 mg.L-1 BAP,0.5 mg.L-1 GA3 and 0.01 mg.L-1 NAA) with respect to P3 (50) which lacked any growth regulators. On the other hand, Myrobolan 29 / C rootstock showed no significant difference in the number of reactive meristems on both P3 (60) and P4 (63) media.

These results confirm the influence of SH macro-element composition on the reactivity of both rootstocks meristems. Fallahpour et al. (2015), described a better reactivity in the media less concentrated in minerals [13], while others in the most concentrated media [14,15]. Moreover, differences between the effects of these culture media used can be explained on the basis of

total ionic concentration, where high levels can have an inhibitory effect on *in vitro* growth in certain woody plant species [16]. Culture medium components (macro-, micro-elements, and vitamins) may also contribute to a difference in the morphogenetic response based on the plant species tested [13]. On the other hand, the positive effect of BAP used on the shootlets number obtained has also been described in several studies [13,15], reflecting the role of cytokinin (BAP) in cell differentiation and division [15]. Besides, the influence of GA3 on plant growth has been demonstrated by the increase in cell division and elongation of the shootlets, while auxins including NAA, act on regulating the patterning at the shoot-apical meristem [17,18].

3.2 Multiplication Phase

To determine the optimal culture medium that resulted in the best multiplication coefficient, and the adequate composition of growth regulators, shootlets established by the end of first phase were transferred into nine multiplication media: P1, P2, P5, P6, P7, P8, P9, P10 and P11, thus constituting the first subculture. Seven successive subcultures were carried out 30 days apart.

3.2.1 Effect of subculture on the multiplication coefficient

The number of shootlets for GF677 and Myrobolan 29/C increased with subcultures (Fig. 2), and a positive subculture effect was observed on all the tested multiplication media (P2, P5, P7, P8, P9, P10, P11), except for P1 and P6 that lacked any growth regulators (Table 4). P10 medium (QL-based with 0.5 mg.L-1 BAP, 0.5 mg.L-1 GA3 and 0.01 mg.L-1 NAA) resulted in the highest multiplication coefficient (9.74 shootlets) with GF677 at the 8th subculture, while P7 medium (DKW-based with 0.5 mg.L-1 BAP, 0.5 mg.L-1 GA3 and 0.01 mg.L-1 NAA) yielded the highest multiplication (12.8) with Myrobolan 29/C at the 8th subculture. A previous study established by Soliman (2012), revealed the effect of subcultures on stone fruits, that remain genetically stable at least up to eight subcultures [19].

3.2.2 Effect of the macro-element on the average number of shootlets

When combining the data obtained during the eight subcultures with the three BAP concentrations, the effect of macro-element composition on the number of shootlets was determined.

3.2.2.1 GF677

The results showed a significant difference between the three-culture media macro-elements composition, with QL medium having the highest multiplication coefficient (4,93) followed by the DKW medium (2.58), and MS (1.81) (Fig. 3A).

The multiplication coefficient obtained (4.93) is two times higher than that obtained in other studies [20,21]. This reflects that the media constituting QL macro-elements is adequate for GF677 propagation. This result is inconsistent with the study of Borkheyli et al. [15], which reported that the maximum number of shootlets was obtained with MS macro-element (5.8), while DKW medium was less efficient for GF677 multiplication. However, this difference can be explained by the variations in the number of subcultures carried out, as well as the incubation period of each subculture (6 weeks in the study of Borkheyli et al. [15]. Moreover, bud multiplication and elongation are strongly affected by the juvenility of explants [22]; In this study, meristems were used as initial source of explants, which doesn't align with Borkheyli et al. where nodal sections of 1 cm were tested [15].

3.2.2.2 Myrobolan 29/C

The results revealed significant differences in the multiplication coefficient between the three-culture media tested. DKW macro-elements resulted in the highest multiplication coefficient (5.33), followed by QL medium (3.31), while MS medium had the lowest coefficient (2.67) (Fig. 3B).

These results are consistent with those obtained by Plopa et al. [23] on Myrobolan Dwarf Plum rootstock, where the QL macro-elements tested showed a higher multiplication coefficient than that of MS. Shabani et al. [24], deduced that the MS and DKW macro-elements constitute the optimal conditions for the micropropagation of 29/C compared to the WPM formula tested in their study.

It is obvious that with the two rootstocks tested, the macro-element composition had a great influence on the multiplication coefficient. These results are in accordance with several subsequent studies [13,14,15,25,26]. Moreover, the differential response obtained is genotype-specific [27].

