Journal of Plant Physiology and Breeding
Print ISSN: 2008-5168 Online ISSN: 2821-0174
Homepage: https://breeding.tabrizu.ac.ir
University of Tabriz

2024, 14(2): 23-38

Evaluation of mother-plant growing beds, explant type, and different disinfection treatments in control of *Sansevieria* rhizome contamination under in vitro conditions

Matin Kazemzadeh Bahnamirei[1], Mostafa K. Sarmast[1*], Mohammad Naghi Padasht Dahkaei[2], and Mahdi Alizadeh[1]

[1]Department of Horticultural Science and Landscape Engineering, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
[2]Guilan Agricultural and Natural Resources Research and Education Center, AREEO, Rasht, Iran.
[*]Corresponding author; Email: mkhsarmast@gau.ac.ir

Article Info
Article type:
Research article

Article history:
Received: September 3, 2024
Revised: November 4, 2024
Accepted: November 11, 2024
Published online: December 31, 2024

Keywords:
Benomyl,
Disinfection,
Explant,
Homogenous growth,
Mercuric chloride,
Rhizome,
Tissue culture

Abstract

Objective: Cloning ornamental plants, particularly chimeric varieties, is a crucial strategy for enhancing competitiveness in the ornamental plant market. Since the only method to propagate clones of chimeric *Sansevieria* varieties is through offset production from rhizome origins, optimizing sterilization techniques for *in vitro* rhizome explants is imperative. A significant drawback of plant tissue culture is the risk of microbial contamination, making the removal of contaminants a foundational step for successful tissue culture. Initial experiments on *Sansevieria trifasciata* rhizome explants revealed that conventional disinfectants were ineffective at eliminating contamination. Consequently, this study aimed to improve culture conditions by evaluating various factors essential for successful plant tissue culture.

Methods: In a preliminary experiment, two types of mother-plant growing media (perlite and conventional greenhouse soil) were compared using a t-test with 50 experimental units within a group. The second experiment was conducted on perlite as the mother-plant growing medium using a factorial arrangement with three factors based on the completely randomized design with three replications. The factors included three rhizome explant types (single node, terminal meristem, and rhizomes with terminal buds), benomyl and carbendazim fungicides at two concentrations of 2% and 10%, and mercuric chloride as the chemical disinfectant at two concentrations of 0.1% and 0.2% for 2 and 20 minutes plus a control without mercuric chloride.

Results: Results indicated that culturing mother plants in the perlite bed significantly reduced microbial loads. The results of the second experiment showed that treatment with 10% benomyl fungicide effectively diminished microbial contamination. Among the disinfectants tested, a 20-minute treatment of explants with 0.2% mercuric chloride resulted in the lowest fungal contamination. Among the explant types, single-node explants exhibited the least fungal contamination.

Conclusion: Pre-treating single-node rhizome explants with 10% benomyl fungicide, followed by a 2-minute exposure to 70% alcohol and a 20-minute treatment with 0.2% mercuric chloride in a laminar flow cabinet, leads to optimal microbial decontamination. These findings underscore the importance of specific sterilization protocols in enhancing the efficacy of plant tissue culture methods for chimeric *Sansevieria* propagation.

Cite this article: Kazemzadeh Bahnamirei M, Sarmast MK, Padasht Dahkaei MN, Alizadeh M. 2024. Evaluation of mother-plant growing beds, explant type, and different disinfection treatments in control of *Sansevieria* rhizome contamination under in vitro conditions. J Plant Physiol Breed. 14(2): 23-38. https://doi.org/10.22034/JPPB.2024.63310.1346

Introduction

Sansevieria trifasciata is an evergreen perennial plant in the family *Asparagaceae*, indigenous to regions such as the tropical Canary Islands and the subtropical areas of Japan, India, Nigeria, Thailand, and Brazil. In addition to its ornamental value, this plant has fibers in its leaves (GarciaHernandez *et al.* 2022), which are primary materials for producing yarn and clothes. Its rhizome extracts and roots are used in medicine (Byrom 1950). Previous research showed that *S. trifasciata* is very effective in absorbing toxins such as benzene, xylene, and formaldehyde (Wolverton *et al.* 1989).

