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Propagation of Clematis 'Warszawska Nike' in In Vitro Cultures

Danuta Kulpa * and Marcelina Krupa-Małkiewicz

Department of Plant Genetics, Breeding and Biotechnology, West Pomeranian University of Technology Szczecin, 71-434 Szczecin, Poland; mkrupa@zut.edu.pl
* Correspondence: danuta.kulpa@zut.edu.pl

Abstract: A micropropagation protocol for growing Clematis 'Warszawska Nike' was developed. The MS medium supplemented with 1 mg·dm^{-3} BAP showed good results in the case of microshoot initiation (80%). The addition of BAP to the medium at higher concentrations resulted in the formation of a large amount of callus tissue at the base of the explant. Of the explants growing on the medium with the lowest cytokinin concentration, 8% flowered. Very quickly, after just 14 days, the explants began to die: some of the leaves that developed in in vitro cultures began to turn yellow and wither. The propagation of shoots was performed in two steps. In the first step, cytokinin BAP and Kin in various concentrations (0.5–2 mg·dm^{-3}) were added to the MS medium. In the second step, MS medium with the combinations of BAP (0.5 and 1 mg·dm^{-3}) with IAA or GA_3 (1 and 2 mg·dm^{-3}) was used. The MS medium with 0.5 mg·dm^{-3} BAP and 2 mg·dm^{-3} GA_3 was the best medium for the multiplication stage of clematis. Plants growing on this medium had the largest number of leaves, shoots, and internodes, and were also heavier compared to plants propagated on other media. The proliferated clematis explants were rooted on MS medium with the addition of IAA or IBA in different concentrations (0.5 to 4 mg·dm^{-3}). Of the concentrations tested, 0.5 mg·dm^{-3} IAA was the most effective one for in vitro root induction. The highest percentage of acclimatized plants (75%) was observed when the shoots were rooted on MS medium with 0.5 mg·dm^{-3} IAA.

Keywords: climbers; auxins; cytokinin; gibberellins; in vitro; rooting

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1. Introduction

One of the most well-known and widely distributed genera of climbers is the clematis (*Clematis* sp.). It has many representatives found in temperate climatic zones. *Clematis* belongs to the buttercup family (*Ranunculaceae* Juss.), and is a medicinally important genus with over 200 species and 400 varieties and hybrids [1,2]. Plants are a rich source of chemical compounds with a broad spectrum of applications in pharmacology, such as antibacterial, anti-inflammatory, anti-cancer, analgesic, and diuretic [3]. Due to their aesthetic value, many cultivars are grown as ornamental plants. However, the shelf life of cut clematis flowers is short. Therefore, in Europe, they are primarily planted outdoors, in contrast to the United States where clematis is cultivated for cut flowers [4].

The Polish breeder of clematis was a Jesuit Father Stefan Franczak (1917–2009), who named over sixty varieties. Several of its varieties, such as 'Blue Angel', 'Polish Spirit', and 'Warszawska Nike' have received the Award of Garden Merit, which is the highest distinction awarded by the Royal Horticulture Society in Great Britain for the best garden plants [5]. One of them, 'Warszawska Nike' (also known as Warsaw Nike), is a resistant, undemanding, very profusely flowering variety. It has velvety, dark purple-violet flowers with a diameter of 12–14 cm. Its values are emphasized by the light background of the wall, leaves, and flowers of other plants. It is recommended for growing in large containers on balconies and terraces. It reaches 2–3 m [6].