Table 3. Meristems' culture establishment results of two prunus rootstocks: GF677 and Myrobolan 29/C (media containing 65 meristem culture per media results of 13 repeats of 5 meristems each)

Rootstock	Media	Number of reactive meristems	Number of regenerated shootlets
GF677	P1	35 ± 0.89 c	35 ± 0.89 c
	P2	38 ± 0.70 bc	38 ± 0.70 bc
	P3	50 ± 0.63 b	50 ± 0.63 b
	P4	63 ± 1.45 a	63 ± 1.45 a
29/C	P1	50 ± 0.49 b	50 ± 0.49 b
	P2	41± 0.99c	41± 0.99c
	P3	60 ± 1.76 a	60 ± 1.76 a
	P4	63 ± 1.59 a	63 ± 1.59 a

Values represented mean values ± Standard Error
*Different letters in the column are significantly different, * P < 0.05 (Duncan test)*

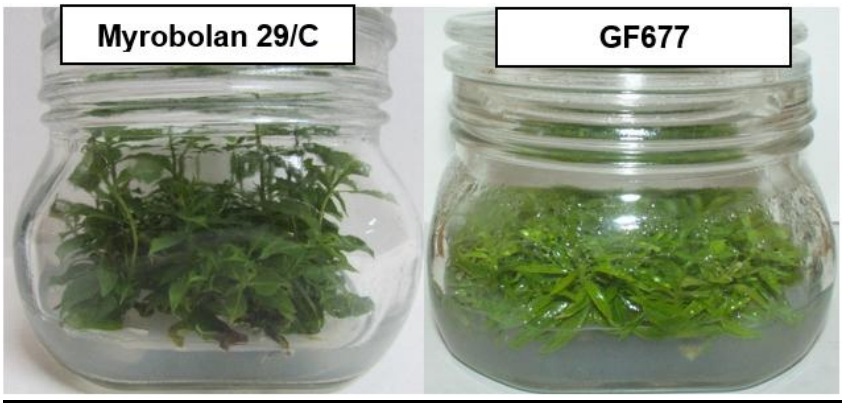

Fig. 2. Myrobolan 29/C and GF677 during subculture 5 of the multiplication phase

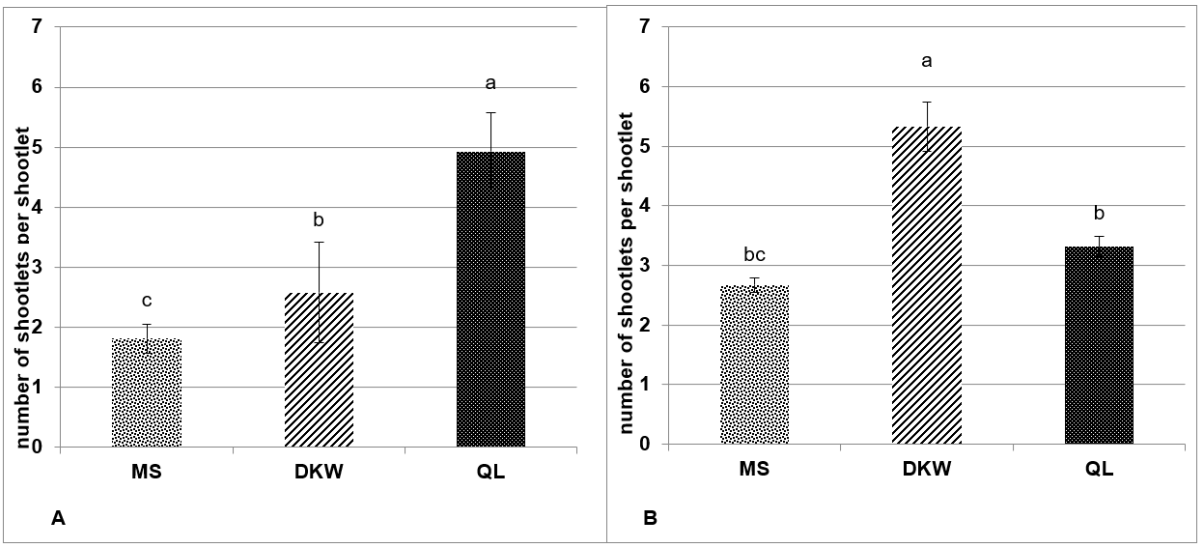

Fig. 3. Effect of macro-elements on the average number of shootlets; A: GF677 and B: Myrobolan 29/C; Subcultures and BAP concentrations are combined
*Bars followed by different letters are significantly different, * P < 0.05 (Duncan test)*

