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MICROPROPAGATION OF PLUM ROOTSTOCK (*PRUNUS DOMESTICA* L.) OF 'WAVIT' VARIETY

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Background. 'Wavit' is a valuable Plum rootstock hybrid of *Prunus domestica* (*P. cerasifera* × *P. spinosa*). It reduces tree vigor and exhibits a high winter hardiness, increases fruit size, shows good compatibility with all types of plums and apricots, and consistently produces high yields. The aim of this study is to propose a suitable *in vitro* propagation protocol for the 'Wavit' rootstock. This includes micropropagation based on the analysis of two base media for shoot proliferation: Driver Kyniyuki Walnut (DKW), and Quorin & Lepoivre (QL) with different combinations of plant growth regulators and two forms of iron chelate. Additionally, the study explores an *in vitro* protocol for rooting with different concentrations of Indole-3-butyric acid (IBA), and an *ex vitro* adaptation period.

Material and Methods. Research was conducted by cultivation under *in vitro* conditions of 'Wavit' explants with following stages: shoot proliferation was exmained by using two basal media DKW, and QL supplemented with Walkey vitamins and different contents of IBA, meta-topolin (MT), 6-benzylaminopurine (6-BAP) and iron chelate: ferric-sodium salt of ethylenediaminetetraacetic acid (FeNaEDTA) and ethylenediamine di-2-hydroxyphenyl acetate ferric (FeEDDHA). After 4 weeks of cultivation shoot length, number of shoots, % of vitrification and multiplication rate were measured.

Rooting medium was consistent with ½ Mourashige & Skoog (MS) medium supplemented with Walkey vitamins and different concentrations of Indole-3-butyric acid. After 4 weeks of cultivation shoot length, root length, number of roots and % of rooted nodal segments were measured.

Acclimatization was conducted in the greenhouse. For the experiments, shoots were divided into 3 groups: unrooted, 1–3 roots, >3 roots, and cultivated for a month after which survival rate was measured.

Results. The research involved the cultivation of 'Wavit' explants under *in vitro* conditions, comprising several stages. At the stage of shoot proliferation after 4 weeks of cultivation, the highest value with a significant difference in shoot length was found in variants DKW 0.5 MT, 0.1 IBA, FeNaEDTA; DKW 0.5 MT, 0.5 BAP, 0.1 IBA, FeNaEDTA; DKW 0.5 MT, 0.5 BAP, 0.1 IBA, FeEDDHA; however, the highest number of vitrified shoots was observed in the last two listed variants. A significant difference was also found in the multiplication coefficient in the variant DKW 0.5 MT, 0.5 BAP, 0.1 IBA, FeEDDHA, which was the lowest among all DKW medium variants.

Subsequently, the data obtained at the rooting stage showed dependency on root formation with increase of IBA concentration in nutrient media. Addition of 1,0, 1,25 and 1.5 mg/L significantly increased percentage of rooted shoots, number of roots and root length, however 1.5 mg/L decreased the shoot length of the explants.

After 1 month of acclimatization, only 25 % of the group without root survived, the survival rate of groups with 1–3 roots and more than 3 roots was 87.5 % and 92 % respectively.

Conclusions. The present study describes a standard in vitro protocol for the mass propagation of a valuable plum 'Wavit' rootstock from stem nodal segments. Driver Kyniyuki Walnut medium supplemented with 0.5 mg/L meta-topolin, or with 0.5 mg/L meta-topolin and 0.1 mg/L indole-3-butyric acid with FeNaEDHHA, showed the overall increased performance during shoot proliferation. For the rooting stage, ½ Mourashige & Skoog with indole-3-butyric acid at concentrations of 1.0 and 1.25 mg/L demonstrated better results. Additionally, we observed the advantage of obtaining rooted plant material before the acclimatization stage, which significantly increased the survival rate of plants.