The traditional method is vegetative propagation, by cuttings, in the early summer, autumn, or winter. This method is more commonly employed than generative propagation due to its efficiency and the production of progeny plants that are genetically identical

to the mother plant. Another notable propagation method is grafting, particularly when *Clematis vitalba* or *C. viticella* are utilized as rootstocks, as this leads to rapid plant growth. Grafting is seldom employed for perennial clematis. Although the method of propagation by grafting is occasionally used on an amateur scale [7], conventional propagation methods may not always be efficient for obtaining sufficient plant quantities as raw materials for the food and pharmaceutical industries. Moreover, the vegetative propagation of some species and their cultivars can be challenging, with cuttings proving difficult to root. This difficulty has prompted the development and commercial use of in vitro methods [1]. The essential objective is to develop and optimize a rapid in vitro culture production technology for generating plants with high economic and medicinal value, such as *Clematis*, all year round and in the desired quantities. In addition, the in vitro technique makes it possible to obtain plant material free of viruses, bacteria, and fungi, which allows for obtaining healthy plant material. This is particularly crucial for early large-flowered varieties [8].

The regenerative capacity depends on the genotype, nutrient solution composition, plant growth regulators (PGRs), and other organic substances [9]. Several studies have been conducted on *Clematis* micropropagation, e.g., Parzymies and Dąbski [10] describe the effect of cytokinin on the in vitro multiplication of *C. viticella* (L.) and *C. integrifolia* 'Petit Faucon'. Chavan et al. [1] achieved a high frequency of propagation of *C. heynei* through nodal bud segments. Izadi Sadeghabadi et al. [11] describe the effects of plant growth regulators on the rooting and growth of the *Clematis orientalis* L. in in vitro culture. Mitrofanova et al. [2] developed a propagation protocol for 13 cultivars of *Clematis* taken in collection plots of the Nikita Botanical Gardens using somatic embryogenesis and in vitro organogenesis. Although in vitro culture methods and conditions are similar for different *Clematis* genotypes, their requirements for growth regulators in culture media are different. A review of the literature showed that an in vitro regeneration protocol for this medicinal climber has not yet been standardized. Therefore, it is important to develop a highly efficient plant regeneration system for each genotype.

This research aims to develop a micropropagation method for *Clematis* 'Warszawska Nike'. The optimal content of plant growth regulators in the media at the initiation, multiplication, and rooting stages will be determined. The influence of auxin content in the media at the rooting stage on the adaptation of clematis to greenhouse conditions will also be determined.

2. Materials and Methods

2.1. Preparation of Plant Material

The materials used in this study were one-year-old *Clematis* 'Warszawska Nike' plants. This cultivar belongs to the *Viticella* section. To establish in vitro cultures, 2–3 cm stem fragments were used, from which the leaves were removed. The stem fragments were rinsed for 15 min under running water and then immersed for 30 s in 70% ethanol. The pre-decorated fragments were immersed in a 7% sodium hypochlorite (NaOCl) solution for 20 min. The next steps were carried out under sterile conditions in a laminar flow cabinet. The NaOCl was discarded and the shoots were rinsed three times with sterile distilled water. The disinfected shoot fragments were dried on sterile tissue paper. Then, the isolated fragments were placed individually in 15 mL test tubes containing 5 mL of mineral medium, according to Murashige and Skoog [12], with the addition of the 6-benzylaminopurine (BAP) (Duchefa Biochemie, Haarlem, The Netherlands) in a concentration from 1 to 3 mg·dm^{-3}.

A total of 50 explants were placed on each type of medium in three repetitions. After 21 days, the number of growth-initiating, dying, and infected cultures was assessed.

2.2. Multiplication and Rooting Stage

Shoot fragments with a length of 2 cm were used to induce organogenesis. The explants were placed on MS medium with the addition of plant growth regulators in different concentrations: 0.5 to 2 mg·dm^{-3} BAP, and 0.5 mg·dm^{-3} to 2 mg·dm^{-3} kinetin (Kin) (Duchefa Biochemie, Haarlem, The Netherlands). In the second experiment, combinations

of MS medium with the addition of 0.5 or 1 mg·dm^{-3} BAP, used simultaneously with 1 or 2 mg·dm^{-3} IAA (indole-3-acetic acid) (Duchefa Biochemie, Haarlem, The Netherlands) or GA_3 (gibberellic acid A_3) (Duchefa Biochemie, Haarlem, The Netherlands) were used. As the control, the MS medium without plant growth regulators was used. After 6 weeks, the morphological characteristics were measured.

