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MICROPROPAGATION OF BLACKBERRIES *IN VITRO* ON A CHEMICALLY STERILIZED MEDIUM USING SODIUM HYPOCHLORITE

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Abstract

Blackberry (*Rubus fruticosus* L.) is a globally important berry crop. The process of micropropagation of plants allows for the production of large quantities of blackberry seedlings, but it is crucial to reduce costs. This research explores the possibility of reducing the cost of micropropagation by replacing the sterilization of the culture medium with chemical sterilization using sodium hypochlorite. In this study, we evaluated the effectiveness of micropropagation of the blackberry variety Brzezina on culture media that had been chemically sterilized with sodium hypochlorite. The microshoots of blackberry obtained from the *In vitro* collection were assessed at different stages of multiplication and rooting, using varying concentrations of active chlorine (0.001% to 0.008%). The study also included a thorough examination of the potential phytotoxicity of the culture media.. Throughout the research, the growth and development of plants *In vitro* were monitored, including the measurement of the effective photochemical quantum yield of photosystem II. The study determined the optimal concentrations of active chlorine (0.001% to 0.002%) that effectively sterilize the medium without negatively impacting the reproduction and rooting indices compared to the control. The fresh weight of new explants and the length of shoots produced during multiplication were significantly higher than in the case of the autoclaved medium. The blackberry seedlings grown on chemically sterilized medium successfully adapted to ex vitro conditions. Therefore, the method of chemically sterilizing nutrient media can be recommended for micropropagation of blackberries to reduce costs and increase productivity.

Key words: Active chlorine; Berry crop; Effective photochemical quantum yield of photosystem II; *In vitro* plant tissue culture techniques; Reducing the cost of plant micropropagation

Introduction

Blackberry (*Rubus fruticosus* L.) is an important berry crop that is cultivated and consumed worldwide (Moyer *et al*., 2002; Milosevic *et al*., 2012; Diamanti *et al*., 2014; Zia-ul-Huq *et al*., 2014). Blackberry propagation is a relatively straightforward process that has traditionally been achieved by cuttings and other vegetative propagation techniques. However, the achievement of high propagation rates and large volumes of high-quality planting material is only possible through the use of *In vitro* micropropagation technologies (Fira *et al*., 2009; Schuchovski & Biasi, 2017; Kefayeti *et al*., 2019; Chandran *et al*., 2020; Clapa *et al*., 2023). Nevertheless, in comparison with conventional plant propagation methodologies, *In vitro* micropropagation technology remains a comparatively costly process (Cardoso *et al*., 2018).

The successful cultivation of a plant organ or tissue in a culture medium is contingent upon the absence of competitors, such as microorganisms. In the event of contamination, plant explants are susceptible to infection by fungi, including yeast, bacteria and bacteria-like organisms, as well as viruses and viroids, which can be pathogenic for plants *In vitro*. These contaminants can infect the explants themselves or act as a reservoir for inoculum, which can then infect other plants or alternative hosts, including humans, following rooting and adaptation (Cassells, 2001). In order to prevent such contamination, it is imperative that all procedures related to *In vitro* micropropagation are conducted under aseptic conditions. This ensures the sterility of the culture vessels and nutrient medium, thereby preventing the spread of pathogens. Historically, the sterilization of the nutrient medium was accomplished through the utilisation of autoclaves or tyndallization techniques (Cherevchenko *et al*., 2008). In large-scale micropropagation, however, the cost of autoclaves and high energy consumption associated with this method renders it very expensive. Moreover, the temperatures attained during standard autoclaving can result in the destruction of certain organic components of the medium. Since the 1970s, there have been several proposals for alternative methods of medium sterilization. These include microwave sterilization, filtration, and chemical sterilization (Latimer *et al*., 1977; Tisserat *et al*., 1992; Teixeira *et al*., 2005). Of these, chemical sterilization has emerged as the most promising method, having been successfully applied by numerous researchers (Teixeira *et al*., 2008; Matsumoto *et al*., 2009; Cardoso & Teixeira, 2012; Brondani *et al*., 2013; Duan *et al*., 2019). Solutions comprising chlorine and iodine-based compounds have been proposed as sterilization agents. However, comparative studies demonstrate that sodium hypochlorite solution is the most effective and cost-effective option (Lepelletier *et al*., 2020; Balla *et al*., 2022; Laksana *et al*., 2023).