Keywords: *Prunus* sp., *in vitro* propagation, plant growth regulators, biostimulators, media composition, adaptation

INTRODUCTION

Plums and apricots belong to a large and diverse group of species related to the *Prunus* genus of the *Rosaceae* family. According to FAO records in 2019, total plum fruit production increased by up to 20 % compared to the previous decade. China remains the leading producer (with 77 % of the world area harvested and 56 % of production), while Ukraine is currently among the top ten producers of plums (CBI, 2020). Data published in 2021 by the State Statistics Service of Ukraine (Prokopenko, 2023) reported a total plum production of 188.3 thousand tons in 2021, reaching its peak in 2019. This represents a 61 % increase compared to the year 2000 and a 28 % increase compared to 2010. Ukraine demonstrates a higher percentage increase in total fruit production per decade compared to the global trend. The introduction of new rootstocks, production of high-quality plant material, and improvements in both *in vitro* and *ex vitro* techniques would further strengthen the market.

Dwarf and semi-dwarf *Prunus* rootstocks are widely used by nurseries, gardens, and plantations to reduce tree vigor, increase yield, and potentially impact heat or cold tolerance, as well as resistance to various pathogens. This allows for manipulation of the planting system (Hoza *et al.*, 2015; Mayer *et al.*, 2020). They are considered

essential in modern agricultural techniques, given their capability to confer the listed features to the scion, along with the diversity of available rootstocks (Dejong *et al.*, 2004; Mestre *et al.*, 2015).

'Wavit' is a plum rootstock clone of *Prunus domestica* (*P. cerasifera* × *P. spinosa*) 'Wangenheim' selected in Austria by Robert Schreiber. According to the information provided by the 'Wavit' license owner (CDB GmbH), it reduces tree vigor, shows high winter hardiness, increases fruit size, exhibits good compatibility with all types of plums and apricots, and consistently produces high yields, as confirmed by initial test results (Stefanova *et al.*, 2009; Murri *et al*., 2013).

Despite high production and demand, cultivating, propagating, and adapting to *ex vitro* conditions pose significant challenges for *Prunus* species. Vegetative propagation yields are low, as shoots without well-formed adventitious roots are unlikely to survive. *In vitro* propagation presents a promising approach for commercial plant cultivation, as it can produce large quantities of high-quality, virus-free material in a relatively short time. However, in vitro cultivation and subsequent acclimatization can be challenging for *Prunus* species, often requiring unique media formulations across various presented varieties (Kazemia & Mohorko, 2017).

P. Druart, the first author to review micropropagation of *Prunus* species in semi-solid media in 1986 (Druart & Gruselle, 1986), emphasized in 1992 the importance of further improving cultivation protocols (Druart, 1992). Despite this, the majority of available protocols still utilize semi-solid media (Ruzic *et al*., 2012; Wolella, 2017; Vujović *et al.*, 2020). However, in recent years, novel protocols for cultivation have also emerged with the aim of enhancing mass production of plant material. These protocols utilize technologies such as liquid-media bioreactors (Gado *et al*., 2022).

It is important to highlight the significance of the rooting stage in *in vitro* plant material. While some authors suggest that rooting may not be crucial for subsequent adaptation to *ex vitro* conditions due to intensive labor involved and high cost (Pincelli-Souza *et al.*, 2018), most researchers continue to explore various media compositions, cultivation techniques, and plant growth regulators (PGR's) interactions to maximize the production of rooted plant material. (Sadeghi *et al.*, 2015; Komakech *et al*., 2020; Lawson *et al*., 2023).

Commercial laboratories have streamlined micropropagation by simultaneously rooting and acclimatizing microcuttings via *ex vitro* rooting. However, for species that are challenging to root directly in a greenhouse, the more conservative approach of *in vitro* rooting may prove useful before transfer to a greenhouse environment (Lawson *et al*., 2023).

The aim of this study is to propose a suitable *in vitro* propagation protocol for the 'Wavit' rootstock. This includes micropropagation based on the analysis of two base media for multiplication (DKW and QL) with different combinations of plant growth regulators (PGR's) and two forms of iron chelate. Additionally, the study investigates an *in vitro* rooting protocol with different concentrations of indole-3-butyric acid (IBA), as well as an *ex vitro* adaptation period in the greenhouse.

MATERIALS AND METHODS

Plant material. *In vitro* plants of 'Wavit' rootstock were taken from CDB (Union of German Nurseries, www.cdb-rootstocks.com) according to the license given for Dolyna Agro LTD from 06.12.2019.