Table 4. Effect of the different culture media on the average number of shootlet, recorded during the 8 subcultures performed on the two rootstocks GF 677 and Myrobolan 29/C

GF 677								
Media	**Subculture 1**	**Subculture 2**	**Subculture 3**	**Subculture 4**	**Subculture 5**	**Subculture 6**	**Subculture 7**	**Subculture 8**
P1	1,17 ± 0,62	1,10 ± 1,90	1,12 ± 0,13	1,28 ± 0,08	1,08 ± 0,08	1.00 ± 0,06	0,96 ± 0,02	0,72 ± 0,03
P2	1,45 ± 0,13	2,20 ± 0,29	2,79 ± 1,60	1,80± 0,10	4,10 ± 0,30	3,00 ± 0,41	2,30 ± 0,08	1,39 ± 0,10
P5	1,23 ± 0,04	2,32 ± 0,99	1,20 ± 0,02	1.20 ± 0,00	3,20 ± 0,04	2,85 ± 0,10	1,45 ± 0,21	1,28 ± 0,09
P6	2,22 ± 1,05	1,35 ± 1,10	2,64 ± 1,30	2,79 ± 1,60	1,79 ± 1,60	2,35 ± 0,21	1.11 ± 0,10	1.30 ± 0,19
P7	2,33 ± 0,65	2,11± 0,04	2,90 ± 0,12	3,20 ± 0,02	3,20 ± 0,02	2,22 ± 0,01	3,40 ± 0,11	2,88 ± 0,14
P8	1,90 ± 0,00	2,22 ± 0,06	3,17 ± 0,01	2,97 ± 0,05	3,97 ± 0,05	3,45 ± 0,42	2,92 ± 0,22	3,21 ± 0,10
P9	1,86 ± 0,60	2,37 ± 1,10	3,64 ± 1,30	4,79 ± 1,60	4,70 ± 1,60	4,70 ± 1,60	2,15 ± 1,00	3,01 ± 0,12
P10	2,22 ± 0.85	4,11± 0,04	6,90 ± 0,12	8,70 ± 0,02	9,48 ± 0,02	8,22 ± 0,05	9,18 ± 0,08	9,74 ± 0,10
P11	2,00 ± 1.02	3,22 ± 0,06	6,10 ± 0,01	6,97 ± 0,05	7,87 ± 0,05	8,2 ± 0,05	7,46 ± 0,03	7,88 ± 0,12
Myrobolan 29 /C								
Media	**Subculture 1**	**Subculture 2**	**Subculture 3**	**Subculture 4**	**Subculture 5**	**Subculture 6**	**Subculture 7**	**Subculture 8**
P1	1,20 ± 0,62	1,44 ± 1,90	0,85 ± 0,13	1,05 ± 0,08	0,73 ± 0,08	1,20 ± 0,04	1,24 ± 0,10	2,22 ± 0,04
P2	1,77 ± 0,13	2,33 ± 0,29	3,44 ± 1,60	3,80± 0,10	4,10 ± 0,30	4,80 ± 0,10	3,20 ± 0,05	3,98 ± 0,02
P5	1,43 ± 0,04	1,33 ± 0,99	2,45 ± 0,02	3.51 ± 0,00	3,99 ± 0,04	4,2 ± 0,07	4,70 ± 0,10	5.07 ± 0,07
P6	2,20 ± 1,05	2,63 ± 1,10	3,73 ± 1,30	3,10 ± 1,60	2,10 ± 1,60	3,00 ± 1,02	3,90± 0.08	4,10 ± 0.10
P7	2,90 ± 0,65	3,20± 0,04	4,70 ± 0,12	7,37 ± 0,02	9,66 ± 0,02	10,20 ± 0,30	11,4 ± 0,40	12,8 ± 0,09
P8	1,25 ± 0,00	2,78 ± 0,06	3,68 ± 0,01	5,77 ± 0,05	5,97 ± 0,05	6,12 ± 0,12	7,20 ± 0,20	8,30 ± 0,10
P9	1,10 ± 0,60	1,25 ± 1,10	2,33 ± 1,30	2,25 ± 1,60	2,31 ± 1,60	1,45 ± 0,20	2,35 ± 0,04	3.21 ± 0,07
P10	1,87 ± 0.85	2,56± 0,04	3,42 ± 0,12	4,88 ± 0,02	5,90 ± 0,02	4,41 ± 0,06	5,61 ± 0,10	4,32 ± 0,07
P11	1,87 ± 0.85	2,98± 0,04	3,66 ± 0,12	3,80 ± 0,02	3,84 ± 0,02	4,10 ± 0,10	5,00 ± 0,08	4,88 ± 0,10

