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SINTEZĂ A CERCETĂRILOR PRIVIND MICROPROPAGAREA PORTALTOILOR SPECIILOR SÂMBUROASE

A REVIEW OF RESEARCHES ON MICROPROPAGATION OF STONE FRUIT SPECIES ROOTSTOCKS

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Abstract

In the context of the rapid development of the biotechnology field and the growing needs of the agricultural market, research regarding to *in vitro* micropropagation technology for rootstocks of the stone fruit species, such as those for plum, preach and apricot is an important step in improving propagation methods for these crops. These studies aim to develop and optimize *in vitro* propagation techniques to rapidly obtain large numbers of high-quality plants adapted to different environmental conditions and resistant to disease and stress. *In vitro* multiplication of rootstocks played an important role in the rapid multiplication of valuable species and the production of healthy plants necessary for nurseries. In last years, various approaches have been carried out for *in vitro* propagation of rootstocks for stone fruit species. Micropropagation using apical buds or nodal segments and understanding the specific requirements at different stages has been comprehensively covered in the specialized literature. In this synthesis is presented the importance of culture media for the proliferation of shoots and root induction, as well as the improvement of protocols for a high rate of multiplication of shoots. The development of a protocol for *in vitro* plant regeneration, which is considered an important step for the successful implementation of various biotechnological techniques used for plant breeding programs, has been adequately addressed in the literature. Overall, the present review provides a consolidated overview of *in vitro* propagation for stone fruit species rootstocks. The most commonly used culture media were Murashige and Skoog medium, including full and half-strength variations, followed by Quoirin and Lepoivre medium.

Cuvinte cheie: portaltoi, *in vitro,* specii sâmburoase.
Key words: rootstocks, *in vitro,* stone fruit species.

1. Introduction

Micropropagation has emerged as a crucial technique in modern fruit cultivation, offering a solution to meet the growing food demands of an increasing world population. This method allows for rapid and intensive mass propagation of varieties and rootstocks under controlled laboratory conditions (Pakyurek, 2019). Rootstocks play an essential role to determining orchard performance of fruit trees. Selecting the right combination of the rootstock and cultivar is important for optimizing fruit quality parameters. Dwarfing is an important agricultural trait for intensive cultivation and effective orchard management in modern fruit orchards. Many of the rootstocks now available to growers of apples, pears, peaches, nectarines, apricots, cherries and plums are listed and described briefly. However, the propagation of these hybrids through conventional means has proven to be difficult. This difficulty has led researchers to explore in vitro propagation as a reliable method for mass clonal propagation of these new rootstocks (Vaghari-Azar et al., 2012).

The requirement for new rootstock genotypes in *Prunus* species is substantial, making micropropagation an indispensable propagation method (Sadeghi et al., 2015). This technique is particularly valuable for rootstocks that exhibit desirable traits such as high adaptability to soil and climatic conditions, good winter hardiness, resistance to overwatered soils, tolerance to drought, and the capacity to grow on heavy soils (Suprun et al., 2024).

Research efforts were focused on developing and optimizing micropropagation protocols for various *Prunus* rootstocks. These studies have investigated different aspects of the process, including the effect of various culture media compositions (Fachinello, 2001; Ostadshariff, 2014), the influence of plant growth regulators (Fachinello, 2001; Ostadshariff et al., 2014), the efficacy of different rooting methods (Ostadshariff et al., 2014). *In vitro* propagation also involves several critical stages, with surface sterilization and *in vitro* culturing of explants being particularly important. These stages present unique

challenges, especially for *Prunus* varieties, as the explants sources are often grown in open lands, increasing the risk of contamination (Ugur, 2020).

The finally goal of these research endeavors is to establish efficient and reproducible protocols for the *Prunus* rootstocks micropropagation that are important from an economic point of view. In this way, researchers aim to provide the fruit industry with a reliable method for producing high-quality planting material on a large scale (Sadeghi et al., 2015; Pakyurek, 2019).

2. Material and methods

The micropropagation starts with plant tissues (explant) from a healthy and vigorous mother plant. Any part of the plant (leaf, apical meristem, bud and root) can be used as explants. The whole process can be summarized into the stages as shown in figure 1.