Culture media and growth conditions. The study utilized DKW (Driver & Kuniyuki, 1984), QL (Quorin & Lepoivre, 1977) media with a vitamin mixture after Walkey (Walkey, 1972), and ½MS (Mourashige & Skoog, 1962) medium with a vitamin mixture after Walkey solidified with 5 g/L plant agar, adjusted to pH 5.8. The medium for shoot proliferation contained 30 g/L sucrose, while the medium for rooting contained 15 g/L sucrose and 50 mg/L of active charcoal (AC). Reverse osmosis purified water was used for media preparation. Composition of plant growth regulators (PGR's) and other ingredients is detailed in subsequent steps.

The stock solutions were stored in the dark at 4–7 °C for approximately 2 months or as recommended by the manufacturer. All media ingredients were sourced from Duchefa Biochemie B.V. Prepared media were sterilized in an autoclave (model VK-75) at 121°C for 20 minutes, and ready-to-use media were stored at 20±2°C for no longer than 2 weeks after preparation. Cultures were incubated in the laboratory at 24±2 °C under a 16/8-hour (light-dark cycle) photoperiod provided with cool white fluorescent light (2500–3000 Lx).

Shoot proliferation. Nodal segments (1–1.5 cm) were excised from initial shoots, ensuring at least 2–3 leaves were retained at the top. Any callus, dead, or vitrified leaves, as well as shoots displaying necrosis signs, were removed. Following the preparation of explants, they were transferred into DKW or QL media with different combinations of 0.5 mg/L meta-topolin (MT), 0.5 mg/L 6-benzylaminopurine (6-BAP), 0.1 mg/L indole-3-butyric acid (IBA), and two forms of iron chelate (37.7 mg/L FeNaEDTA and 100 mg/L FeEDDHA). A total of 12 media compositions were examined in this study. After four weeks of incubation, shoot length, the number of shoots per explant, and the percentage of vitrified shoots were measured and recorded.

***In vitro* rooting.** Shoots measuring at least 1.5–2.5 cm in length were separated from the rosette. Similar to the multiplication process, the lower leaves were removed, leaving at least 2–3 leaves at the top. The prepared shoots were then inserted into ½MS medium with vitamins after Walkey, supplemented with different concentrations of (IBA) (0.1, 0.25, 0.5, 0.75, 1.0, 1.5 mg/L). After four weeks of incubation, the shoot length, number of roots, root length per explant, and percentage of rooted shoots were measured and recorded.

Acclimatization. After growth on the rooting medium, the plantlets were transferred to a greenhouse. The shoots were carefully removed from the nutrient medium and washed in distilled water to remove any residues. The explants were then divided into three groups based on root development: 1) shoots with more than 3 roots, 2) shoots with 1–3 roots, and 3) shoots without roots. These groups were planted in multiplates containing a substrate composed of peat soil (70 %) and coconut shavings (30 %), with a pH of 5.5.

The seedlings were grown for one month on racks, maintaining a 16/8-hour (light-dark cycle) photoperiod provided with cool white fluorescent light (2500–3000 Lx) and maintaining high humidity at a temperature of 25±2 °C.

After two months, the survival rate of the explants and the length of the shoots were analyzed.

Statistical analysis. The experiment followed a completely randomized design (CRD) with factorial arrangement and included 3 replications per treatment. Statistical

analysis of the data was performed using Microsoft Excel software. Means were compared using Tukey's Multiple Range Test (Nanda *et al*., 2021) with significance levels set at $p \leq 0.05$ and $p \leq 0.01$.

RESULTS AND DISCUSSION

Shoot proliferation. The nutrient media composition selected for micropropagation presents one of the most important roles in establishing the plant *in vitro* culture, and can have direct effects on plant growth performance (Kazemia & Mohorko, 2017).

In this study, DKW medium has shown overall better performance than QL medium at the micropropagation phase with a significant shoot length increase (**Table 1**, **2**). The combination of MT and BAP increased shoot number on both media, but it also increased the percentage of vitrificated shoot which potentially leads to an increased amount of discarded material decreasing multiplication rate.