Values represented mean values ± Standard Error

3.2.3 Effect of the BAP on the average number of shootlets

To evaluate the effect of the growth regulator (BAP) on the multiplication coefficient, the average number of shootlets was calculated for each culture medium having different mineral composition along the eight subcultures. For GF677 and Myrobolan 29/C (Fig. 4), the highest number of shootlets was obtained with 0.5 mg.L-1 BAP (4.05 and 5.11 respectively), when compared to the 1 mg.L-1 BAP (3.61 and 4.1 respectively). However, a strong significant difference in the multiplication coefficient was observed in the media lacking any growth regulators (2.15 and 2.12 respectively). These results demonstrate the importance of using growth regulators to determine the number of shootlets compared to hormone-free media. This effect of BAP on GF677 propagation, has been illustrated in several studies; Tatari and Mousavi [21] obtained the optimal results using 0.6 mg.L-1 BAP; Thorpe et al. [25], reported that the best propagation with BAP concentrations ranging between 0.5 to 2.5 mg.L-1; Besides, Nazary and Yadollahi [20], obtained the best results using 1 mg.L-1 BAP. On the other hand, for Myrobolan 29/C, Plopa et al., Nasri et al. and Guney et al. [23,28,29], reported that optimal multiplication coefficients, were observed at moderate concentrations of BAP. Hence, cytokinin stimulates the initiation and the activity of axillary meristems, leading to cell division, shoot multiplication and axillary bud formation [30]. Additionally, the influence of cytokinins on tissue or organ cultures may differ based on the crop type, the plant variety, and the explants' age [27].

3.3 Rooting Phase

Nine culture media were tested for this phase (P12, P13, P14, P15, P16, P17, P18, P19 and P20), differing by macro-elements composition (MS, DKW or QL) and IBA concentration (0, 1 or 2 mg.L-1). The obtained results are presented in Table 5. For the GF677 rootstock, P19 medium (QL-based macro-element composition with 1mg.L-1 IBA), recorded the highest percentage of rooted plants (85%), the highest number of roots/plantlet (4.33), and a root length of 1.9 cm (Fig. 5). P16 medium (DKW-based macro-element composition with 1 mg.L-1 IBA) had the longest roots (2.9 cm), with only 25% of plants rooted.

On the other hand, the superior values for rooting parameters of 29/C were obtained with P16 medium, having100% rooted plants, an average number of 5.34 of roots/plantlet and an average root length of 1.83cm (Fig. 5). However, the longest roots of 3.73cm were obtained with P18 (QL-based macro-element lacking any growth regulators). These results are comparable to those obtained with Shabani et al. [24], where the optimal rooting percentage (100%) for 29/C, was recorded with DKW medium. Similarly, several studies have recommended moderate IBA concentrations for optimal rooting of both GF677 and Myrobolan 29/C rootstocks [13,15,29].