To induce in vitro rooting, the multiplied shoots were placed on a rooting MS medium with the addition of auxins, IAA or IBA (indole-3-butric-acid) (Duchefa Biochemie, Haarlem, The Netherlands), in concentrations from 0.5 to 4 mg·dm^{-3}. The MS medium without the addition of plant hormones was the control. After 6 weeks, the morphological characteristics were measured.

2.3. Culture Conditions

The experiment on the multiplication and rooting stage was carried out in 10 replicates of 6 explants in a 200 mL jar filled with 30 mL of the respective medium. At each stage, the MS medium was supplemented with 30 g·dm^{-3} sucrose, 0,1 g·dm^{-3} myo-inositol (Duchefa Biochemie), and 8 g·dm^{-3} agar. The pH of the medium was set at 5.7, using 0.1 M solutions of HCl and NaOH. The medium was sterilized in an autoclave at 121 °C and 1 MPa for 20 min. The cultures were placed in a phytotron at a temperature of 24 °C and relative humidity of 70–80%, under a 16 h photoperiod with a photosynthetic photon flux density (PPFD) of 40 µmol·m^{-2}·s^{-1} PAR (photosynthetically active radiation).

2.4. Adaptation to Ex Vivo Conditions

The rooting shoots were transferred to multiple pots filled with an organic-mineral substrate based on peat and perlite in a ratio of 3:1 at pH 6, and a polymer hydrogel additive of 2.5 g·dm^{-3} containing nitrogen (N) 0.64% (amide form 0.46%, nitrate form 0.18%) and potassium oxide (K_2O) 0.43% soluble in water.

After 2 weeks, mineral fertilization was applied with a liquid organic-mineral fertilizer with the following composition: total nitrogen (N) 1.00%, total phosphorus (P_2O_5) 0.50%, and total potassium (K_2O) 1.74%. Fertilization was applied at a rate of 10 mL·dm^{-3}. At the same time, Thiram Granuflo 80 WG protection products were applied at a rate of 0.6 g·dm^{-3} against grey mold.

After 4 weeks, the plantlets were transferred to the greenhouse, where they were transplanted into pots filled with a mixture of peat, sand, shredded pine bark, and compost in a ratio of 2:1:1:1 (pH of 6.0). ENTEC fertilizer (25 g·dm^{-3}) was added to the substrate with the following composition: total nitrogen (N) 14%, including nitrate nitrogen 5.5% and ammonium nitrogen 8.5%, total phosphorus (P_2O_5) 7%, total potassium (K_2O) 17%, magnesium (MgO) 2%, sulfur (S) 10%, and micronutrients such as boron (B) 0.02% and zinc (Zn) 0.01%.

From each rooting medium, 20 explants were planted in three repetitions. The survival rate (%) was evaluated 2 months after the beginning of the acclimatization.

2.5. Statistical Analysis

All statistical analyses were performed using Statistica 13.0 (StatSoft, Cracow, Poland). The statistical significance of the differences between means was determined by testing the homogeneity of variance and normality of distribution, followed by ANOVA with Tukey's post hoc test. The significance was set at $p < 0.05$. When effects were expressed as percentages, data were arcsin-square-root-transformed before analysis.

3. Results and Discussion

One of the factors influencing the initiation of explants under in vitro conditions is the type and concentration of plant growth regulators in the culture medium. Low concentrations of growth regulators are essential for the induction and regulation of key physiological and morphogenetic factors. For each species and cultivar, the optimal concentrations and combinations of plant hormones in the medium should be selected individually. According