Plant material. Various rootstocks have been the subject of micropropagation studies, including: 'Okinawa' (*P. persica*), 'Nemared' [(*P. persica* × *P. davidiana*) × *P. persica*], 'Garnem' (*P. dulcis* × *P. persica*) (Eliwa, 2024) (Alhady et al., 2018), 'Tetra' (*Prunus empyrean 3*) (Sadeghi et al., 2015), 'Adaptabil' (*Prunus besseyi* x open pollination) (Plopa et al., 2012), 'Mirobolan dwarf' (Plopa et al., 2012), 'HS314', a hybrid between almond (*Prunus amygdalus*) and peach (*P. persica*) (Dejampour, 2011), 'Adafuel' (*Prunus dulcis x P. persica*) (Marin et al., 2009), 'Myrobalan' (*Prunus cerasifera* Ehrh.) plum rootstock (Hammerschlag, 1982), 'Nemaguard' (*Prunus persica x Prunus davidiana*) peach rootstock (Reeves et al., 1983).

Explants Types. Different types of explants were used by the researchers, such as nodal segments (Dejampour, 2011; Sadeghi et al., 2015), shoot tips (Hammerschlag, 1982), axillary buds (Eliwa et al, 2024), meristems (Plopa et al., 2012 b).

Culture Media. Different media compositions were tested and the effects of macronutrients, micronutrients and hormonal balances on growth parameters were evaluated. The most used media were:

- Murashige and Skoog medium (MS, 1962), including full and half-strength variations (Hammerschlag, 1982; Fotopoulos and Sotiropoulos, 2005; Marin et al., 2009; Ak et al., 2021);
- Linsmaier Skoog medium (LS) (Arab et al., 2018);
- Quoirin and Lepoivre medium (QL, 1977) (Radmann et al., 2009; Plopa et al., 2012 a, b);
- Driver J., Kuniyuki A. medium (DKW, 1984) (Plopa et al., 2012 a);
- YAS medium (optimized for G×N15 by Arab et al., 2016;
- ME medium (specifically created by Sadeghi et al., 2015);
- Mc Cown Woody Plant Medium (WPM, 1981) (Vaghari-Azar et al., 2012).

Growth regulators. Various plant growth regulators were used in different combinations and concentrations:

- BA (benzyladenine): 0.5-2.0 mg/l (Ak et al., 2021);
- BAP (6-Benzylaminopurine): 0.5-2.5 mg/l (Plopa et al., 2012 a);
- IBA (Indole-3-butyric acid): 0.01-2.0 mg/l (Plopa et al., 2012 a; Sadeghi et al., 2015; Arab et al., 2018; Ak et al.,2021);
- GA3 (Gibberellic acid): 0.01-0.1 mg/l (Plopa et al., 2012 a);
- NAA (Naphthaleneacetic acid): 0.2 mg/l (Plopa et al., 2012 b);
- IAA (Indole-3-acetic acid), (Hamerschlag, 1982).

3. Results and discussions

Explant establishment. The success appears to be highly dependent on species and cultivar-specific factors, including explant choice, timing of collection, culture medium composition, and growth regulator combinations (Table 1).

In accordance with studies mentioned, the explant types showed different regeneration capacities. Nodal cuttings of the 'Garnem' hybrid rootstock demonstrated high regeneration potential, especially when cultured on MS medium with 2.0 mg/l BA (Ak et al.,2021). 'Tetra' (*Prunus empyrean* 3) rootstock record a good evolution when in vitro culture was initiate using nodal segments on WPM (woody plant medium) without growth regulators (Sadeghi et al., 2015). Hammerschlag (1982) utilized shoot tips (5 mm) on a modified Murashige and Skoog (MS) medium for initial culture establishment of 'Myrobalan' plum (*Prunus cerasifera* Ehrh.). According to Plopa et al., (2012 b) regeneration capacity in case of 'Mirobolan dwarf' increase with explant size, their recomandation being that the explant size for good results must be minimum 0.8 mm.

The choice of explant and timing of collection, significantly influenced regeneration success. For 'Myrobalan' plum shoot tips with 5 mm size were productive, while nodal explants collected in spring showed the best results for apricot × plum hybrid rootstocks (Vaghari-Azar et al., 2012). Plum rootstock 'PK SK 1' demonstrated highest regeneration rates (78.1–93.8%) when explants were collected during

active shoot growth in early to mid-May (Suprun et al., 2024). This demonstrate the importance of timing and explant source in initial culture establishment.

As stated by Dejampour et al., (2011), nodal segments of 'HS314' rootstock (*Prunus amygdalus* × *P. persica*) were successfully established in a modified DKW medium containing 3% sucrose, 100 mg l-1 of Phloroglucinol, 0.7% plant agar, and 0.5 mg l-1 BAP. Nodal cuttings of the 'Garnem' hybrid rootstock demonstrated high regeneration potential, particularly when cultured on MS medium with 2.0 mg/l BA (Ak et al.,2021).