In all experimental variants with the DKW medium (**Table 1**), the number of shoots did not exceed the level of significance at $p \leq 0.01$; however, differences were observed in all other parameters. The highest value with a significant difference in shoot length was found in variants DKW 0.5 MT, 0.1 IBA, FeNaEDTA; DKW 0.5 MT, 0.5 BAP, 0.1 IBA, FeNaEDTA; DKW 0.5 MT, 0.5 BAP, 0.1 IBA, FeEDDHA; however, the highest number of vitrified shoots was observed in the last two listed variants. A significant difference was also found in the multiplication coefficient in the variant DKW 0.5 MT, 0.5 BAP, 0.1 IBA, FeEDHHA, which was the lowest among all DKW medium variants.

When examining indicators on the QL (**Table 2**) medium, it was noticed that the shoot length and multiplication coefficient were significantly lower compared to DKW; however, the highest shoot number was obtained in variants QL 0.5 MT, 0.5 BAP, 0.1 IBA, FeNaEDTA; QL 0.5 MT, FeEDDHA; QL 0.5 MT, 0.1 IBA, FeEDDHA. Additionally, the highest significantly meaningful number of vitrified shoots was observed in QL 0.5 MT, BAP 0.5, 0.1 IBA, FeEDDHA. Shoots with necrotic symptoms and vitrification are unsuitable for further rooting, and such shoots are not used for further micropropagation. The reason for such changes could be the excessively high concentration of growth regulator or the composition of the basal medium.

Necrosis of shoots often occurs due to the absence or insufficient amount of growth regulators of the cytokinin group (Martin *et al*., 2007). A reduction of vitrification and necrosis can be achieved by reducing the cultivation time, changing cultivation conditions, including temperature, humidity, and lighting, as well as the mineral composition of the nutrient medium (Nezami *et al*., 2015; Wiszniewska *et al*., 2016). However, a clear definition of the factors causing necrosis and adjustment of cultivation conditions for explants of the 'Wavit' rootstock require further research.

***In vitro* rooting.** The inability, or challenges to induce the formation of adventitious roots is often a limiting factor in plant tissue culture aimed for commercial micropropagation. There are several reports that *in vitro* rooting for Prunus species had difficulty with rhyzogenezis, but formed a large callus tissue (Sadeghi *et al*., 2015; Lawson *et al*, 2023).

The highest shoot length was observed in media containing 0.1 and 0.25 mg/L IBA (**Table 3**). However, in these variants, root length, number of roots, and percentage of rooted shoots observed were the lowest. Further increases in IBA concentration improved root development, adventitious root formation, and their length. This data

Table 1. **Growth characteristics of 'Wavit' shoots on the DKW medium variants**

Variants	Shoot length (cm)	Shoot number (n)	Vitrificated shoots (%)	Multiplication rate (n)
DKW 0.5 MT, FeNaEDTA	3.67±0.04 Aa	2.56±0.07 Aa	6.52±0.04 Aa	3.6±0.05 Aab
DKW 0.5 MT, 0.1 IBA, FeNaEDTA	3.64±0.04 Aa	2.39±0.06 Aa	5.59±0.03 Aa	3.4±0.04 aBb
DKW 0.5 MT, 0.5 BAP, 0.1 IBA, FeNaEDTA	3.78±0.04 aBb	2.54±0.06 AaBb	14.98±0.06 Aab	3.7±0.06 Aa
DKW 0.5 MT, FeEDDHA	3.41±0.03 BbCc	2.42±0.06 AaBb	6.61±0.04 aBb	3.4±0.03 aBb
DKW 0.5 MT, 0.1 IBA, FeEDDHA	3.86±0.04 Bb	2.32±0.06 abC	12.93±0.05 aBbc	3.6±0.04 Aab
DKW 0.5 MT, 0.5 BAP, 0,1 IBA, FeEDDHA	3.16±0.03 Cc	2.57±0.06 AaBbc	16.49±0.06 abCc	3.1±0.04 bCc

Note: * – presented data describes mean ± SE, different uppercase letters in the same column indicate significant difference at a = 0.05. Different lowercase letters in the column indicate significant difference at a = 0.01

Table 2. **Growth characteristics of 'Wavit' shoots after 4 weeks on the QL media**