Sodium hypochlorite exerts its effects upon the substrate through the process of oxidative chlorination. In an aqueous solution at a pH close to neutral, sodium hypochlorite dissociates according to the following reaction: NaOCl = Na+ + OCl-. In this instance, the concentrations of hypochlorite ions and hypochlorous acid are approximately equal. The resulting metastable system is capable of generating a number

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of compounds and particles with a strong antimicrobial effect, including: O2 - singlet molecular oxygen; ClO* - hypochlorite radical; C1* - chlorine radical (atomic chlorine); O- - atomic oxygen; OH* - hydroxyl radical. The action of these active compounds and particles disrupts the activity of enzymes that catalyse oxidation-reduction processes in bacterial cells, thereby initiating the disinfection process (Shvetsov *et al*., 2009). The antimicrobial action of sodium hypochlorite necessitates a temporal element, the duration of which is contingent on the concentration level of the sterilizer (Clifford, 1999; Achmit *et al*., 2018). To calculate the modes of chemical sterilization, it is necessary to determine the concentration of the so-called active chlorine. Active chlorine is defined as the sum of free chlorine (Cl2), hypochlorous acid (HOCl) and hypochlorite ions (OCl-) (Egorova & Bobina, 2012).

The objective of this study was to evaluate the effectiveness of chemical sterilization of the nutrient medium using sodium hypochlorite and its effect on the development of blackberry *In vitro* using the Brzezina variety as an example.

Material and Methods

The studies were conducted at the laboratories of microclonal propagation of plants of the Don State Technical University and the Botanical Garden of the Southern Federal University (Rostov-on-Don).

The efficiency of sodium hypochlorite as a chemical sterilizer of the medium was assessed by testing various sterilization modes. The objective of this study was to identify the most effective method by determining the following parameters: the initial concentration of sodium hypochlorite (% of active chlorine) in the culture medium, and the exposure time of the medium (settling) prior to the inoculation of the plants. Settling of the medium is necessary for the release and evaporation of active chlorine. All culture vessels were subjected to preliminary heat treatment in a drying cabinet at a temperature of 200°C for a duration of 2 hours. The source of sodium hypochlorite was a 5% solution of this substance under the commercial name Belizna. The preparation was then added to the Murashige-Skoog culture medium at the stage of melting the agar at a temperature of approximately 80°C. Thereafter, the medium was expeditiously transferred into 100 ml culture vessels in portions of 20 ml. These vessels were then covered with aluminium foil and left to cool completely. Three blocks of experiments were carried out. Initially, it was imperative to ascertain the minimum concentration of sodium hypochlorite required for complete sterilization of the nutrient medium. To this end, a series of concentrations of Belizna solution containing equivalent levels of active chlorine were subjected to rigorous testing. These concentrations included 0.0001%, 0.0003%, 0.0005%, 0.0007%, 0.001%, 0.002%, 0.003%, 0.004%, 0.005%, 0.006%, 0.007%, and 0.008%. The development of micro-organisms in culture vessels was monitored over a period of six weeks, with observations including turbidity of the medium and colony formation. Control 1 comprised a thermally sterilised medium that had undergone autoclaving at 121°C for a duration of 20 minutes. Control 2 comprised MS nutrient medium without autoclaving or chemical sterilization. The experiments were carried out in 25 replicates.

The second series of experiments was designed to assess the efficacy of the culture medium following chemical sterilization for the cultivation of blackberries at two main stages of *In vitro* micropropagation: propagation and rooting. Samples of the *In vitro* shoot culture of Brzezina blackberry were obtained from the collection of the SFedU Botanical Garden, where they were at the elongation stage. Initially, all shoots were cultivated on Murashige-Skoog medium (Murashige and Skoog, 1962) with the addition of 1 mg/l thiamine, 0.5 mg/l pyridoxine, 0.5 mg/l nicotinic acid, 100 mg/l inositol, 30 g/l sucrose, and without phytohormones for a period of six weeks (photo). In all stages of micropropagation, experiments were carried out on the basic MS medium of the same modification and the corresponding phytohormones. The control was constituted by an experiment with a heat-treated medium by autoclaving at 121°C for 20 min. A comparison was made with chemical sterilization variants by adding different amounts of sodium hypochlorite. In all variants, the pH of the medium was adjusted to 5.8 using a 1N NaOH solution before sterilization. At each stage of *In vitro* micropropagation, a number of concentrations of Belizna solution equivalent to active chlorine were tested: 0.001% (GN1), 0.002% (GN2), 0.003% (GN3), 0.004% (GN4), 0.005% (GN5), 0.006% (GN6), 0.007% (GN7), 0.008% (GN8) ml/l over 6 weeks of cultivation. The sterilization variants are represented by the symbols (Table 1). The experiments were conducted in 25 replicates. It was hypothesised that the plants would be cultivated in a medium devoid of active chlorine residues. To this end, for each sterilizer concentration, the time of complete "volatilization" of active chlorine was determined by chemical analysis using a test system. The explant tubes were placed in a growth room. Growth room conditions were maintained at 60% humidity, 25 ± 2 °C, 16 h photoperiod with PPFD 50 µmol m−2 s−1 using cold white fluorescent lamps (6 500 K).