These observations highlight the complex nature of regeneration capacity in stone fruit species rootstocks micropropagation.

Shoot multiplication. Studies have also highlighted in this case differences depending on genotype, culture medium, hormonal balance and subcultures number. So, literature shows that the composition of the culture media influences in significantly mode the influences of the success from *in vitro* culture. While MS medium and its modifications were commonly used in the studies, the culture media specific optimized showed superior results. For exemple, YAS medium get the better of standard media for G×N15 (Arab et al., 2016), and ME medium was most efficiency for 'Tetra' rootstock which achieved the highest reported multiplication rate with 30.4 shoots per explant (Sadeghi et al., 2015).

Vaghari-Azar et al. (2012) achieved the greatest shoot proliferation using 4 mg/l BAP + 0.5 mg/l GA3 supplemented in WPM medium . Suprun et al. (2024) found that for 'PK SK 1' rootstock, MS medium with 1 mg/L 6-BAP and either Fe-EDTA or Fe-EDDHA was effective, producing an average of 11.7 to 12.3 shoots per explant after the fourth passage.

Ostadsharif et al. (2014) reported that MS medium supplemented with BAP and IBA at concentrations of 0.5 and 0.1 mg/l, respectively, produced the highest number of healthy shootlets (2.125 per explant) for 'Saint Julien A' rootstock.

BAP concentrations between 3-5 mg/L showed similar proliferation rates across different rootstocks: 'Okinawa' - 3.77-6.11 shoots per explant, 'Nemared' - 4.33-8.88 shoots per explant, 'Garnem' - 3.33-7.44 shoots per explant. The combination of 5.0 mg/L BAP with 0.2 mg/L IBA significantly increased proliferation, resulting in 96.29% shoot proliferation and 7.48 shoots per explant. These results demonstrate the synergistic effect of combining cytokinins and auxins for enhanced shoot proliferation. (Eliwa et al., 2024).

Proliferation rates differed among species. 'Myrobalan' plum achieved a 10-fold multiplication rate every 4–6 weeks (Hammerschlag, 1982), whereas plum rootstock 'PK SK 1' produced 11.7-12.3 shoots per explant after the fourth passage (Suprun et al., 2024). The use of liquid medium for short periods (4-7 days) was found to be beneficial for 'Myrobalan' plum, a technique not reported for other species (Hammerschlag, 1982). This suggests that optimizing physical culture conditions, in addition to medium composition, can significantly impact proliferation rates.

QL medium showed versatility across different rootstocks. For the 'Adaptabil' rootstock, QL medium supplemented with 0.1 mg/l GA3, 1.5 mg/l BAP, and 0.2 mg/l ANA achieved an optimal multiplication rate of 1/8. QL medium with Walkey vitamins, 0.01 mg/l GA3, and 1 mg/l IBA resulted in an impressive 98% rooting for 'Adaptabil' (Plopa et al., 2012) . For *Prunus* sp. 'GxN-9' rootstock, QL medium produced 3 shoots per explant, but also showed a 55% vitrification rate. Interestingly, WPM medium did not induce vitrification in the same rootstock. This observation suggests that media composition plays a crucial role in managing vitrification, and that lower salt concentrations might be beneficial in reducing this physiological disorder (Arab et al., 2016). This highlights the importance of optimizing media composition for each specific genotype (Table 2).

Rooting. Rooting success varied significantly among rootstocks. 'Adaptabil' achieved an impressive 98% rooting on QL medium with specific growth regulators (Plopa et al., 2012 a) , while 'Garnem' showed 42.8% rooting on ½ MS medium (Ak et al., 2021), PR 204/84 exhibited increased rooting percentage on ½ MS medium with 2.5 µM IBA, for 'Adafuel', rooting was achieved on ½ MS salts with 5 µM IBA, though the exact percentage was not specified (Marin et al., 2009). This variability emphasizes the need for rootstock-specific rooting protocols and suggests that rooting ability might be genetically determined (Marin et al., 2009).

'Myrobalan' plum achieved 100% rooting in dark conditions with IAA (Hammerschlag, 1982), while light was inhibitory (Hammerschlag, 1982). In contrast, plum rootstock 'PK SK 1' rooted on hormone-free medium (Suprun et al., 2024). The addition of chlorogenic acid improved rooting in light conditions for 'Myrobalan' plum (Hammerschlag, 1982), indicating the potential role of phenolic compounds in overcoming light inhibition of rooting.