to several authors [2,3,13], the preferred medium for *Clematis* initiation is based on the mineral composition of Murashige and Skoog [12]. Raja Naika and Krishna [3] inoculated the stem explants on MS with the addition 2,4-D (2,4-dichlorophenoxyacetic acid) with FAP (6-furfurylamino purine). Still, the organogenic response was noticed only when the MS medium was augmented with 3 to 5 mg·dm^{-3} FAP. In contrast, Parzymies and Dąbski [10] concluded that KIN at a concentration of 10 mg·dm^{-3} or 5 mg·dm^{-3} 2iP (isopentenyl adenine) was the best for micropropagation of *Clematis viticella* shoot tips. In the present study, BAP was used as the main cytokinin at the initiation stage of clematis in vitro. Single microshoot formations were observed in the first week of culturing, on all media types. The largest number (80%) of explants initiated growth on MS medium supplemented with 1 mg·dm^{-3} BAP. The least (36%) of the plants initiated growth on MS medium with the addition of 3 mg·dm^{-3} BAP (Figure 1a,b). In explants placed on medium with the addition of 0.5 and 2 mg·dm^{-3} BAP, growth was initiated by 42 and 50%, respectively. Of the explants growing on the medium with the lowest cytokinin concentration, 8% flowered. Very quickly, after just 14 days, the explants began to die: some of the leaves that developed in in vitro cultures began to turn yellow and wither.

At the multiplication stage of clematis 'Warszawska Nike', the experiment was divided into two steps. In the first step, cytokinin BAP and Kin (Kinetin) in various concentrations (0.5–2 mg·dm^{-3}) were added to the MS medium (Table 1, Figure 1c,d).

Table 1. The influence of the cytokinins BAP and Kin in MS medium on the morphological traits of clematis 'Warszawska Nike'.

Morphological Traits	Control		Cytokinins					
			Kin (mg·dm^{-3})			BAP (mg·dm^{-3})		
			0.5	1	2	0.5	1	2
Number of leaves	4.75	abc *	3.83 bc	3.75 bc	2.75 c	7.75 a	6.50 ab	3.16 c
Shoot length (cm)	2.51	a	1.83 ab	1.29 b	1.54 ab	1.83 ab	2.16 ab	1.62 ab
Number of nodals	2.00	ab	1.41 abc	1.08 bc	1.08 bc	1.66 abc	2.25 a	0.83 c
Number of shoots	1.33	d	1.47 d	1.68 cd	2.02 bc	2.45 ab	2.88 a	2.03 bc
Fresh weight (g)	0.10	b	0.03 b	0.04 b	0.07 b	0.18 b	0.24 b	0.59 a

* Means followed by different letters in columns are significantly different at the 5% level according to Tukey's multiple ranges.

The addition of kinetin to MS medium regardless of the concentration used, had an inhibitory effect on the growth of plants (Table 1). The addition of BAP to the medium, especially at higher concentrations, resulted in the formation of a large amount of callus tissue at the base of the explant (Figure 1d). As the results of our research showed, plants growing on the medium with the addition of 1 mg·dm^{-3} BAP had the largest number of leaves, shoots, and internodes and were also heavier compared to plants propagated on other media. Only the length of the shoots was lower than in the case of plants propagated on a medium without plant growth regulators (Table 1). Shoots developed by explants growing on the medium with the highest BAP concentration (2mg·dm^{-3}) were distorted and vitrified.