Propagating chimeric cultivars with the help of leaf cuttings leads to the production of plants with green leaves (Dolati *et al.* 2023). Currently, the most reliable way to propagate chimeric varieties is to use offset shoots and rhizomes. Some varieties of *S. trifasciata* can be propagated by seeds, leaf cuttings, and rhizomes (Arnold 2004). However, seed propagation is not feasible due to the slow growth rate and the unavailability of seeds. Our previous research on leaf explants showed that *in vitro* techniques failed to produce true-to-type plants from leaf explants (Sarmast *et al.* 2018). For commercial production and yield increase, we must have a method that can produce plants in a short period. Using offsets is an important method of vegetative propagation for *Sansevieria* plants. Offsets are taken from the end of short stolons and constitute an effective method of vegetative propagation in producing perennial plants (Carey 2008).

Micropropagation allows the production of many high-quality plants in a relatively short period (Tarinejad and Amiri 2019; Ahmadi *et al.* 2022). The biggest problem with this technique is contamination (Altan *et al.* 2010). Many types of microorganisms (mainly fungi and bacteria),

microarthropods (thrips), and viruses have been identified as contaminants in plant tissue culture (Altan *et al.* 2010). Different tissues have different contamination levels and need a series of pretreatments before culture establishment. For example, because of the protective layer, the seeds easily tolerate stronger disinfectants and also for a longer period or are naturally less contaminated. Other tissues, especially those that are close to the soil, have a high microbial load, which makes it difficult to control the contamination of these tissues (Bach and Sochacki 2012). Some underground organs, such as pseudo-bulbs, rhizomes, etc., are the only or perhaps the most effective way of propagating a number of cultivars mostly because of their fast reproduction rate and genetic stability (Podwyszyńska 2012; Ngezahayo and Liu 2014). Rhizomes have a higher direct regeneration potential than other explants and are easier to obtain (Ma and Gang 2006). However, the level of contamination in tubers and other underground organs can reach 95-100% (Yasmin *et al.* 2013). Marinescu *et al.* (2013) evaluated the disinfection efficiency of various lily (*Aphylla* L.) explants. The contamination rate of rhizomes was 86%, and the contamination rate of leaves was 20%. Several factors determine the effectiveness of disinfection. Among these factors are planting conditions, physiological state of the plant, size, age, type of explant, type of disinfectant and its concentration, sowing time, and temperature exposed to disinfectants (Teixeira da Silva *et al.* 2015). Since mother plants are the sources of explants, they should be kept under controlled conditions such as a greenhouse or growth chamber, to minimize the microbial load (Reed and Tanprasert 1995).

Yang *et al.* (2015) used 75% alcohol for 60 seconds and then 0.1% mercury chloride for 12-15 minutes to disinfect the explants obtained from the saffron corm. To establish corms, Yasmin *et al.* (2013) placed the saffron explants in 0.1% carbendazim and 0.2% mancozeb along with Tween-20 for 10 minutes. Soon after, explants were exposed to 50% sodium hypochlorite for 10 minutes. Finally, explants were treated again with 1.6% mercury chloride for 5 minutes. Marinescu *et al.* (2013) immersed one-centimeter explants of *Iris* rhizomes in 70% alcohol for two minutes, washed them three times, and exposed them to 6% calcium hypochlorite. More than 80% of the samples were successfully disinfected.

To our knowledge, there has been no scientific report of sexual or asexual reproduction of S. trifasciata chimeric varieties that would lead to true-to-type plants in vitro. Since it is commercially valuable to use methods that can reproduce true-to-type S. trifasciata chimeras, this research aimed to demonstrate an optimal disinfection protocol and the most suitable S. trifasciata rhizome explants, free from fungal and bacterial diseases for further tissue culture research.

Materials and Methods

Plant materials

In this research, *S. trifasciata* "Laurentii" was used. All mother plants of *Sansevieria* were procured from a commercial greenhouse in Mazandaran province, north of Iran, and transferred to the greenhouse at the Department of Horticultural Sciences, Faculty of Plant Production, Gorgan University, Iran. Prior to transplanting the mother plants, we verified that they were free from any contamination. The plants selected for transplantation varied in size and were chosen at random.