Vaghari-Azar et al. (2012) obtained success with a high-concentration IBA dip followed by culture on hormone-free medium, while Suprun et al. (2024) achieved good results with hormone-free MS medium for 'PK SK 1'. Sadeghi et al. (2015) reported 100% rooting with a low concentration of IBA in the medium for 'Tetra' rootstock. This variation suggests that rootstocks may have different auxin requirements for optimal rooting.exact percentages were not provided (Fotopoulos and Sotirpoulos, 2005).

The study on 'HS314' rootstock (*Prunus amygdalus* × *P. persica*) provides detailed information on rooting. Microshoots were transferred to DKW medium supplemented with different concentrations of auxins for root induction: IBA or NAA: 0.5, 1, 2, or 4 mgL-1. The highest root number and the greatest root length were achieved on a medium containing 2 mgL-1 IBA. Rooting percentage was improved from 66% to more than 85% by reducing the concentration of DKW salts to half strength (Dejampour et al., 2011).

For 'Nemaguard' rootstock was obtained 90% rooting with 3.0 mg/L IBA, producing an average of 5.8 roots per shoot. The use of half-strength MS medium supplemented with 3.0 mg/L IBA or NAA resulted in maximum root lengths of 8.5 cm and 7.2 cm, respectively. For 'Nemaguard' and 'Okinawa' rootstocks, the combination of NAA and IBA at 1.0 mg/L each recorded the maximum root number and root length (Alhady, 2018). The 'Tetra' (*Prunus empyrean* 3) rootstock achieved 100% in vitro rooting on ½ strength MS medium with 0.5 mg/L IBA, 1.6 mg/L thiamine and 150 mg/L iron sequestrene. For the rootstock 'Cadaman', rooting rate improved with the addition of the chelated form of iron FeEDDHA at 150 mg/L. These findings suggest that both auxin type and concentration, media strength, and additional supplements influence rooting outcomes, and that optimal conditions can vary significantly between different *Prunus* rootstocks (Balla and Mansvelt, 2006) (Table 3).

Ostadsharif et al. (2014) used a pulsing method for root induction for 'Saint Julien A' (*Prunus domestica* spp. *insititia*) rootstock. The pulsing method refers to a technique where shoots are exposed to a high concentration of auxin for a short period before being transferred to a hormone-free or low-hormone medium. In this study, they compared two different methods of auxin application: gradual pre-application and pulsing. The results showed that the pulsing method was more effective, resulting in 77.28% rooting and an average of 1.8 roots per shoot. This method demonstrated a significant improvement in rooting efficiency compared to other conventional methods tested in the study (Table 3).

Acclimatization. Different approaches to acclimatization were reported. 'Myrobalan' plum was successfully transferred directly to soil with no losses (Hammerschlag, 1982), while other species required more controlled conditions. Acclimatization success varied depending on the substrate used. For 'Garnem', a peat: perlite mixture yielded the highest survival rate (47.2%) (Ak et al., 2021), while other substrates showed lower success rates. The 'Adafuel' rootstock was successfully acclimatized, but specific rates were not provided (Marin et al., 2009). These results indicate that optimizing the acclimatization substrate is crucial for each rootstock (Ak et al., 2021; Marin et al., 2009). For 'Nemaguard', only 4 out of 10 rooted shoots survived transfer to soil (Reeves et al., 1983).

High survival rates (over 90%) were reported for 'Nemaguard' when plantlets were transferred to greenhouse conditions. The replacement of sucrose with glucose in the rooting medium improved survival rates during acclimatization for some peach cultivars and rootstocks. This indicates that the composition of the rooting medium can impact subsequent ex vitro survival (El-Razek and Abdel-Aziz, 2014).

Acclimatization success rates varied across studies. Suprun et al. (2024) reported a high success rate of 87.5% for plum rootstock 'PK SK 1', while Ostadsharif et al. (2014) achieved 60% success for Saint Julien A (*Prunus domestica* spp. *insititia*) rootstock. This variation suggests that post-*vitro* adaptation remains a critical area for improvement in *Prunus* rootstock micropropagation (Table 4).

4. Conclusions

In *Prunus in vitro* propagation all studies constant responding to regarding to the importance of genotype. The 'Garnem', 'Adaptabil' and *Prunus* sp. 'GxN-9' rootstocks each showed unique optimal conditions for proliferation and rooting. This expresses the importance of developing adapted protocols for each *Prunus* rootstock. 'Myrobalan' plum showed rapid propagation *in vitro*, just like other plum rootstock 'PK SK 1' and apricot × plum hybrid rootstocks. This confirms the potential of micropropagation as a viable method for mass clonal propagation of plum species, to significantly enhance the production of high-quality planting material for fruit tree nurseries.