Variants	Shoot length (cm)	Shoot number (n)	Vitrificated shoots (%)	Multiplication rate (n)
QL 0.5 MT, FeNaEDTA	3.10±0.03 Aa	2.32±0.05 cDd	7.46±0.04 dEe	3.1±0.06 Aa
QL 0.5 MT, 0.1 IBA FeNaEDTA	2.83±0.03 Bb	2.03±0.05 dEe	7.05±0.03 Ee	2.3±0.08 Cc
QL 0.5 MT, 0.5 BAP, 0.1 IBA FeNaEDTA	2.89±0.03 Bb	2.97±0.06 aBb	12.12±0.05 bCc	2.8±0.07 AaBb
QL 0.5 MT, FeEDDHA	3.16±0.03 Aa	3.29±0.06 Aa	10.46±0.06 CcDd	3.1±0.07 Aa
QL 0.5 MT, 0.1 IBA, FeEDDHA	2.93±0.02 aBb	3.10±0.06 AaB	16.36±0.07 aBb	2.3±0.09 Cc
QL 0.5 MT, 0.5 BAP, 0.1 IBA, FeEDDHA	2.34±0.03 Cc	2.76±0.07 bCc	19.47±0.07 Aa	2.3±0.08 Cc

Note: * – presented data describes mean ± SE, different uppercase letters in the same column indicate significant differnce at a = 0.05. Different lowercase letters in the column indicate significant difference at a = 0.01

Table 3. **Growth characteristics of 'Wavit' shoots after 4 weeks on the rooting media**

Media composition	Shoot length (cm)	Root length (cm)	Root number (n)	Rooted shoots (%)
½MS, 0.1 IBA	4.68±0.05Aa	2.51±0.11Aa	1.46±0.07Aa	26 %
½MS, 0.25 IBA	4.83±0.04Aa	4.29±0.14aB	1.6±0.07aBb	38 %
½MS, 0.5 IBA	4.45±0.04aB	6.13±0.1bC	1.81±0.08bCc	47 %
½MS, 0.75 IBA	4.2±0.03Bb	7.84±0.12cDd	2.21±0.09cDd	59 %
½MS, 1.0 IBA	4.33±0.06aB	8.37±0.18DdE	2.68±0.1dEe	76 %
½MS, 1.25 IBA	3.634±0.03Cc	8.28±0.11DdEe	3.61±0.16Ff	81 %
½MS, 1.5 IBA	3.45±0.03Cc	7.11±0.11Cc	4.19±0.14fGg	82 %

Note: * – presented data describes mean ± SE, different uppercase letters in the same column indicate significant differnce at a = 0.05. Different lowercase letters in the column indicate significant difference at a = 0.01

confirms that auxin hormones play a significant role in rooting initiation, the formation of adventitious roots, and overall plant growth and development (Kang *et al*., 2018).

Variants with 1.0 mg/L and 1.25 mg/L showed the highest performance in root formation and development with the highest significantly different numbers of root length, number of roots and percentage of rooted shoots. Variant with addition of IBA 1.5 mg/L showed a significantly different decrease in root length, but with a significant increase in the number of formed adventitious roots. Nevertheless, the decreased shoot length in these variants may pose challenges during acclimatization. Explants with undeveloped or small shoots, despite being rooted, have a lower chance of surviving *ex vitro* conditions (Quambusch *et al*., 2017).

Addition of 0.1 mg/L, 0.25 mg/L and 0.5 mg/L didn't seem to be viable IBA concentration in the rooting media, because the percentage of rooted shoots was less than 50 % which potentially will lead to a higher amount of discarded material at the adaptation to *ex vitro* condition in the future.

In comparison with the authors who conducted similar tests on 'Wavit' *in vitro* microshoots, their numbers of rooted plant material did not exceed 16 % using both half-strength MS media and full strength MS media with addition from 1 to 3 mg/L of IBA (Yaremenko *et al*., 2023). It is worth mentioning that the effectiveness of rhizogenesis is also determined by cultivating conditions, quality and manufacturer of reagents used in media preparation, prerequisite steps of cutting microshoots (Wiszniewska *et al*., 2016; Kazemia & Mohorko, 2017; Lawson *et al*, 2023).