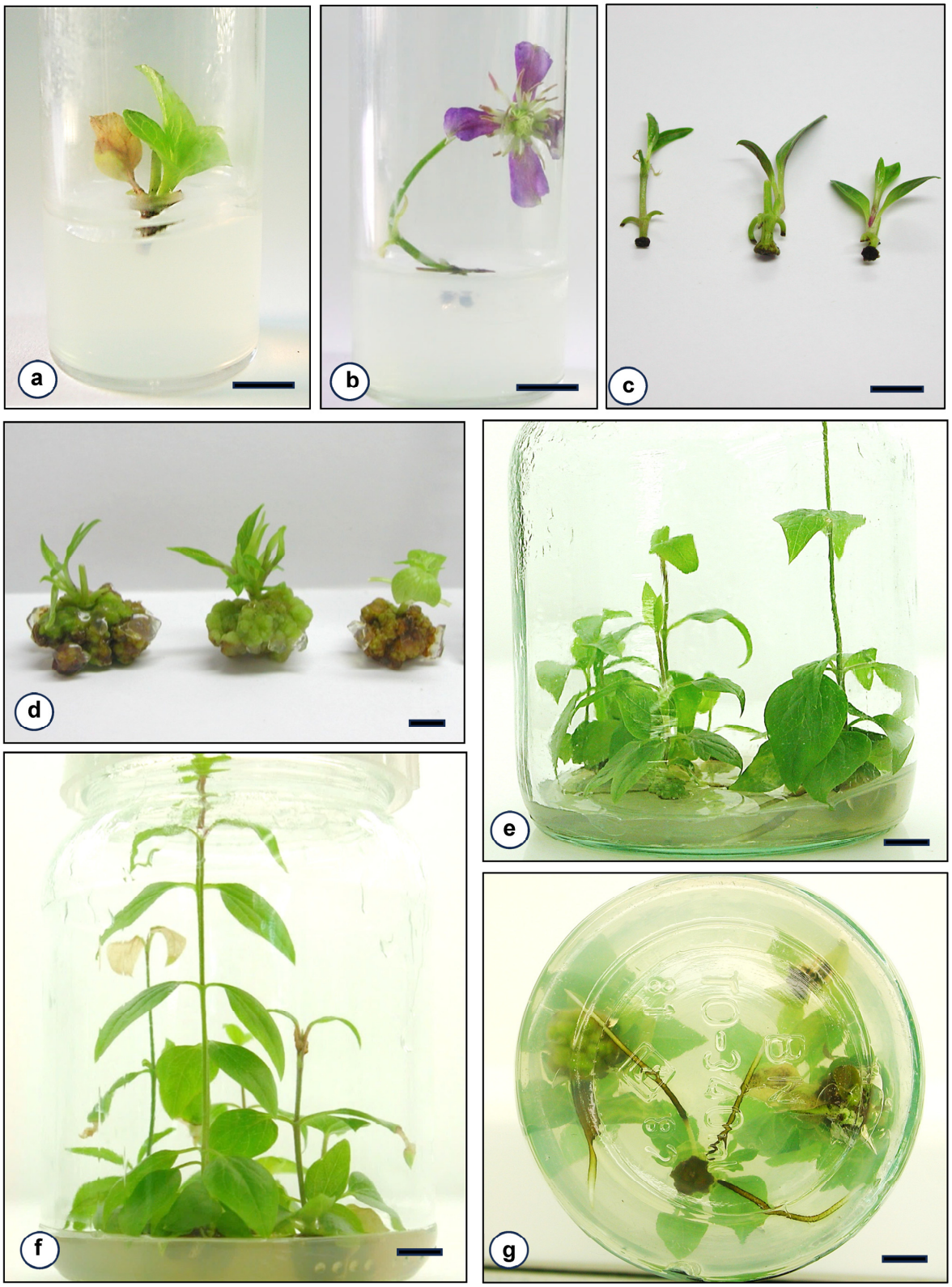

Figure 1. Micropropagation of *Clematis* 'Warszawska Nike': (**a**) initiation of explant on the medium supplemented with 3 mg dm^{-3} BAP and (**b**) 0.5 mg dm^{-3} BAP; (**c**) explants propagated for 6 weeks on control medium (**d**) and medium with the addition of 3 mg·dm^{-3} BAP with a large amount of callus tissue; (**e**) explants propagated on MS medium with the addition of 0.5 mg·dm^{-3} BAP with IAA (0.5 mg·dm^{-3}) (**f**) and with GA_3 (2 mg·dm^{-3}); (**g**) roots of plants on rooting medium (0.5 mg·dm^{-3} IBA). Scale bar = 1 cm.