Althought the studies primarily focused on research aspects, providing valuable insights and identified areas for future research. These include further optimization of culture conditions, investigation of long-term field performance of micropropagated rootstocks, and exploration of genetic factors influencing in vitro responses.

These studies the complex and genotype-specific nature of *Prunus* rootstock micropropagation. They emphasize the need for careful optimization of all stages of the micropropagation process, from media composition and growth regulator balance to acclimatization conditions, to achieve successful and commercially viable protocols.

While significant progress has been made, there is still a need for further optimization of protocols, particularly for recalcitrant genotypes. Additionally, more research is needed on improving acclimatization techniques to enhance the overall efficiency of the micropropagation process.

In conclusion, these studies collectively provide a strong foundation for the micropropagation of various *Prunus* rootstocks. The developed protocols offer promising avenues for commercial-scale

production of high-quality planting material, contributing significantly to the advancement of the fruit tree industry.

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Tables and Figures

Table 1. Growth regulators used in the initiation of *in vitro* cultures

Species/ Cultivar	Explant Type	Culture Medium	Growth Regulators	Results	References
0	1	2	3	4	5
Plum rootstock PK SK 1	Not specified	MS with Fe-EDTA or Fe-EDDHA	1 mg/L 6-BAP	Regeneration reaches 78.1–93.8% when collected during first and second decade of May	Suprun et al., 2024
Garnem	Node explants	MS medium	2.0 mg/L BA	Highest number of shoots and proliferation	Ak et al., 2021
Marianna GF 8/1, Myrobolan B, Myrobolan 29-C, St Julien A	Shoot tips	MS medium	1 mg L-1 BAP + 0.5 mg L-1 IBA + 0.25 mg L-1 GA3	Best propagation	Pakyurek and Hepaksoy, 2019
G×N15 (Garnem)	Not specified	LS medium	1 mg/L IBA, 1.6 mg/L Thiamine, 100-200 mg/L Fe-EDDHA	Optimized rooting medium	Arab et al., 2018
G×N15 (Garnem)	Not specified	YAS medium	NH4+ 14 mM, NO3- 27.5 mM, Ca2+ 5 mM, K+ 25.9 mM, Mg2+ 0.7 mM, PO42- 1.1 mM, SO42- 4.7 mM, Cl− 0.96 mM	Best proliferation rate	Arab et al., 2016
Tetra (*Prunus empyrean* 3)	Nodal segments	WPM (Woody Plant Medium)	None	Enhanced culture establishment	Sadeghi et al., 2015
Saint Julien A (*P. domestica* spp. *insititia*)	Nodal segments	MS medium	0.5 mg/l BAP + 0.1 mg/l IBA	2.125 healthy shootlets per explant	Ostadsharif et al., 2014
Apricot × plum hybrid rootstocks (HS405 and HS706)	Nodal explants	WPM	None	Highest number of active buds obtained	Vaghari-Azar et al., 2012
Apricot × plum hybrid rootstocks (HS405 and HS706)	Not specified	Not specified	200 mg/l Na-Cefotaxime or 100 mg/l nano silver	Positive effect on controlling indigenous contaminations	Vaghari-Azar et al., 2012
'Adaptabil' rootstock (*Prunus besseyi* x open pollination)	Apically buds	QL	0.1 mg/l GA3, 0.01 mg/l IBA	Efficient culture initiation	Plopa et al., 2012
Mirobolan Dwarf	Buds	QL	0.01 mg/l IBA+0.1 mg/l GA3	100% explant diferentiation	Plopa et al., 2012