Treatment of the mother plants

At first, 100 plants with bare roots were housed in pots without damaging the surface of the roots and rhizomes. The underground parts were exposed to running water for half an hour to remove the soil and debris. Then, they were placed on a sterile bed for 24 hours to dry. After drying, the plants were cultivated in equal numbers in two beds of perlite and conventional greenhouse soil (10% perlite, 30% compost, 30% leaf soil, 20% sand, and 10% decomposed manure). The cultivated plants were placed in greenhouse conditions at 27 °C, 50% humidity, and 5000 lux light intensity for one month to facilitate acclimatization. Immediately after adaptation, five fungicide treatments were applied to the plants to reduce the microbial load in the greenhouse. The treatments included carbendazim 2%, carbendazim 10%, benomyl 2%, benomyl 10%, and the control. Each of these fungicide treatments was repeated in their respective treatment groups at four stages with a time interval of 15 days. In applying the fungicide treatment, each pot received 100 ml of fungicide solution distributed evenly across the pot. The control pots were watered with distilled water. After 60 days, rhizome samples were collected from the pots for surface disinfection in the subsequent phase of the study.

Surface disinfection

In this experiment, the rhizomes obtained from the mother plants were categorized into three types for disinfection treatments. The first explant type was prepared as a single node from the rhizome (N), the second type included a single node along with the terminal meristem (M), and the third type consisted of a single node along with the leaf and terminal bud (MN) (Figure 1). After preparing the explants, the nodes were washed for 10 minutes with a solution of water and dishwashing liquid (three drops per liter), and then placed under running tap water for 120 minutes. The micro-samples were then transferred to a laminar flow hood for further processing. All explants were treated with 70% alcohol for two minutes. Subsequently, each explant was treated with sodium hypochlorite and 15%

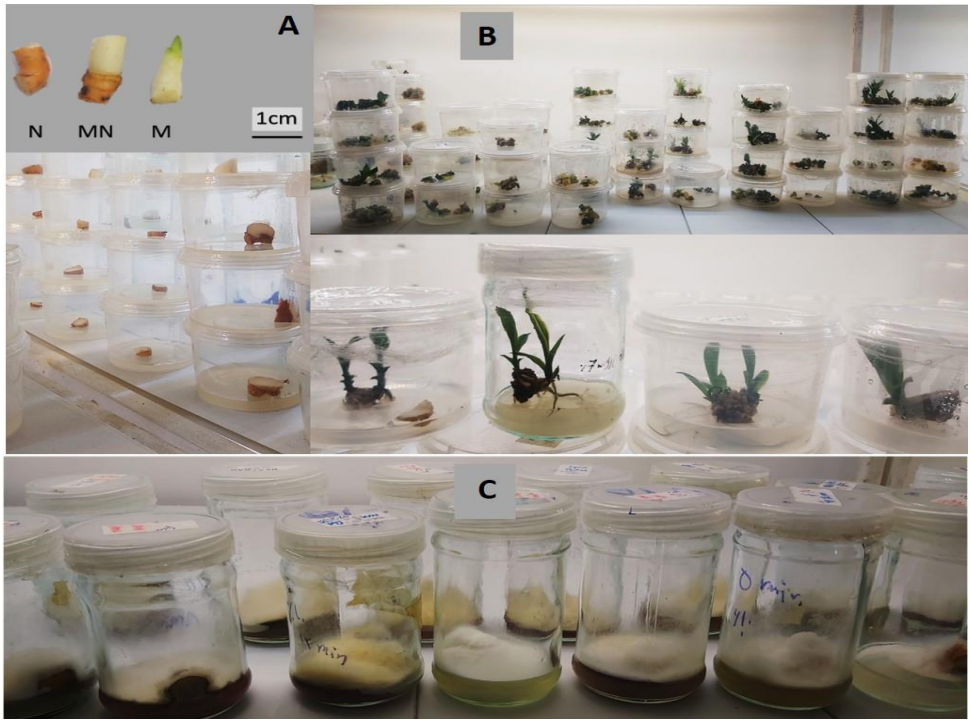

Figure 1. A) Three types of rhizome explants: Single node explant (N), terminal meristem explant (M), and rhizome explant with leaves together with terminal meristem (MN), B) Different regenerated and unregenerated *Sansevieria trifasciata* rhizome explants, C) Fungal contamination of rhizomes.

oxidine (Aral Chemical Company, Iran) at concentrations of 10, 20, 30, 40, and 50% for 10 and 20 minutes.