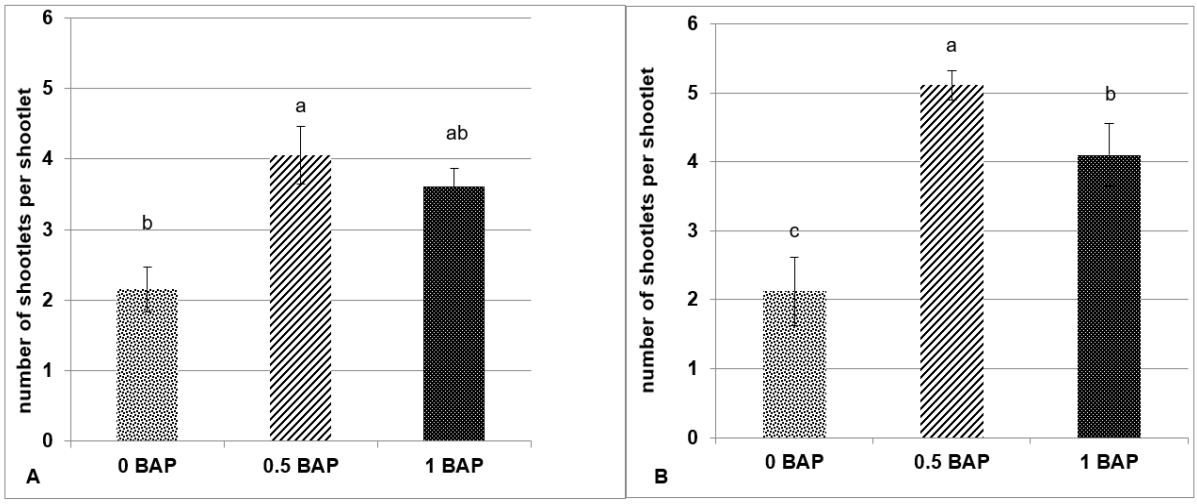

Fig. 4. Effect of BAP concentration on the average number shootlets. A: GF677 and B: Myrobolan 29/C; Subcultres and macro-elements are combined

*Bars followed by different letters are significantly different, * P < 0.05 (Duncan test)*

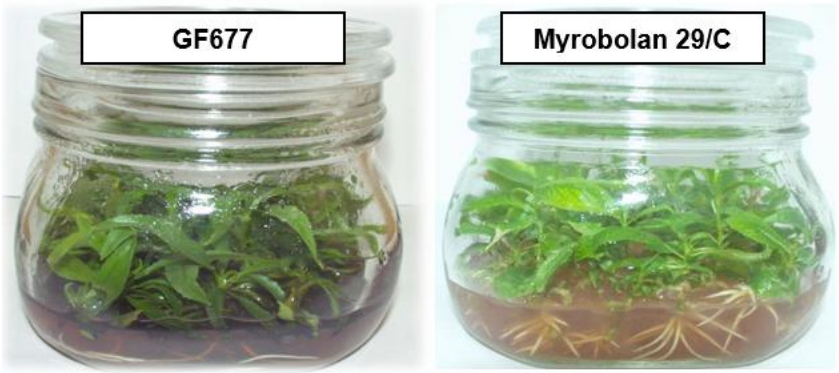

Fig. 5. GF677 (P19 medium) and myrobolan 29/C (P16 medium) during rooting phase

Table 5. Effect of different media (P12, P13, P14, P15, P16, P17, P18, P19 and P20) on the rooting of GF677 and Myrobolan 29/C shootlets

Media	Percentage of rooted plantlets	Average number of roots / plantlets	Average lengths of roots (cm)
		GF677	
P12	1.0 ± 0.03 e	1.0 ± 0.3 cde	2.03 ± 0.08 ab
P13	40.33 ± 4.23 c	1.7 ± 0.24 c	1.03 ± 0.11 cd
P14	43.67 ± 9.87 c	3.13 ± 0.02 b	2.1± 0.09 ab
P15	0.0 ± 0.0 e	0.0 ± 0.0 f	0.0 ± 0.0 e
P16	25 ± 8.5 d	1.5 ± 0.1 cd	2.9 ± 0.1 a
P17	17 ± 3.98 d	0.83 ± 0.05 de	1.4 ± 0.3 bc
P18	1.0 ± 0.5 e	0.33 ± 0.03 ef	0.26 ± 0.02 de
P19	85 ± 11.2 a	4.33 ± 03 a	1.9 ± 0.25 bc
P20	68.5 ± 9.56 b	2.65 ± 0.2 b	1.10 ± 0.15 cd
		Myrobolan 29 /C	
P12	3.7 ± 1.3 f	1.5 ± 0.57 c	2.4 ± 1.3 b
P13	94.43 ± 14 b	4.76 ± 1.24 ab	2.1 ± 0.75 b
P14	61.12 12.1 e	5.46 ± 1.2 a	2.9± 0.07 ab
P15	4.17 ± 2.2 f	1.8 ± 0.85 c	3.67 ± 1.8 ab
P16	100 ± 3.41 a	5.34 ± 0.9 a	1.83 ± 0.91 bc
P17	83.33 ± 12.3 c	4.53 ± 0.5 ab	2.77 ± 1.1 ab
P18	3.13 ± 1.1 f	3.8 ± 0.8 b	3.73 ± 1.1 a
P19	83.3 ± 11.2 c	4.27 ± 1.1 ab	2.0 ± 0.5 b
P20	66.7 ± 13.1 d	3.76 ± 0.8 b	0.83 ± 0.4 c