Table 1. The main indicators of the growth and development of the Brzezina blackberry culture at the multiplication stage depending on the mode of chemical sterilization of the culture medium using active chlorine.

Sterilization variant code	Active chlorine concentration, %	Number of new shoots per explant	Fresh mass of explant with new shoots, g	Average length of shoots per explant, cm	Vitrification degree, %	Contamination, %	Y(II), Relative units
Control	Autoclave	16.95 ± 2.17a	0.4 ± 0.33a	1.4 ± 0.38a	3	0	0.73 ± 0.03a
GN1	0.001	18.7 ± 3.17a	0.76 ± 0.41b	1.8 ± 0.21b	5	5	0.71 ± 0.02a
GN2	0.002	17.15 ± 2.55a	0.95 ± 0.49b	2.1 ± 0.28b	0	0	0.71 ± 0.05a
GN3	0.003	16.85 ± 2.93a	0.46 ± 0.39a	1.7 ± 0.39b	0	0	0.72 ± 0.03a
GN4	0.004	14.54 ± 1.94a	0.51 ± 0.43a	0.55 ± 0.41a	0	0	0.69 ± 0.05a
GN5	0.005	15.48 ± 3.05a	0.65 ± 0.59a	1.5 ± 0.19a	0	0	0.71 ± 0.02a
GN6	0.006	14.82 ± 3.27a	0.65 ± 0.94a	1.52 ± 0.28a	0	0	0.69 ± 0.03a
GN7	0.007	10.26 ± 2.11b	0.41 ± 0.36c	1.47 ± 0.33c	0	0	0.48 ± 0.08b
GN8	0.008	5.3 ± 1.95c	0.39 ± 0.33c	1.1 ± 0.19d	0	0	0.49 ± 0.05b

*Means followed by the same letter in the column are not statistically different in Tukey's test at 5% level

Multiplication stage: In order to induce axillary and adventitious shoots, 0.5 mg/l of benzylaminopurine (BAP) was added to the Murashige and Skoog (MS) medium in accordance with the protocol for micropropagation of blackberries proposed by Clapa *et al*., (2011). Explants in the form of microshoots with two nodes were inoculated onto the culture medium with five shoots per vessel. Following six weeks of cultivation, the number of new axillary or adventitious shoots per explant (multiplication coefficient), the fresh weight of the explant with the formed new shoots (weight of fresh shoots/explant), and the average length of all new shoots formed from one explant (average length of shoots per explant) were counted. The degree of vitrification was then visually assessed through the percentage of vitrified shoots from the total number. Finally, cases of detection of contamination in vessels associated with contamination of explants or unsuccessful work in a laminar flow hood were taken into account.

Rooting and *Ex vitro* acclimatization: The shoots obtained at the multiplication stage were transferred to a rooting medium based on MS with the addition of 1 mg/l IBA. Five explants were inoculated per vessel. Following a period of four weeks, a comprehensive assessment was conducted to determine the percentage of rooted shoots, the number of formed roots per shoot, the average length of roots per shoot, and the functional state of the plants.

The adaptation of shoots with roots was then carried out on a mixture of peat and perlite (5:1) under *Ex vitro* conditions using microgreenhouses. Seedlings cultivated on chemically sterilized media were compared with seedlings cultivated on autoclaved media. At this stage of the experiment, only seedlings obtained under the identified optimal chemical sterilization modes were tested: GN1, GN2, GN3. Following a period of six weeks during which the plants were acclimatised, an assessment was made of the percentage of plants that had undergone acclimatization and their functional state.

Active chlorine analysis: The concentration of residual active chlorine in the culture medium was measured 1 hour after the agar had completely solidified. This measurement was then taken every 12 hours for a period of 6 days. The culture medium was maintained in culture vessels at a temperature of 25°C. A volume of 1 ml of distilled water at a temperature of 25°C was added to 20 ml of the solidified medium, and the mixture was then agitated for 10 seconds at 2000 rpm using a Luyan FSH-2A homogenizer (China) until the mixture was homogeneous. The concentration of active chlorine was measured using the method described in GOST 18190-72 (2009).