In the main experiment, mercury chloride (0.1 and 0.2%) was applied to all micro-samples for 2 and 20 minutes. After surface sterilization, all micro-samples were washed at least six times with sterile distilled water. Using a sterile scalpel, N, M, and MN rhizome explants were cut open and inoculated onto MS medium (Murashige and Skoog 1962). We measured the percentages of fungi, bacteria, burn spots, and the growth rates of fungi and bacteria in centimeters (Figure 1).

Data collection was conducted 45 days after the commencement of cultures.

Experimental design and statistical analysis

The experiment was carried out as factorial based on a completely randomized design with three replicates using four explants per experimental unit. The means were compared using Duncan's multiple range test. Also, a t-test was conducted to compare the effects of conventional greenhouse soil and perlite on rhizome microbial contamination using fifty replications per group. Data analysis was performed using SPSS software (version 22).

Results

Effects of the type of mother-plant growing beds on controlling the microbial load of rhizome explants

In the investigation that was conducted on two different types of mother-plant growing beds (conventional greenhouse soil and perlite), a significant difference appeared in controlling fungal contamination in the two media. The perlite substrate significantly reduced the amount of fungal contamination by more than half. It was also effective in reducing the amount of bacterial contamination. The type of mother-plant growing bed did not affect the burn percentage of explants. The perlite substrate significantly reduced the growth rate of fungi and bacteria. The number of regenerated shoots (2.5) and leaf number (6) in rhizomes originating from the perlite substrate were significantly higher than that of conventional greenhouse soil (Table 1). Therefore, all follow-up *in vitro* experiments were carried out on rhizomes obtained from the perlite bed.

Table 1. Effects of growing substrate (perlite and conventional greenhouse soil) on controlling the microbial contamination of *Sansevieria trifasciata* rhizome explants and the regenerated shoots and leaves.

Growing substrate	Fungi (%)	Bacteria (%)	Burns (%)	Fungal growth rate (cm)	Bacteria growth rate (cm)	Number of regenerated shoots	Leaf number per explant
Conventional greenhouse soil	74.4	12.72	5.74	5.26	0.50	0	0
Perlite	34.8	0.54	5.70	1.66	0.04	2.5	6
Significance	**	**	ns	**	**	**	**

ns, and **: Non-significant and significant at 0.01 probability level based on t-test.

Effects of chemical disinfectants, fungicides, and explant types of rhizomes on microbial load in vitro

Analysis of variance of the effects of the three aforementioned factors on disinfection in *Sansevieria* plants is presented in Table 2. All three factors had a significant effect on the fungi load, fungi growth rate, number of regenerated shoots, and number of leaves. However, only bacterial load and bacterial growth rate were affected significantly by the fungicides, and burn percentage by explant type and chemical disinfectants. Most interactions among the factors were not significant, except explant type × fungicide for fungi load, fungi growth rate, and burn percentage, chemical disinfectant × explant type for fungi load and burn percentage, and chemical disinfectant × fungicide and chemical disinfectant × explant type × fungicide for fungi load.

Among the two types of fungicides used, the benomyl fungicide was the most effective when used along with 0.2% mercuric chloride for 20 minutes on N explants. By applying to perlite-cultured

plants at four intervals, 15 days apart, over two months, it controlled fungal contamination completely compared to the control (Table 3). Benomyl (10%) also showed the lowest fungal growth rate and low burn percentage on N explants (Table 4). Carbendazim, especially at 10% concentration, had minimal impact on the fungal control (Table 3). Benomyl also showed zero bacterial load and bacterial growth rate and significantly differed from the control (Table 5). Also, the rhizome explants that were treated with the benomyl fungicide (10%) produced a higher number of shoots and leaves compared to other fungicide treatments (Table 5, Figure 2).

Table 2. Analysis of variance of chemical disinfectants, fungicides, explants, and their interaction on controlling the microbial contamination of *Sansevieria trifasciata* rhizome explants and the regenerated shoots and leaves.

Sources of variation	**df**	**Fungi**	**Bacteria**	**Burn**	**Fungi growth rate**	**Bacterial growth rate**	**Number of regenerated shoots**	**Leaf number**
Chemical disinfectants (C)	3	10745**	203	318**	23.9**	0.08	20.1**	33.1**
Explants (E)	2	20722**	166	6540**	68.6**	0.05	25.1**	65.2**
Fungicides (F)	4	11444**	500*	19.6	57.4**	0.13*	55.2**	57.3**
C × E	6	3166**	92	318**	4.7	0.03	4.3	5.1
C × F	12	592*	303	5.2	3.0	0.08	3.5	4.0
E × F	8	861**	166	19.6**	14.1**	0.05	8.0	10.0
C × E × F	24	898**	92	5.2	2.2	0.03	2.0	3.5
Error	120	255	166	7.2	4.4	0.053	5.0	5.5
(CV)		10	12	13	11	14	10	14

* and **Significant at 5% and 1% probability levels, respectively.