The effect of cytokinin on *Clematis* multiplication was also described by Kreen et al. [7]. They compared five cultivars of clematis belonging to the section *Atragene*. Based on their results, they concluded that the optimal medium for the propagation of this species was MS with the addition of 1 mg·dm^{-3} BAP. The efficacy of BAP and Kin during shoot induction and proliferation has been reported for *C. heynei* by Chaval et al. [1]. It was observed that the frequency of axillary shoot proliferation and the number of shoots per explant increased with increasing concentrations of BAP (4 mg·dm^{-3}) and Kin (3 mg·dm^{-3}) in the MS medium. The opposite results of our study were obtained by Raja Naika and Krishna [3]. They used MS medium supplemented with a 6-furfuryl amino purine (FAP) and BAP for the propagation of *Clematis gouriana* Roxb. Of the concentrations of FAP and BAP used, adventitious shoot organogenesis was observed only on the medium supplemented with FAP, whereas the use of low concentrations of BAP (0.5–1.5 mg·dm^{-3}) resulted in the development of callus tissue. Also, Khanbabaeva et al. [14] observed that BAP in a concentration of 0.3 mg·dm^{-3} and 0.4 mg·dm^{-3} for the propagation of 'Terry' varieties of *Clematis* plants allows for obtaining a high multiplication factor.

Several authors [1–3,13] recommend the addition of cytokinin in combination with gibberellin or lower auxin to the medium to induce organogenesis in *Clematis* plants. According to Gabryszewska [15], gibberellins and cytokinin play a crucial role in the activation of axillary buds (control of apical dominance) in lilacs (*Syringa vulgaris)*. Gibberellins belong to a group of plant growth regulators with effects on the control of cell division and elongation, as well as on the control of apical dominance (para-dormancy). On the other hand, the use of higher auxin concentrations in the multiplication stage may potentially inhibit cytokinin, affecting shoot shortening.

During the multiplication stage on media with the addition of KIN and BAP, despite the growth of the shoots, we did not obtain satisfactory results due to their poor quality: a large amount of callus tissue and signs of vitrification were observed. In the second step of the multiplication stage, BAP in combination with IAA or GA_3 was added to the MS medium, in different concentrations (Table 2). It was observed that the number of leaves per plant increased with rising concentrations of BAP and IAA in the medium and decreased with increasing concentrations of BAP and GA_3 (Table 2). *Clematis* on MS medium supplemented with 1 mg·dm^{-3} BAP and 2 mg·dm^{-3} IAA, as well as on MS with 0.5 mg·dm^{-3} BAP + 2 mg·dm^{-3} GA_3 developed the largest number of leaves (114% more leaves compared to the control (4.75)) and shoots (3.21). On the other hand, elevated concentrations of cytokinin in combination with auxin or cytokinin with gibberellin in the MS medium exerted an inhibitory effect on the shoot length of *Clematis* explants. The shoot lengths were lower than the control (2.54 cm), from 33% to 42% in the MS medium with BAP + IAA, and from 49% to 12% in the MS medium with BAP + GA_3. Additionally, it was observed that a higher concentration of IAA or GA_3 relative to cytokinin BAP stimulated internode formation. The explants on MS medium supplemented with 0.5 mg·dm^{-3} BAP and 2 mg·dm^{-3} GA_3 developed the most internodes (2.83) and shoots (3.67). However, for both the shoot length and number of internodes, these differences were not statistically significant. The number of nodes is a commercially significant parameter that significantly influences the profitability of the conducted production. Only in two scientific reports published so far has the impact of adding plant growth regulators on its formation been determined for the *Clematis* species. Parzymięs and Dąbski [10] found that *Clematis viticella* (L.) propagated on MS medium had 2.2 nodes, while when cytokinins (TDZ, BAP, 2iP, and Kin) were added to it, the number ranged from 2.3 to 3.4. Mitrofanova et al. [2] investigated the influence of BAP (0.5 to 2 mg·dm^{-3}) and TDZ (at concentrations of 0.66 to 1.98 mg·dm^{-3}) on the propagation of 13 *Clematis* varieties, including the number of nodes. A statistical analysis was not conducted between media within the same variety; however, the differences in the number of nodes between the lowest and highest cytokinin concentrations were at most twofold. In our recent studies, we obtained similar results: the number of nodes for plants propagated on MS medium was 2.2, while on the medium supplemented with BAP and IAA or GA_3, it was similar and ranged from 1.66 to 2.83.