The third series of experiments was aimed at identifying the phytotoxicity of the nutrient medium with residual active chlorine. It should be noted that only media with the optimal concentration of active chlorine were included in the testing process. In this series of experiments, blackberry microshoots with several nodes for elongation were inoculated onto this culture medium. The MS medium, which is of the same composition, was utilised in this instance, albeit without the presence of phytohormones. The medium was allowed to settle at different intervals, with active chlorine being gradually evaporated. Consequently, media with varying current concentrations of active chlorine (0.0005%, 0.001%, 0.002%) were examined. Visual morphological deviations and the functional state of the plants were noted.

The determination of the functional state of plants *In vitro* and *Ex vitro*: The functional state of plants *In vitro* and *Ex vitro* conditions was diagnosed by assessing the effective photochemical quantum yield of photosystem II - Y(II) (Kalaji *et al*., 2014; Smolikova *et al*., 2015). The measurement was carried out using a DIVING PAM II fluorometer (Germany) in five replicates.

Statistical analysis

All data obtained during this study were statistically analysed using Excel 2016 and an online software resource. Website www.socscistatistics.com [accessed 20 December 2024]. Each experiment was performed in triplicate as an independent experiment. 25 explants or shoots were used for each treatment. Normality was tested using the Shapiro-Wilk test. For multiple group comparisons, an ANOVA test was performed and significant differences between means were calculated using Tukey's HSD test.

Results

Efficiency test for chemical sterilization: When testing the media with different initial concentrations of sodium hypochlorite compared to the control, the following was observed. The heat-treated medium showed no signs of contamination throughout the observation period. On the medium without chemical and heat treatment, the appearance of microbial colonies was observed on the 11th day of observation (Fig. 3a). The active chlorine concentration of 0.0005% ensured sterilization in only 60% of the cases. The medium with 0.001% active chlorine and higher remained without signs of contamination throughout the observation period (Fig. 1).

Tests for residual active chlorine in media containing different concentrations of sodium hypochlorite have shown that active chlorine is no longer detectable in the GN1 medium after 80 hours of settling, in the GN2 medium after 120 hours, and so on. At higher concentrations, active chlorine can be detected even after one week (Fig. 2).

An evaluation of the influence of the modes of chemical sterilisation on the cultivation of blackberries *In vitro* has shown the following results.

Multiplication stage: At the multiplication stage, critical and optimal modes of chemical sterilisation for blackberry propagation were identified (Table 1). It was found that active chlorine affected the propagation coefficient only in variants GN7 and GN8. In these cases, the propagation coefficient values were significantly lower (10.26 and 5.3, respectively) than the control (16.95) at $p<0.01$. Other concentrations of active chlorine did not significantly affect the propagation coefficient values compared to the control ($p>0.05$). The coefficient varied between 14.54 and 18.7. The fresh mass of the explant with new shoots was higher on GN2 (0.76 g), GN3 (0.95 g) media compared to the control (0.4 g) at $p<0.05$ (Fig 3d, e, f). On GN3-GN6 media, shoot mass was not different from the control ($p>0.05$). On GN7 (0.41 g), GN8 (0.39 g) media, shoot weight showed lower results compared to the control ($p<0.05$). Average shoot length per explant was higher than the control (1.4 cm) on GN1 (1.8 cm), GN2 (2.1 cm), GN3 (1.7

cm) media at $p<0.05$. On GN4-GN6 media, this indicator did not differ significantly from the control, while on GN7 (1.47 cm), GN8 (1.1 cm) media it decreased compared to the control ($p<0.05$). Several cases of shoot vitrification were observed in all variants of this experiment. On GN2 medium, vitrification was observed at a level of 5%, while in the control it was 3%. No vitrified shoots were found in the other variants. Signs of contamination were observed in 5% of the culture vessels on GN1 medium, while this phenomenon was not observed on the other variants. Measurements of the effective photochemical quantum yield of photosystem II (YII) showed that the functional state of plants on GN1-GN6 media (from 0.69 to 0.72) was not significantly different from the control (0.73) at $p>0.05$. On GN7 (0.48) and GN8 (0.49) media, this indicator decreased by a statistically significant difference ($p<0.01$) compared to the control.