Acclimatization. The acclimatization of rooted *in vitro* produced plantlets is one of the key factors in their subsequent survival in the field (Hazarika *et al*., 2006). This is because *in vitro* cultured plants tend to have abnormal morphologies, anatomies, and physiologies and so need time to acclimatize in the greenhouse before they are exposed to *ex vitro* environmental conditions (Pospíšilová *et al*., 1999). After 1 month of cultivation, only 25 % of the group without root survived, which confirms our previous mention of the importance of the *in vitro* rooting as a prerequisite step for further acclimatization in the greenhouse. In the groups with 1–3 roots and more than 3 roots, the survival rate was 87.5 % and 92 % respectively (**Fig. 1**, **2**).

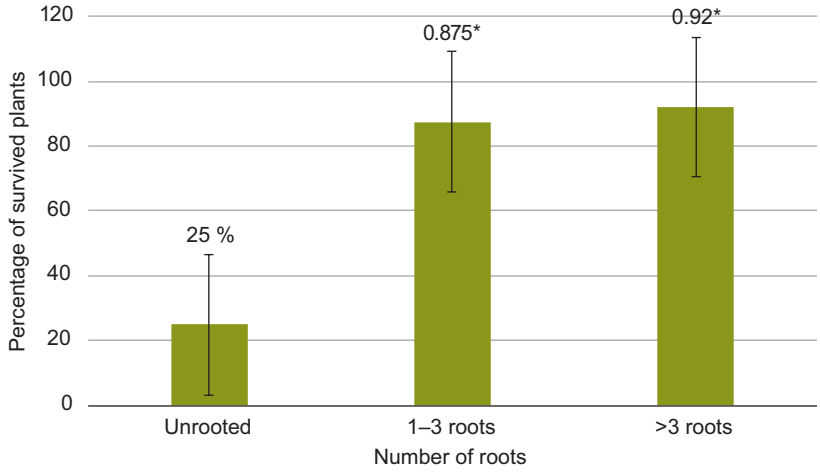

Fig. 1. Percentage of survived plants after 1 month of cultivation in the greenhouse. Presented data describes mean ± SE, * – significant difference at a = 0.05

Fig. 2. Plantlets after 1 month of cultivation in the greenhouse, where: ***A*** — plantlets without roots; ***B*** – 1–3 roots; ***C*** – > 3 roots

CONCLUSION

The present study outlines a standardized *in vitro* protocol for the mass propagation of the valuable plum rootstock 'Wavit' from stem nodal segments. DKW media supplemented with 0.5 mg/L MT, or with 0.5 mg/L MT and 0.1 IBA with FeNaEDTA, demonstrated the best overall performance in terms of shoot length, multiplication rate, and the lowest incidence of vitrified shoots. In contrast, QL media exhibited inferior performance compared to DKW. Supplementing QL media with FeEDDHA and 0.5 BAP, 0.5 MT, and 0.1 IBA resulted in the highest incidence of vitrified shoots, leading to a significant amount of discarded material.

Increasing the IBA concentration resulted in higher root numbers, root length, and percentage of rooted plants, thereby enhancing overall root development. However, this increase in IBA concentration corresponded with a decreased shoot length, potentially complicating subsequent acclimatization. Nonetheless, further studies on *in vitro* proliferation, rooting, and acclimatization of plum rootstock 'Wavit', encompassing a broader range of media compositions and growth protocols, are recommended to enhance the efficiency of production of this valuable rootstock.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [S.B.]; methodology [S.B.]; formal analysis [S.B.; M.K.]; investigation [S.B.; M.K.]; resources [S.B.; M.K.]; data curation [S.B.; M.K.] writing – original draft preparation [S.B.]; writing review and editing [S.B.; M.K.]; visualization [S.B.; M.K.]; supervision [M.K.]; project administration [S.B.; M.K.]; funding acquisition [-].

All authors have read and agreed to the published version of the manuscript.