The results obtained from surface disinfection indicated that using oxidine and sodium hypochlorite could not control the surface contamination of rhizome explants, and all samples exposed to oxidine and sodium hypochlorite became contaminated in less than a week. However, applying 0.2% mercuric chloride for 20 minutes effectively controlled fungal infections when used with the benomyl fungicide (2% or 20%) on the N explants, as none of the samples became infected (Table 3). Burning symptoms were also lowest when 0.2% mercuric chloride was applied to N explants for 20 minutes (Table 6). Furthermore, using 0.2% mercuric chloride for 20 minutes resulted in a zero fungal growth rate and a higher number of shoots and leaves per explant (Table 7).

Table 3. The effect of interaction of explant types, chemical disinfectants, and fungicides on fungal contamination of *Sansevieria trifasciata* rhizome explants.

Explant types	Fungicides	Chemical disinfectant	Fungi (%)
M	HgCl20.1 % 2 minutes	Control	33.3^{c}
		Carbendazim 2%	66.6^{b}
		Carbendazim 10%	100^{a}
		Benomyl 2%	33.3^{c}
		Benomyl 10%	1.4^{h}
M	HgCl2 0.1 % 20 minutes	Control	33.3^{c}
		Carbendazim 2%	33.3^{c}
		Carbendazim 10%	33.3^{c}
		Benomyl 2%	33.3^{c}
		Benomyl 10%	5.7^{e}
M	HgCl2 0.2 % 2 minutes	Control	33.3^{c}
		Carbendazim 2%	1.4^{h}
		Carbendazim 10%	2.8^{g}
		Benomyl 2%	7.1^{d}
		Benomyl 10%	1.4^{h}
M	HgCl2 0.2 % 20 minutes	Control	33.3^{c}
		Carbendazim 2%	1.4^{h}
		Carbendazim 10%	5.7^{e}
		Benomyl 2%	1.4^{h}
		Benomyl 10%	7.1^{d}
MN	HgCl20.1 % 2 minutes	Control	100^{a}
		Carbendazim 2%	66.6^{b}
		Carbendazim 10%	100^{a}
		Benomyl 2%	33.3^{c}
		Benomyl 10%	33.3^{c}
MN	HgCl2 0.1 % 20 minutes	Control	100^{a}
		Carbendazim 2%	66.6^{b}
		Carbendazim 10%	66.6^{b}
		Benomyl 2%	66.6^{b}
		Benomyl 10%	33.3^{c}
MN	HgCl2 0.2 % 2 minutes	Control	66.6^{b}
		Carbendazim 2%	33.3^{c}
		Carbendazim 10%	66.6^{b}
		Benomyl 2%	33.3^{c}
		Benomyl 10%	7.1^{d}
MN	HgCl2 0.2 % 20 minutes	Control	66.6^{b}
		Carbendazim 2%	66.6^{b}
		Carbendazim 10%	66.6^{b}
		Benomyl 2%	33.3^{c}
		Benomyl 10%	2.8^{g}
N	HgCl20.1 % 2 minutes	Control	33.3^{c}
		Carbendazim 2%	33.3^{c}
		Carbendazim 10%	33.3^{c}
		Benomyl 2%	33.3^{c}
		Benomyl 10%	33.3^{c}
N	HgCl2 0.1 % 20 minutes	Control	33.3^{c}
		Carbendazim 2%	2.8^{g}
		Carbendazim 10%	4.2^{f}
		Benomyl 2%	2.8^{g}
		Benomyl 10%	1.4^{h}
N	HgCl2 0.2 % 2 minutes	Control	33.3^{c}
		Carbendazim 2%	33.3^{c}
		Carbendazim 10%	33.3^{c}
		Benomyl 2%	33.3^{c}
		Benomyl 10%	5.7^{e}
N	HgCl2 0.2 % 20 minutes	Control	66.6^{b}
		Carbendazim 2%	33.3^{c}
		Carbendazim 10%	66.6^{b}
		Benomyl 2%	0^{i}
		Benomyl 10%	0^{i}

Mean values with the same letters are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Table 4. The growth rate of fungi and burn percentage, influenced by the interaction of fungicides and *Sansevieria trifasciata* rhizome explant type.