Rooting stage: The shoots obtained at the multiplication stage, which did not differ in their parameters from the control, were transferred to the medium for rooting. On GN1-GN6 media, the percentage of rooting was not significantly different from the control ($p>0.05$) and varied from 92.8% to 94.1% (Table 2). As the concentration of active chlorine increased during sterilisation, the rooting percentage decreased. On GN7 (83.7%) and GN8 (65.2%) media, this parameter was significantly lower than the control (94.1%). The average root length did not show significant differences from the control (4.57 cm) on GN1-GN4 media (from 4.32 to 5.12 cm), whereas on GN5-GN8 media the roots developed more weakly (from 2.55 to 3.22 cm). The average number of roots per explant on GN1-GN6 media (4.81-5.81) was not statistically different from the control (5.23), and on GN7, GN8 media (2.71-2.74) this indicator was significantly lower than the control (at $p<0.01$). A relatively low percentage of contamination was observed in all experimental variants. Only GN1 and GN4 media showed a small percentage of jars with microorganism colonies. The YII indicator, which reflects the functional state of blackberries *In vitro*, on GN1-GN6 media (0.69-0.73) was not statistically significantly different from the control (0.71). On GN7, GN8 media this indicator decreased to 0.59 ($p<0.01$).

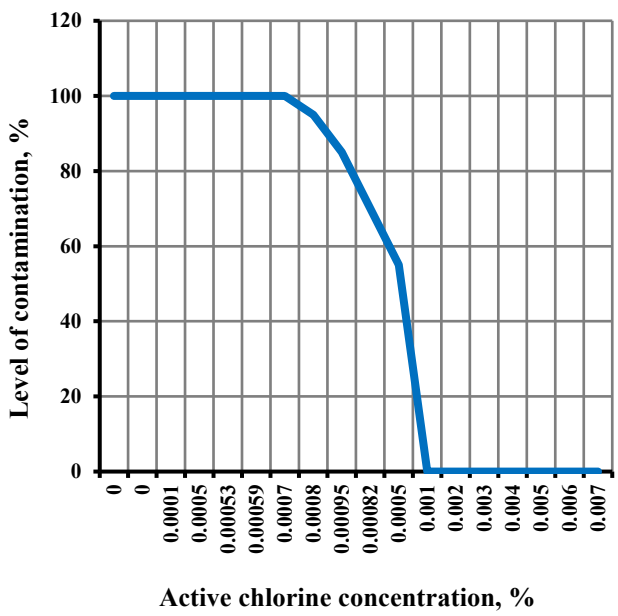

Fig. 1. The influence of active chlorine on the level of contamination of the culture medium.

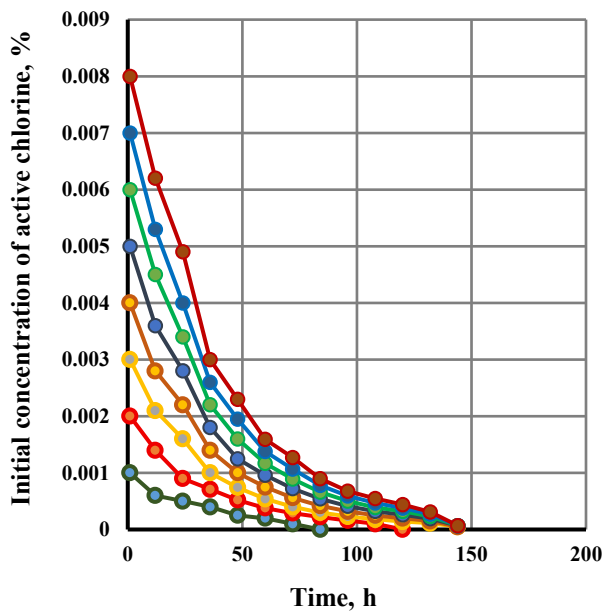

Fig. 2. Dynamics of reduction of different concentrations of active chlorine over time.

Table 2. The main indicators of the growth and development of the Brzezina blackberry culture at the rooting stage depending on the mode of chemical sterilization of the culture medium using active chlorine.