Table 2. The influence of the combinations of BAP with IAA or GA_3 in the MS medium on the morphological traits of clematis 'Warszawska Nike'.

Morphological Traits	Control	BAP + IAA (mg·dm^{-3})				BAP + GA_3 (mg·dm^{-3})			
		0.5 + 1	0.5 + 2	1 + 1	1 + 2	0.5 + 1	0.5 + 2	1 + 1	1 + 2
Number of leaves	4.75 ab *	4.75 ab	6.91 ab	6.83 ab	10.16 a	4.37 ab	10.16 a	6.29 ab	7.20 ab
Shoot length (cm)	2.54 a	1.70 a	1.66 a	1.66 a	1.47 a	1.48 a	2.23 a	1.37 a	1.29 a
Number of nodals	2.00 a	2.16 a	2.08 a	1.66 a	2.25 a	2.16 a	2.83 a	2.12 a	2.08 a
Number of shoots	1.33 e	1.67 de	1.89 d	2.00 d	1.89 d	3.21 bc	3.67 a	3.46 ab	3.01 c
Fresh weight (g)	0.10 b–d	0.10 b–d	0.48 a	0.40 ab	0.22 b–d	0.38 abc	0.31 a–d	0.15 b–d	0.18 a–d

* Means followed by different letters in columns are significantly different at the 5% level according to Tukey's multiple ranges.

It was observed that the fresh weight of the plants decreased with increasing concentrations of IAA or GA_3 in the medium (Table 2). At IAA concentrations of 1 mg·dm^{-3} and low BAP concentrations (0.5 mg·dm^{-3}), the explants achieved nearly five times higher fresh weight compared to the control (0.1 g). A synergistic effect of cytokinin in combination with auxins was reported for *Clematis heynei* M.A. Rau [1], and *Stevia rebaudiana* Bertoni [16,17]. However, Gao et al. [18] achieved an effective adventitious bud proliferation of *Clematis* 'Julka' using MS medium supplemented with 1 mg·dm^{-3} BAP + 0.2 mg·dm^{-3} IBA and 0.2 mg·dm^{-3} GA_3. Raja Naika and Krishna [3] observed that the addition of auxin IBA (indole-3-butyric acid) in a higher concentration (above 0.8 mg·dm^{-3}) and FAP above 5 mg·dm^{-3} inhibited shoot organogenesis.

During the multiplication stage, the explants of *Clematis* 'Warszawska Nike' were unable to produce roots. Therefore, the multiplied shoots of clematis were transferred on rooting media supplemented with auxin IAA or IBA at different concentrations (Table 3, Figure 1e). Among the tested concentrations, 0.5 mg·dm^{-3} was the most effective for in vitro root induction (Figure 1f). After 6 weeks of culture, *Clematis* explants developed, at the base of the end of the shoots, an average of 1.75 roots per plant, with an average length of 3.10 cm and 4.41 cm, respectively. Upon comparison, the roots developed on the MS medium supplemented with IAA were whitish, brittle, and shorter compared to IBA. However, plants on the 0.5 mg·dm^{-3} IAA medium were taller compared to plants on the 0.5 mg·dm^{-3} IBA medium (2.48 cm). No statistically significant differences were observed in fresh mass when plantlets were rooted on MS medium supplemented with IAA or IBA, regardless of their concentrations. Despite this, the highest fresh weight was noted in plantlets grown on culture media with the lowest concentrations (0.5 mg·dm^{-3}) of auxins IAA and IBA (0.3 g and 0.25 g, respectively). According to Figiel-Kroczyńska et al. [19], IBA and IAA are often used for in vitro root initiation and to increase root number and length. The process of rooting stimulated by the presence of auxins in the medium influences the elongation of root hair cells by importing auxins into non-root-forming epidermal cells. The effectiveness of IAA in rooting has been reported by Raja Naika and Krishna [3] for *Clematis gouriana* Roxb. Explants rooted well on MS without plant growth regulators as well as on MS with the addition of 0.1–0.5 mg·dm^{-3} IAA. Chavan et al. [1] noticed that the MS medium with the addition of 1 mg·dm^{-3} IBA was able to produce about 8-9 roots per *Clematis heynei* M. A. Rau. plant.