Values represented mean values ± Standard Error
*Different letters in the column are significantly different, * P < 0.05 (Duncan test)*

Fig. 6. Shootlets of GF 677 and Myrobolan 29/C rootstocks acclimatized in the greenhouse

3.4 Acclimatization

The rooted shootlets were transferred to the greenhouse, then examined a week later. Signs of survival or loss started to appear (Fig. 6). A few shootlets rapidly withered away, as a result of foliage drying out or root system asphyxia, while others began to develop new leaves, indicating the success of acclimatization in the greenhouse and their transition from the heterotrophic (in vitro) to the autotrophic stage.

After 30 days, the survival rate marked the survival of 66% of the transferred GF677 plantlets and 70% of 29/C plantlets, thus reflecting the success of the acclimatization phase and indicating the effectiveness of the rooting protocol carried out in the previous step.

4. CONCLUSION

Meristem culture is the most valid production method used for disease-free, clonal and mass production of plants. This study aimed to develop an efficient protocol for *in vitro* propagation of two *Prunus* rootstocks GF677 and Myrobolan 29/C. To this end, meristems isolated from these two rootstocks were cultured. Different culture media with different macro-element mineral compositions were tested for each micropropagation phase. Different growth regulators were also tested and the effect of successive subcultures was evaluated. The results of this research will markedly improve the micropropagation of *Prunus* rootstocks in Lebanon. In this context, we recommend using SH medium based macro-element combined with 0.5 mg.L-1 BAP, 0.5 mg.L-1 GA3 and 0.01 mg.L-1 NAA in the establishment phase for both rootstocks GF677 and Myrobolan 29/C, QL based macro-element with 0.5 mg.L-1 BAP for the multiplication of GF677, and DKW based macro-element with 0.5 mg.L-1 BAP for the multiplication of Myrobolan 29/C. Furthermore, we propose using QL and DKW macro-elements supplemented with 1 mg.L-1 IBA for the rooting of GF677 and Myrobolan 29/C respectively. Given the records collected for meristem culture establishment, the coefficient of multiplication and the rooting stage, working further on improving acclimatization conditions to reach the 100% hardening survival rate for both rootstocks is fundamental. Consequently, optimizing the micropropagation protocol can induce a positive impact on stone fruits cultivation in Lebanon, ensuring the local production of true-to-type and virus free material. Adopting a certification program by the Ministry of Agriculture in collaboration with the private sector is an urgent need in order to enhance the quality of rootstocks and cultivars in the fruit trees sector.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing, or producing any section of this manuscript. The whole manuscript content is the result of human effort, research, and analysis.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Azzi A. Lebanese agricultural sector at a glance. Blominvest Bank; 2021. Accessed 27 September 2024. Available:https://blog.blominvestbank.com/wp-content/uploads/2022/10/Lebanese-Agricultural-Sector-at-a-Glance.
2. Das B, Ahmed N, Singh P. Prunus diversity-early and present development: A review. International Journal of Biodiversity and Conservation. 2011;3(14):721-734.
3. Dhurve L, Mathew D, Kumar A, Joseph VA, Mehara H.Rootstocks: Importance in Fruit Crop Improvement. International Journal of Environment and Climate Change. 2023;13(11):4479-4490.
4. Kumar A, Bhuj BD, Dhar S. New approaches of root stocks in fruit production: a review. Journal of Botanical Insights. 2024;2(1).
5. Albacete A, Martínez-Andújar C, Martínez-Pérez A, Thompson JA, Dodd CI, Pérez-Alfocea F. Unravelling rootstock×scion interactions to