Sterilization variant code	Rooting ratio, %	Root length, cm	Root number average, pcs	Contamination, %	Y(II), Relative units
Control	94.1 ± 3.25a	4.57 ± 2.15a	5.23 ± 3.21a	0	0.71 ± 0.01
GN1	93.7 ± 2.35a	4.32 ± 1.89a	5.81 ± 2.94a	3.5	0.73 ± 0.02
GN2	93.9 ± 1.70a	5.12 ± 2.05a	5.19 ± 3.72a	0	0.69 ± 0.01
GN3	94.1 ± 4.15a	5.09 ± 1.95a	4.81 ± 2.98a	0	0.70 ±0.03
GN4	93.7 ± 4.25a	4.42 ± 2.35a	5.11+3.08a	2.0	0.72 ± 0.03
GN5	93.5 ± 3.6a	3.18 ± 1.42b	4.94 ± 2.77a	0	0.69 ± 0.02
GN6	92.8 ± 3.25a	3.22 ± 1.35b	4.90+2.41a	0	0.70 ±0.05
GN7	83.5 ± 5.55b	3.15 ± 2.56b	2.74 ± 1.75b	0	0.59 ± 0.06
GN8	65.2 ± 5.75c	2.55 ± 1.95c	2.71 ± 2.58b	0	0.59 ± 0.07

*Means followed by the same letter in the column are not statistically different in Tukey's test at 5% level

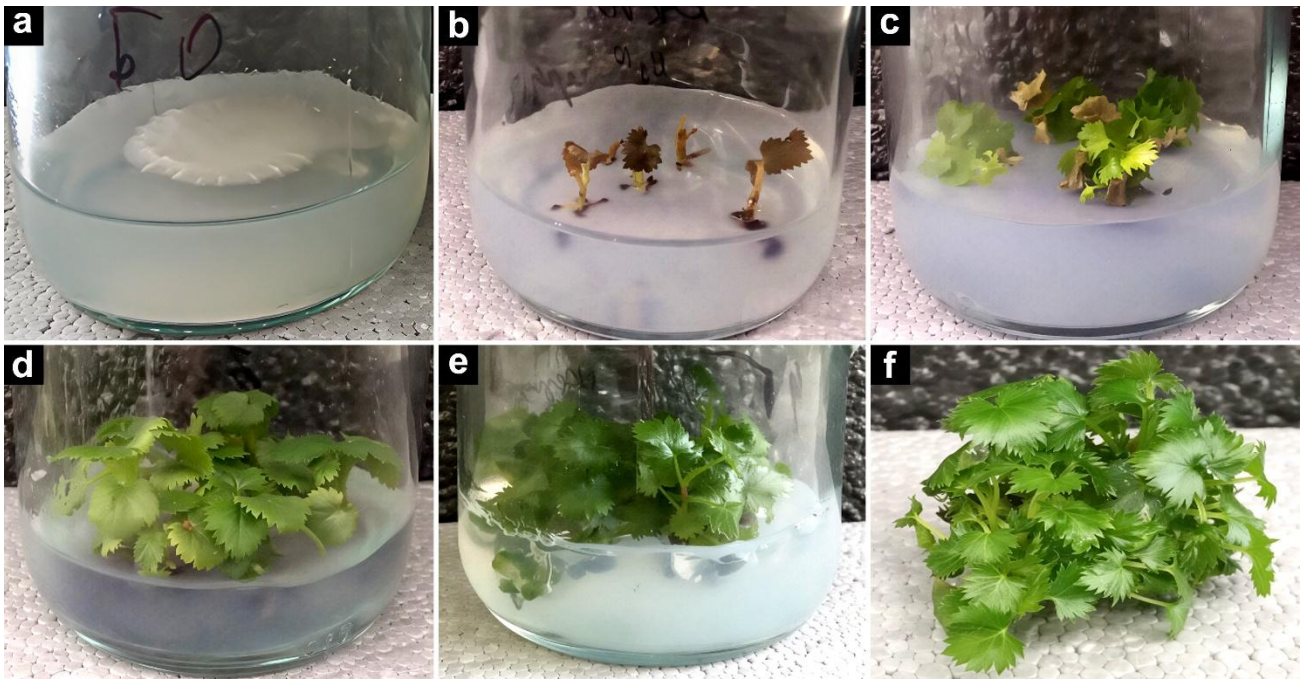

Fig. 3. Using chemically sterilized medium for micropropagation of blackberry Brzezina. **a)** A culture medium that has not been sterilised; **b)** Blackberry shoots cultivated in a medium that contains 0.002% active chlorine without any settling of the medium; **c)** Blackberry shoots cultivated in a medium that contains 0.0005% active chlorine; **d)** Multiplication of blackberry shoots on an autoclaved medium in the sixth week of cultivation; **e, f)** Multiplication of blackberry shoots on a medium after sterilization with 0.002% active chlorine in the sixth week of cultivation.