0	1	2	3	4	5
HS314 rootstock (*Prunus amygdalus* × *P. persica*)	Nodal segments	Modified DKW medium with 3% sucrose, 100 mgL-1 Phloroglucinol, 0.7% plant agar	0.5 mgL-1 BAP	Maximum shoot production with 2 mgL-1 BAP	Dejampour et al., 2011
Prunus sp. 'GxN-9' rootstock	Basal explants	QL and WPM	Not specified	80% shooting percentage	Radmann et al., 2009
Prunus sp. 'GxN-9' rootstock	Basal explants	QL	Not specified	3 shoots per explant (highest)	Radmann et al., 2009
Prunus sp. 'GxN-9' rootstock	Basal explants	QL	Not specified	55% vitrification	Radmann et al., 2009
Prunus sp. 'GxN-9' rootstock	Basal explants	WPM	Not specified	No vitrification	Radmann et al., 2009
Prunus sp. 'GxN-9' rootstock	Not specified	Not specified	0.5 mg/dm³ BAP	Reduced vitrification, increased shoot growth	Radmann et al., 2009
PR 204/84 (*Prunus persica x P. amygdalus*)	Not specified	1/2 MS medium	2.5 µM IBA	Increased rooting percentage	Fotopoulos and Sotopoulos, 2005
G x N22, GF 677, Mr.S 2/5, Marianna, Mirabolano	Nodal segments	MS and MS ¾	BAP (0.1-0.7 mg.L-1)	Varied responses; Marianna best in MS with 0.7 mg.L-1 BAP, Mirabolano in MS ¾ with 0.5 mg.L-1 BAP	Fachinello, 2001
Nemaguard (*Prunus persica x Prunus davidiana*)	Not specified	Modified MS medium	Not specified	Best survival, growth, and condition at pH 5.8	Reeves et al., 1983
Myrobalan (*Prunus cerasifera* Ehrh.)	Shoot tips (5 mm)	Modified MS medium	0.01 mg/L IBA, 0.26 mg/L BA	Initial culture establishment	Hamerschlag, 1982
Myrobalan (*Prunus cerasifera* Ehrh.)	Not specified	MS medium	2.5 – 5.0 mg/L IAA	100% rooting at 4 weeks (dark incubation)	Hamerschlag, 1982
Pixy (*P. insititia*) and F 12/1 (*P. avium*)	Not specified	Not specified	Not specified	Successful adaptation of methods from apple rootstocks	Jones and Hopood, 1979

Table 2. Proliferation and multiplication rate results

Species/Cultivar	Culture Medium	Growth Regulators	Results	References
0	1	2	3	4
Okinawa (*P. persica*)	MS	3-5 mg/L BAP	3.77-6.11 shoots per explant	Eliwa et al., 2024
Nemared ((*P. persica* × *P. davidiana*) × *P. persica*)	MS	3-5 mg/L BAP	4.33-8.88 shoots per explant	Eliwa et al., 2024
Garnem (*P. dulcis* × *P. persica*)	MS	3-5 mg/L BAP	3.33-7.44 shoots per explant	Eliwa et al., 2024
PK SK 1 (Plum rootstock)	MS medium with Fe-EDTA or Fe-EDDHA	1 mg/L 6-BAP	11.7-12.3 shoots per explant (after 4th passage)	Suprun et al., 2024
Garnem	MS medium	2.0 mg/l BA	Highest number of shoots and	Ak et al., 2021
Garnem	MS medium	0.5 mg/l BA	Most effective for shoot length elongation	Ak et al., 2021
Marianna GF 8/1, Myrobolan B, Myrobolan 29-C, St Julien A	MS	1 mg L-1 BAP + 0.5 mg L-1 IBA + 0.25 mg L-1 GA3	Best propagation (specific numbers not provided)	Pakyurek and Hepaksoy, 2019
Nemaguard	MS	3.0 mg/L BAP + 0.5 mg/L 2iP	Maximum of 7.0 shoots per explant	Alhady, 2018
G×N15 (Garnem)	YAS medium	NH4+ 14 mM, NO3- 27.5 mM, Ca2+ 5 mM, K+ 25.9 mM, Mg2+ 0.7 mM, PO42- 1.1 mM, SO42- 4.7 mM, Cl− 0.96 mM	Best proliferation rate	Arab et al., 2016
Tetra (*Prunus empyrean* 3)	ME medium	0.8 mg/l BAP, 0.05 mg/l IBA	30.4 shoots per explant	Sadeghi et al., 2015, 2015
Saint Julien A (Prunus domestica spp. *insititia*)	MS	0.5 mg/l BAP + 0.1 mg/l IBA	2.125 healthy shootlets per explant	Ostadsharif et al., 2014
Nemaguard and Okinawa	MS	0.5 mg/L BA	Enhanced shoot proliferation compared to control	El-Razek et al., 2014