improve food security. J Exp Bot. 2015; 66(8): 2211–2226.
6. Aris S, Hooghvorst FJ, Gort AA. In vitro Propagation of Prunus Rootstocks: A Review. HortScience. 2017;52(1):7-13.
7. Felipe AJ, Gómez-Aparisi J, Socías R, Carrera M. The almond x peach hybrid rootstocks breeding program at Zaragoza (Spain). Journal of Horticultural Science & Biotechnology. 2003; 78(5):765-772.
8. Garagurbanly I, Suleymanova S, Hafizov G. Rootstocks GF677, Garnem 15, Maxma 14 and Myrobalan29C: from introduction to culture in vitro to adaptation of planting material ex vitro. Modern Approaches in Engineering and Natural Sciences. 2023; 040008:1-7.
9. MurashigeT, and Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 1962;15:473-97.
10. Schenk RU and Hildebrandt AC. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 1972;50: 199-204.
11. Driver and Kuniyuki. In vitro propagation of Paradox walnut rootstock. HortScience. 1984;19(4): 507-9.
12. Quoirin M, Lepoivre P. Etude de milieux adaptes aux cultures in vitro de Prunus. Acta Hort. 1977;78:437-42.
13. Fallahpour M, Miri SM, and Bouzari N. In vitro propagation of Gisela 5 rootstock as affected by media and plant growth regulators. Journal of Horticultural Research. 2015;23(1):57-64.
14. Gallo CM, Radmann EB, Ritterbusch CW, Feijó A, Bianchi VJ, and Peters JA. The effects of the culture media components by in vitro multiplication of rootstock Ciência Agrícola, Rio Largo. 2017;15(1):9-16.
15. Borkheyli MM, Miri SM, and Nabigol A. In vitro multiplication and rooting of GF677 rootstock. Journal of horticulture and postharvest research. 2021;4(2):243-252.
16. Bell RL, Srinivasan C, Lomberk D. Effect of nutrient media on axillary shoot proliferation and preconditioning for adventitious shoot regeneration of pears. In Vitro Cellular & Developmental Biology – Plant. 2009;45:708.
17. Vernoux T, Besnard F, Traas J. Auxin at the shoot apical meristem. Cold Spring Harb Perspect Biol. 2010;2(4):a001487.
18. Paiva PDO, Silva DPCD, Silva BRD, Sousa IP, Paiva R, Reis MVD. How Scarification, GA_3 and Graphene Oxide Influence the In Vitro Establishment and Development of Strelitzia. Plants (Basel). 2023;12(11):2142.
19. Soliman H. *In vitro* Propagation of Apricot (*Prunus armeniaca* L.) and Assessment of Genetic Stability of Micropropagated Plants Using RAPD Analysis. World Applied Sciences Journal. 2012; 19(5): 674-687.
20. Nazary MAR, Yadollahi A. Micropropagation of GF 677 Rootstock. Journal of Agricultural Science. 2012;4(5): 31-138.
21. Tatari M, Mousavi SA. Optimization of in vitro culture in Tetra, Nemaguard and GF677 clonal rootstocks. Journal of Crops Improvement. 2014;15(3):103-115.
22. Naghmouchi S, Khouja ML. and Boussaid M. Effect of growth regulators and explant origin on in vitro propagation of *Ceratonia siliqua* L. via cuttings. Biotechnol. Agron. Soc. Environ. 2008; 12(3):251-258.
23. Plopa C, Dutu I, Isac V, Mazilu C, and Ancu S. Factors influencing in vitro propagation of Myrobolan Dwarf Plum rootstock. Acta Horticulturae. 2012;968 (968):153-158.
24. Shabani Z, Moghadam EG, Abedi B, and Tehranifar A. Effect of media and regulators of plant growth on micro propagation of Myrobalan 29C rootstock. Journal of Horticulture and Forestry. 2015; 7(3):57-64.
25. Aftabi M, Mozaffari J, Hossein Ava S, Miri SM. Selection an appropriate medium for in vitro proliferation of hazelnut genotypes. 8th National Horticultural Science Congress of Iran. Hamedan, Iran. 2013; 2628-2631.
26. Fallahpour M, Miri SM, Bouzari N. Effects of media cultures and plant growth regulators on micropropagation of CAB-6P cherry semi-dwarf rootstock. Iranian Journal of Horticultural Science. 2019;50 (1):187-196.
27. Thorpe T, Stasolla C, Yeung EC, and De Klerk. Plant Growth Regulators. Dordrecht, Netherlands: Springer. 2008;3(1):115-173.
28. Nasri A, Baklouti E, Romdhane AB, Maalej M, and Schumacher HM. Large-scale propagation of Myrobolan (Prunus cerasifera) in RITA bioreactors and ISSR-based assessment of genetic conformity. Scientia Horticulturae. 2019;245(6):144-153.

29. Guney M. Development of an in vitro micropropagation protocol for Myrobalan 29C rootstock. Turk J Agric For. 2019; 43(6):569-575.

30. Dobránszki J, and Teixeira da Silva JA. Micropropagation of apple – A review. Biotechnology Advances. 2010;28(4):462-488.

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