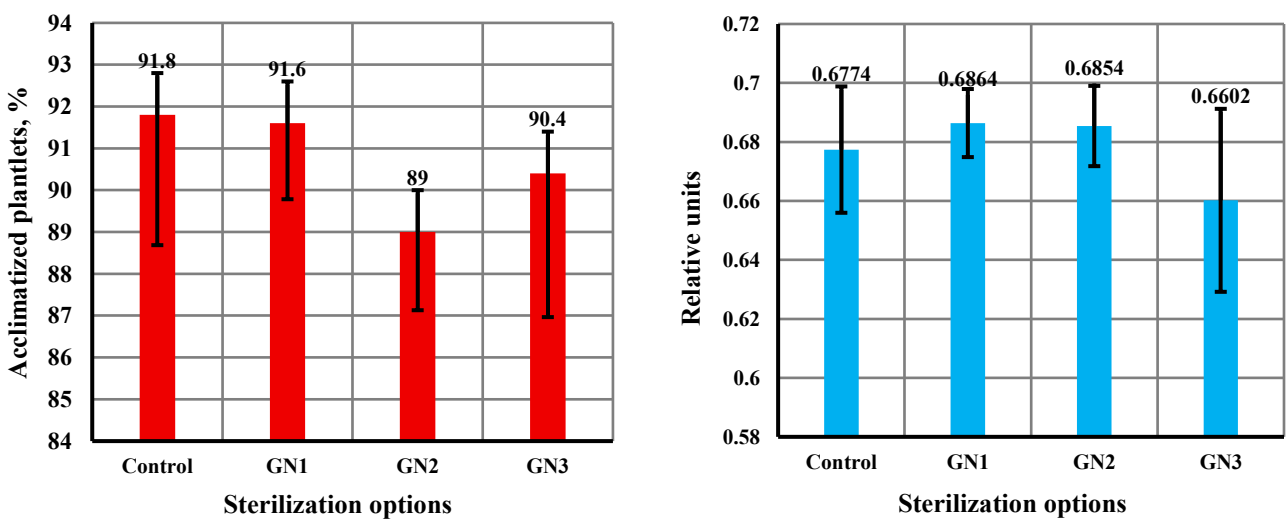

Fig. 4. Efficiency of blackberry Brzezina adapting to *Ex vitro* conditions after thermal and chemical sterilisation.

Acclimatization: Regenerated plants following *In vitro* rooting were subsequently adapted to *Ex vitro* conditions. Shoots exhibiting high rates of reproduction and rooting, which had been obtained on GN1-GN3 and control media, were sent for adaptation. The proportion of shoots acclimatized to *Ex vitro* conditions in all experimental variants did not significantly differ from the control (91.8%) and varied from 89% to 91.6%. The values of the YII indicator also fell into the same statistical group with the control and were within the range from 0.66 to 0.68, demonstrating successful adaptation (Fig. 3).

The evaluation of the phytotoxic potential of the culture medium: The present study assesses the growth and development of blackberry shoots inoculated onto GN2 medium at different stages of medium settling. It was established that if active chlorine remained in the medium at a concentration close to the initial one, the explants quickly perished (Fig. 3b). After approximately 50 hours, when the concentration decreased to 0.0005%, survival of the plants was observed, but with the presence of noticeable burns (Fig. 3c). After a period of five days had elapsed, during which the medium underwent settling, active chlorine was no longer detected in the nutrient medium. On this medium, the functional state of the plants did not statistically significantly differ from that of the control. Concurrently, no discernible morphological discrepancies were observed.