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0	1	2	3	4
Nemaguard and Okinawa	MS	0.1 mg/L IAA	Enhanced shoot proliferation compared to control	El-Razek et al., 2014
Nemaguard and Okinawa	MS	2.0 mg/L BA + 2.0 mg/L 2iP	Highest shoot number compared to other treatments	El-Razek et al., 2014
Apricot × plum hybrid rootstocks (HS405 and HS706)	WPM medium	4 mg/l BAP + 0.5 mg/l GA3	Greatest shoot proliferation (rate not specified)	Vaghari-Azar, et al., 2012
'Adaptabil' rootstock (*Prunus besseyi* x open pollination)	QL	0.1 mg/l GA3, 1.5 mg/l BAP, 0.2 mg/l ANA	Optimal multiplication rate of 1/8	Plopa et al., 2012
Mirobolan Dwarf	QL	1 mg/l BAP+0.2 mg/l NAA	The multiplication rate grow from 1.88 to 3.77	Plopa et al., 2012
HS314 rootstock (*Prunus amygdalus* × *P. persica*)	Modified DKW medium with 3% sucrose, 100 mgL-1 Phloroglucinol, 0.7% plant agar	0.5, 1, or 2 mgL-1 BAP and 0, 0.1, or 0.5 mgL-1 IBA	Maximum number of shoots produced on medium containing 2 mgL-1 BAP	Dejampour et al., 2011
Prunus sp. 'GxN-9' rootstock	QL	Not specified	3 shoots per explant	Radmann et al., 2009
Prunus sp. 'GxN-9' rootstock	QL and WPM	Not specified	80% shooting percentage	Radmann et al., 2009
Marianna	MS	0.7 mg.L-1 BAP	Best results (specific number not provided)	Fachinello, 2001
Mirabolano	MS ¾	0.5 mg.L-1 BAP	Best results (specific number not provided)	Fachinello, 2001
G x N22 and Mr.S 2/5	MS ¾	BAP (concentration not specified)	Better results compared to full MS (specific number not provided)	Fachinello, 2001
Myrobalan (*Prunus cerasifera* Ehrh.)	Stage II multiplication medium (Stage I medium with modifications)	1.0 mg/liter BA, 0.6% agar	10-fold multiplication rate every 4-6 weeks	Hamerschlag, 1982

Table 3. *In vitro* rooting results

Species/Cultivar	Culture Medium	Growth Regulators	Results	References
0	1	2	3	4
PK SK 1 (Plum rootstock)	Hormone-free MS with Fe-EDDHA	None	57.1–61.8% rooting, 2.4 roots per shoot, 1.2-5.1 cm long	Suprun et al., 2024
Myrobalan 29C	1/2 MS	1.0 mg/l IBA	90% rooting with well-developed roots	Yaremko, 2023
Myrobalan 29C	MS	0.75 mg/l NAA	100% rooting, but weAK et al.,er root system	Yaremko, 2023
Garnem	½ MS medium	2 mg/l IBA	42.8% rooting	Ak et al., 2021
Marianna GF 8/1, Myrobolan B, Myrobolan 29-C, St Julien A	½ MS	2 mg L-1 IBA	Best rooting (specific percentage not provided)	Pakyurek and Hepaksoy, 2019
Nemaguard	MS	3.0 mg/L IBA	90% rooting, maximum average root number/shoot (5.8)	Alhady, 2018
Nemaguard	1/2 MS	3.0 mg/L IBA	Maximum root length (8.5 cm)	Alhady, 2018
Nemaguard	1/2 MS	3.0 mg/L NAA	Maximum root length (7.2 cm)	Alhady, 2018
G×N15 (Garnem)	LS medium	1 mg/L IBA, 1.6 mg/L Thiamine, 100-200 mg/L Fe-EDDHA	Optimized rooting medium	Arab et al., 2018
Tetra (*Prunus empyrean* 3)	½ strength MS	0.5 mg/l IBA, 1.6 mg/l thiamine, 150 mg/l iron sequestrene	100% rooting	Sadeghi et al., 2015, 2015
Nemaguard and Okinawa	MS	NAA + IBA (1.0 mg/L each)	Maximum root number and root length	El-Razek et al., 2014
Saint Julien A (*Prunus domestica* spp. *insititia*)	Not specified	IBA and NAA (gradual pre-application and pulsing)	Pulsing method: 77.28% rooting, 1.8 roots per shoot	Ostadsharif et al., 2014
'Adaptabil' rootstock (*P. besseyi* x open pollination)	QL with Walkey vitamins	0.01 mg/l GA3, 1 mg/l IBA	98% rooting	Plopa et al., 2012