After completing the rooting stage, *Clematis* seedlings rooted on media with different IAA or IBA content were transferred directly to pots filled with an appropriate substrate. The acclimatization rate was measured after two months. The percentage of plants acclimatized to the greenhouse conditions varied from 0 (for plantlets from MS + 2 mg·dm^{-3} IBA) to 75% (for plantlets from MS + 0.5 mg·dm^{-3} IAA). Moreover, a positive correlation was observed between the in vitro rooting rate and the acclimatization rate. In the case of IBA concentrations used, plant survivability was lower with a mean of 18.75% (Table 4). The highest percentage of acclimatized plants in this group (50%) was observed when MS with the addition of 0.5 mg·dm^{-3} IBA on the rooting stage was used (Figure 2). In contrast,

on MS medium supplemented with IAA, the average percentage of acclimatized plants was 60.42%. The obtained results were also influenced by the conditions in the greenhouse during adaptation where the main problem was maintaining adequate humidity for the acclimatized plants. This issue was noted by Kreen et al. [7], who found that microcuttings require higher relative humidity (100%) than seedlings (80–90%) during acclimatization. This elevated humidity for stem cuttings increases their susceptibility to grey mold.

Table 3. In vitro rooting of clematis 'Warszawska Nike'.

Morphological Traits	Control	AUXIN (mg·dm^{-3})							
		IAA				IBA			
		0.5	1	2	4	0.5	1	2	4
Shoot length (cm)	2.54 a*	2.95 a	1.57 a	1.39 a	1.93 a	2.48 a	1.70 a	1.66 a	1.71 a
Fresh weight (g)	0.10 a	0.3 a	0.29 a	0.06 a	0.13 a	0.25 a	0.05 a	0.08 a	0.11 a
Number of roots	0.00 b	1.75 a	0.41 ab	0.16 b	0.75 ab	1.75 a	0.83 ab	0.33 ab	0.91 ab
Root length (cm)	0.00 b	3.10 ab	2.00 ab	0.18 b	3.19 ab	4.41 a	0.16 b	0.16 b	0.58 ab

* Means followed by different letters in columns are significantly different at the 5% level according to Tukey's multiple ranges.

Table 4. Percentage of plants [%] adapted to greenhouse conditions depending on the auxin content in the rooting medium.

Auxin	Concentrations (mg·dm^{-3})				Mean
	0.5	1	2	4	
IAA	75.0	58.3	50.0	58.3	60.42
IBA	50.0	16.7	0.0	8.3	18.75
Mean	62.5	37.5	25.0	33.3	39.60

Figure 2. Clematis 'Warszawska Nike' plants rooted on MS medium with the addition of 0.5 mg·dm^{-3} IAA after 4 months of growth in greenhouse conditions.

4. Conclusions

This paper presents a complete micropropagation protocol for *Clematis* 'Warszawska Nike'. The MS medium supplemented with 1 mg·dm^{-3} BAP showed good results in the case of shoot initiation (80%). The MS medium supplemented with 1 mg·dm^{-3} BAP and the MS medium with the addition from 0.5 mg·dm^{-3} BAP and 2 mg·dm^{-3} GA_3 proved to be very effective for the rapid proliferation/multiplication and growth of clematis explants. Well-rooted plants on MS medium with the addition of 0.5 mg·dm^{-3} IAA were adapted to field conditions and showed a 75% survival rate with normal morphology and growth characteristics. The developed in vitro regeneration protocol for *Clematis* 'Warszawska Nike' can help optimize the micropropagation of other plants belonging to the species under study and may be useful, for example, for the commercial propagation and ex situ conservation of this medicinal plant.

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