Discussion

In this study, the concentrations of active chlorine were evaluated at two levels: initial and current. This is due to the chemical properties of sodium hypochlorite. The rate of hydrolysis of sodium hypochlorite is relatively high, while complete sterilization of the medium is achieved by prolonged exposure to active chlorine (Clifford, 1999). Concurrently, the concentration of active chlorine in the medium gradually diminishes (Shvetsov *et al*., 2009). Consequently, both the initial and current concentrations of active chlorine emerge as pivotal parameters when selecting a chemical sterilization mode for the nutrient medium. The results of our studies demonstrated that when the initial concentration of active chlorine was 0.001% or higher, complete sterilization of the medium was achieved. Pais *et al*., (2016) further corroborate this finding, reporting that active chlorine concentrations ranging from 0.002% to 0.003% were optimal for sterilizing the medium used for the propagation of gerbera. As stated by Teixeira *et al*., (2008), a concentration of 0.005% was utilised for the micropropagation of eucalyptus. Earlier, Teixeira *et al*., (2005) reported that they achieved good results with a chlorine concentration an order of magnitude lower – 0.0003%. However, a notable lacuna in these studies is the absence of any mention concerning the concentration of residual active chlorine in the medium prior to the inoculation of plants. This is a crucial omission, as residual active chlorine has been demonstrated to exert deleterious effects on explants. Analysis of the prepared medium for active chlorine during settling showed that GN1 and GN2 media are ready for use only after 80 and 120 hours, respectively. Inoculation of plants prior to these timeframes has been observed to result in either plant death or tissue burns, along with substantial inhibition of plant growth. Pais *et al*., (2016) reported that to avoid negative effects, they kept the culture vessels open for 10 min to remove residual active chlorine before inoculating plants. In contrast, in our experimental setup, the inoculation of shoots was initiated immediately after opening the jars, a strategy enabled by the medium's pre-settling period. It is plausible that residual active chlorine may have been responsible for the inhibition of micropropagation in crops such as blueberries, cranberries, mulberries, and chokeberries (Siekierzyńska & Litwińczuk, 2018).

The sterilisation of the nutrient medium by chemical means, using sodium hypochlorite, is accompanied by the interaction of active chlorine particles with organic components of the nutrient medium, including vitamins, amino acids, and phytohormones. This process can lead to the degradation of these components by oxidation (Al-Nu'ayrat *et al*., 2020). The effect of active chlorine on the components of the medium can be indirectly gauged by the main indicators of blackberry growth and development at different stages of micropropagation.

The ensuing experiments yielded the identification of optimal initial concentrations of active chlorine in the nutrient medium (ranging from 0.001% to 0.003%), at which two phenomena occurred: firstly, the complete death of all microorganisms, and secondly, the maximum reproduction rates were recorded. A reliable increase in the fresh mass of new shoots and their average length at active chlorine concentrations of 0.001% - 0.002% clearly indicates that certain components of the medium were subject to less change during chemical sterilization than during thermal sterilization. Consistent findings have been reported by other researchers. In this study, an increase in the growth rates of the aboveground part of Gerbera was observed when using a chemically sterilized medium with chlorine dioxide (ClO2) in comparison to an autoclaved medium (Cardoso *et al*., 2018). Moreover, an increase in the average number of *Bucephalandra* and *Philodendron* shoots has been reported on sodium hypochlorite-treated medium (Wamaedeesa *et al*., 2020; Laksana *et al*., 2023). Our research has demonstrated that chemical sterilization with active chlorine concentrations ranging from 0.003% to 0.006% yields results that are statistically indistinguishable from those of autoclaved medium. Conversely, concentrations above 0.007% have been observed to inhibit explants. Concurrently, there was a marked decline in growth and development indicators, as well as the Y (II) index.

Blackberry rooting was successfully achieved in media treated with active chlorine concentrations ranging from 0.001% to 0.004%, while higher concentrations resulted in inhibition of root formation. Ribeiro *et al*., (2022) also noted successful rooting of shoots *In vitro* using the example of *Gerbera hybrida* cv. Essandre on chemically sterilized media.

It is our opinion that a chemical sterilization regime should include the initial concentration of active chlorine, and the exposure time. The latter parameter is of great importance for the organisation of laboratory work and logistical connections. It is evident that the attainment of maximum sterilization rates is preferable, which is associated with the maximum concentrations of sodium hypochlorite. Nevertheless, our focus was on concentrations of 0.002%, which have been identified as optimal. This is due to the instability of commercial sodium hypochlorite solutions over extended periods of time. Blackberry shoots obtained on chemically sterilized medium were successfully adapted to *Ex vitro* conditions. The high level of adaptation observed is indicative of the efficacy of the sterilization method for the propagation of blackberry.

Conclusions

The chemical sterilisation of the culture medium intended for micropropagation of blackberries has been shown to be an effective method of reducing the costs of obtaining high-quality seedlings. The present study demonstrates, for the first time, that the stages of multiplication and rooting of the Brzezina blackberry variety can be carried out using chemically sterilized nutrient media with sodium hypochlorite. The study demonstrated that the efficacy of this method is contingent on the parameters of chemical sterilization, including the initial concentration of sodium hypochlorite in the medium and the duration of the medium's settling time until the complete release of active chlorine. The method of chemical sterilization of the medium described in this study is a promising tool for laboratories engaged in the production of blackberry seedlings through *In vitro* technologies.

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