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0	1	2	3	4
Mirobolan Dwarf	½ strength MS	1.5 mg/l IBA	Maximum rooting percentage of 100%	Plopa et al., 2012
Apricot × plum hybrid rootstocks (HS405 and HS706)	Hormone-free WPM	1000 mg/l IBA dip for 20 seconds	Best rooting results (rate not specified)	Vaghari-Aza et al., 2012
HS314 rootstock (*Prunus amygdalus* × *P. persica*)	DKW medium	0.5, 1, 2, or 4 mgL-1 IBA or NAA	Highest root number and greatest root length gained on medium containing 2 mgL-1 IBA; Rooting percentage improved from 66% to more than 85% by reducing DKW salts to half strength	Dejampour et al., 2011
'Adafuel' (*Prunus dulcis x P. persica*)	½ MS salts	5 µM IBA	Rooting achieved (percentage not specified)	Marin et al., 2009
Cadaman	Not specified	Not specified + 150 mg/L FeEDDHA	Improved rooting rate	Balla and Mansvelt, 2006
PR 204/84 (*Prunus persica x P. amygdalus*)	½ MS medium	2.5 µM IBA	Increased rooting percentage	Fotopoulos and Sotirpoulos, 2005
PR 204/84 (*Prunus persica x P. amygdalus*)	MS medium (full and half strength)	0-10 µM IBA	Increased mean root number per shoot with increasing IBA concentration	Fotopoulos and Sotirpoulos, 2005
Myrobalan (*Prunus cerasifera* Ehrh.)	MS medium	2.5 – 5.0 mg/liter IAA	100% rooting at 4 weeks (dark incubation for 2 weeks)	Hamerschlag, 1982
Myrobalan (*Prunus cerasifera* Ehrh.)	MS medium	IAA	Significantly better rooting than IBA (dark incubation for 2 weeks)	Hamerschlag, 1982
Pixy (*Prunus insititia*)	Not specified	Not specified	Roots readily formed (specific percentage not provided)	Jones and Hopgood, 1979

Table 4. Acclimatization results

Species/Cultivar	Substrate	Duration	Survival Rate	References
PK SK 1 (Plum rootstock)	Agrobalt plant nutrient soil, vermiculite, and perlite (3:1:1)	4 weeks	87.5%	Suprun et al, 2024
Garnem	Peat:perlite (1:1)	8 weeks	47.2%	AK et al., 2021
Garnem	Perlite only	8 weeks	32.8%	AK et al., 2021
Garnem	Peat only	8 weeks	23.6%	AK et al., 2021
Nemaguard	Not specified	Not specified	Over 90%	Alhady, 2018
Saint Julien A (*Prunus domestica* spp. *insititia*)	Mixture of perlite and pitmass (3:1)	Not specified	60%	Ostadsharif et al., 2014
'Adafuel' (*Prunus dulcis x P. persica*)	Peat:vermiculite (1:1)	2 weeks	Successful (percentage not specified)	Marin et al., 2009
Peach rootstocks (general)	Not specified	Not specified	High survival when transferred to greenhouse conditions	Balla and Mansvelt, 2006
Nemaguard (*Prunus persica* × *Prunus davidiana*)	Soil	Not specified	4 out of 10 rooted shoots	Reeves et al., 1983
Myrobalan (*Prunus cerasifera* Ehrh.)	Soil	Not specified	Successfully transferred	Hamerschlag, 1982
Pixy (*P. insititia*) and F 12/1 (*P. avium*)	Not specified	Not specified	Successfully grown-on in pots (specific percentage not provided)	Jones and Hopgood, 1979

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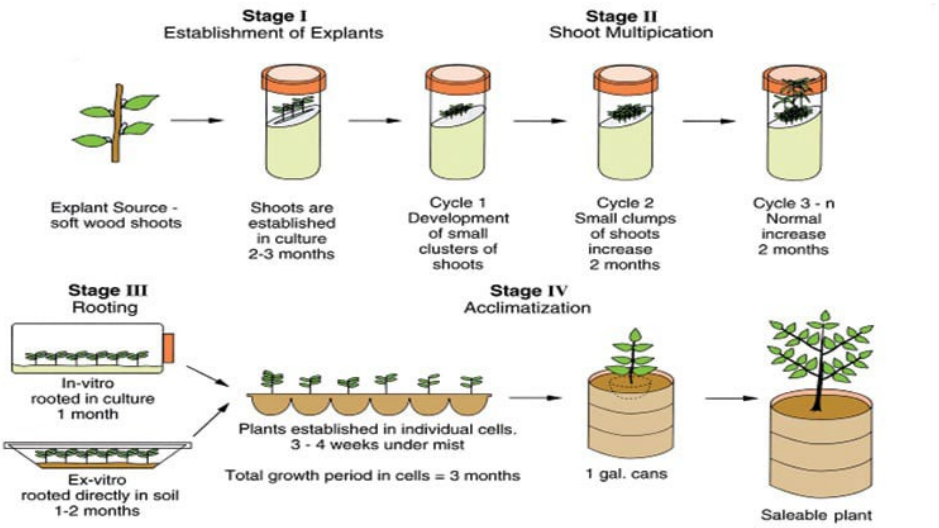

Fig. 1. The stages of micropropagation
Image Source: University of Florida.
(https://microbenotes.com/micropropagation-stages-types-applications-advantages-limitations/)