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КЛОНАЛЬНЕ РОЗМНОЖЕННЯ ПІДЩЕПИ СЛИВИ (*PRUNUS DOMESTICA*) СОРТУ 'WAVIT'

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Вступ. 'Wavit' – цінний гібрид сливи-підщепи *Prunus domestica* (*P. cerasifera* × *P. spinosa*). Сповільнює ріст у висоту, виявляє високу морозостійкість, збільшує розмір плодів, сумісний з усіма видами сливи й абрикосів, стабільно дає високі врожаї. Мета цього дослідження – запропонувати відповідний протокол розмноження *in vitro* для підщепи 'Wavit'. Це включає клональне розмноження на основі аналізу двох базових середовищ для клонального розмноження: Driver Kyniyaki Walnut (DKW) і Quorin & Lepoivre (QL) з різними комбінаціями регуляторів росту

рослин і двох форм хелату заліза. Крім того, у дослідженні розглянуто протокол укорінення *in vitro* з різними концентраціями індол-3-масляної кислоти (ІМК) та період адаптації *ex vitro*.

Матеріали та методи. Дослідження проводили методом культивування в умовах *in vitro* експлантів 'Wavit' з такими етапами. Проліферацію пагонів досліджували за допомогою двох різних типів живильних середовищ DKW і QL, доповнених вітамінами за Walkey та різним складом ІМК, мета-тополіну (МТ), 6-бензиламінопурину (6-БАП) та двох форм хелату заліза: Ферум-натрієва сіль етилендіаміну тетраоцтової кислоти (FeNaEDTA) і Ферум етилендіамін 2-дигідроксифенілацетату (FeEDDHA), після 4 тижнів культивування визначали довжину пагонів, кількість пагонів, % вітрифікації та коефіцієнт мікророзмноження.

Середовище для укорінення складалося з ½ Mourashige & Skoog (MS), вітамінами за Walkey та різними концентраціями ІМК, через 4 тижні культивування спостерігали довжину пагона, довжину кореня, кількість коренів і % укорінених вузлових сегментів.

Акліматизацію проводили в теплиці, для досліду пагони поділяли на 3 групи: неукорінені, 1–3 корені, >3 коренів і культивували протягом місяця, після чого спостерігали виживаність саджанців.

Результати. Дослідження включали культивування експлантатів Wavit® в умовах *in vitro*, що складалося з кількох етапів. На стадії проліферації пагонів через 4 тижні культивування найвище значення зі значущою різницею довжини пагонів виявлено у варіантів DKW 0,5 МТ, 0,1 ІВА, FeNaEDTA; DKW 0,5 МТ, 0,5 ВАР, 0,1 ІВА, FeNaEDTA; DKW 0,5 МТ, 0,5 ВАР, 0,1 ІВА, FeEDDHA; однак найбільшу кількість вітрифікованих пагонів спостерігали у двох останніх перелічених варіантах. Суттєву різницю виявлено також у коефіцієнті розмноження у варіанті DKW 0,5 МТ, 0,5 ВАР, 0,1 ІВА, FeEDDHA, який був найнижчим серед усіх середніх варіантів на середовищі DKW.

Згодом отримані дані на етапі укорінення показали залежність утворення коренів від збільшення ІМК у живильних середовищах. Додавання 1,0, 1,25 та 1,5 мг/л значно підвищувало відсоток укорінених пагонів, кількість коренів і довжину коренів, однак 1,5 мг/л знижувало довжину пагонів експлантатів.

Через 1 місяць акліматизації вижило тільки 25 % саджанців у групі без коренів. У групах з 1–3 коренями та більше 3 коренів виживаність становила 87,5 % і 92 % відповідно.

Висновки. У цьому дослідженні описується стандартний протокол *in vitro* для клонального розмноження підщепи сливи сорту 'Wavit' зі сегментів пагонів. Середовище DKW з 0,5 мг/л МТ або 0,5 мг/л МТ та 0,1 мг/л ІМК з FeNaEDTA продемонструвало загалом кращу ефективність під час стадії клонального розмноження пагонів. Для стадії вкорінення ½ MS з ІМК в концентраціях 1,0 та 1,25 мг/л продемонстрували кращі результати. Крім того, визначено важливість отримання вкоріненого рослинного матеріалу до етапу акліматизації, що значно підвищує приживлюваність рослин у подальшому.

Ключові слова: *Prunus* sp., мікророзмноження *in vitro*, регулятори росту рослин, біостимулятори, поживне середовище, адаптація

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