Explant type	Fungicide	Fungi growth rate (cm)	Burn (%)
M	Control	1.6^{e}	3.3^{b}
	Carbendazim 2%	1.08^{h}	2.0^{b}
	Carbendazim 10%	1.4^{f}	3.3^{b}
	Benomyl 2%	0.5^{i}	2.3^{b}
	Benomyl 10%	0.3^{j}	3.0^{b}
MN	Control	6.8^{a}	8.3^{a}
	Carbendazim 2	2.4^{c}	10.3^{a}
	Carbendazim 10%	3.3^{b}	9.5^{a}
	Benomyl 2%	1.2^{g}	10.6^{a}
	Benomyl 10%	0.4^{j}	11.6^{a}
N	Control	2.08^{d}	2.0^{b}
	Carbendazim 2%	1.08^{h}	2.5^{b}
	Carbendazim 10%	1.1^{h}	3.3^{b}
	Benomyl 2%	0.75^{i}	2.5^{b}
	Benomyl 10%	0.1^{k}	3.0^{b}

Mean values with the same letters are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Table 5. Effects of different fungicides on reducing the microbial load and regeneration efficiency in *Sansevieria trifasciata* rhizome explants grown in perlite beds.

Fungicides	Bacteria (%)	Bacterial growth rate (cm)	Number of regenerated shoots	Leaf number per explant
Control	8.3^{a}	0.15^{a}	0^{d}	0^{d}
Carbendazim 2%	2.7^{a}	0.08^{a}	1^{c}	1^{c}
Carbendazim 10%	0^{a}	0^{a}	0^{d}	0^{d}
Benomyl 2%	0^{a}	0^{a}	2^{b}	2^{b}
Benomyl 10%	0^{a}	0^{a}	3^{a}	5^{a}

Mean values with the same letters are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

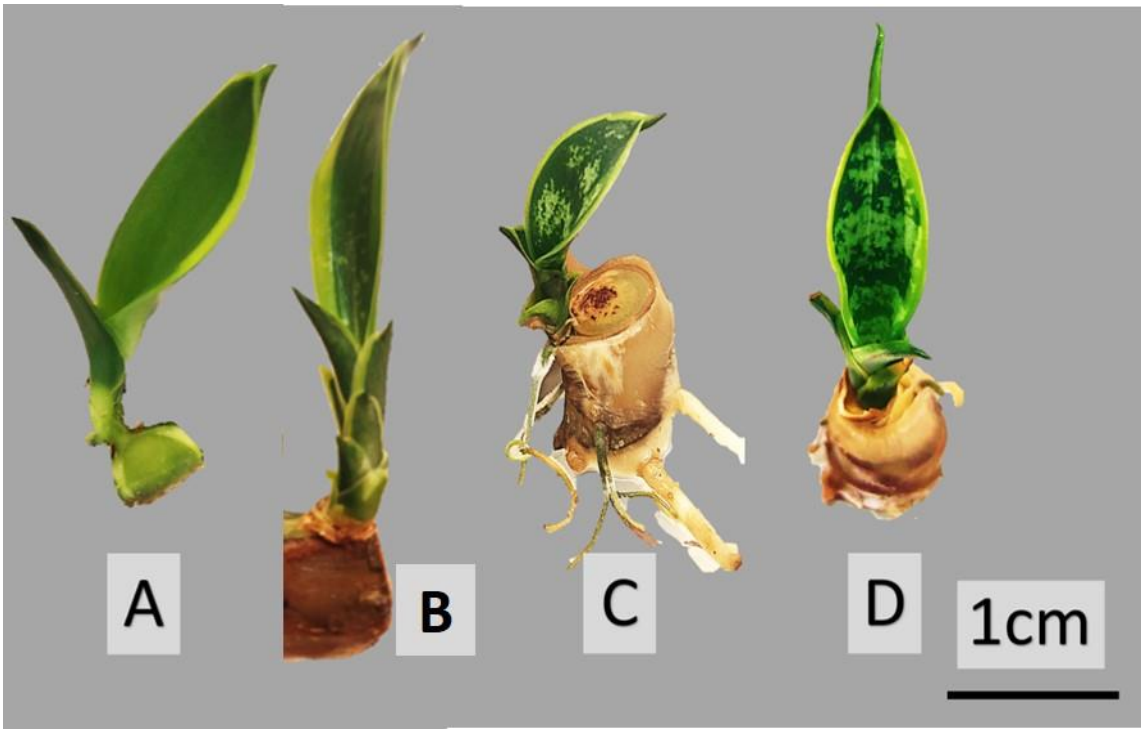

Figure 2. Successfully in vitro-established rhizome explant revealing original chimeric pattern; A and B) Single node explant, c) Terminal meristem explant, D) Rhizome explant with leaves together with terminal meristem.

Table 6. The burning percentage of rhizome explants, influenced by the interaction of chemical disinfectant and *Sansevieria trifasciata* rhizome explant types.

Chemical disinfectant	Explant type	Burn (%)
HgCl2 0.1% 2 minutes	M	1.3^{h}
	MN	8.3^{d}
	N	2.6^{f}
HgCl2 0.1% 20 minutes	M	1.9^{g}
	MN	15.6^{c}
	N	1.3^{h}
HgCl2 0.2% 2 minutes	M	8.8^{d}
	MN	21.3^{b}
	N	2.2^{f}
HgCl2 0.2% 20 minutes	M	4.4^{e}
	MN	27^{a}
	N	1.1^{i}

Mean values with the same letters are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

As mentioned above, the N-type explants had zero fungal contamination when the benomyl fungicide along with 0.2% mercuric chloride was applied for 20 minutes (Table 3). The fungal growth rate was also lowest on this explant under the influence of the benomyl fungicide (Table 4). In terms of bacterial load and bacterial growth rate, there was no significant difference among the explant types (Table 8). The MN explant had a high degree of burn injury. In the rest of the explants, the burn percentage was zero (Table 8). The nodal-originated shoots (3) and leaves (4) in the N explant were significantly higher than other rhizome explants (Table 8).

Table 7. Effects of chemical disinfectants on surface contamination and number of regenerated shoots and leaves in *Sansevieria trifasciata* rhizome explants.

Chemical disinfectants	Fungi growth rate (cm)	Number of regenerated shoots	Leaf number per explant
HgCl20.1 % 2 min	2.5a	1d	1d
HgCl2 0.1 % 20 min	1. 5ab	2c	2c
HgCl2 0.2 % 2 min	0.32b	3b	5b
HgCl2 0.2 % 20 min	0b	4a	7a

Mean values with the same letters are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Table 8. The comparison of the microbial contamination of *Sansevieria trifasciata* rhizome explants and the regenerated shoots and leaves in three different rhizome explants.

Explant type	Bacteria (%)	Bacterial growth rate (cm)	Number of regenerated shoots	Leaf number per explant
N	0^a	0^a	3^a	4^a
M	1.6^a	0.05^a	2^b	3^b
MN	4.9^a	0.13^a	1^c	2^c

Mean values with the same letters are not significantly different according to Duncan's multiple range test ($p \leq 0.05$).

Discussion

Using leaf cuttings cannot maintain the chimeric features in the micropropagation of *Sansevieria* plants. However, using *Sansevieria* rhizomes as explants can regenerate chimeric *Sansevieria* plants (Sarmast *et al.* 2023). On the other hand, since rhizomes grow and develop under the soil for a long time, they tend to have high fungal contamination, which incentivizes the *in vitro* propagation of this plant using rhizome microexplants. Therefore, obtaining a suitable disinfection protocol for rhizome explants is very important. Microbial contamination in explants depends on various factors such as

species, age, sampling season, and weather conditions (Read and Preece 2014). Therefore, controlling contamination in plant tissue culture is one of the most important steps, and the propagator should be careful not to damage the tissues when controlling the contamination. In this research, we used different treatments to obtain the best disinfection protocol. At first, we evaluated two perlite beds and conventional greenhouse soils. The results showed that the perlite substrate reduced the percentage of fungal contamination of the explants by more than half, and it also contributed to the effective control of bacterial and fungal growth rates. According to the obtained results, the perlite substrate was very effective in reducing the microbial load of the *Sansevieria* rhizome due to its sterile nature and being free of organic substances, as well as the pore spaces, which are very suitable for air circulation in the root environment and do not allow room for excess moisture in the substrate (Maloupa *et al.* 1992).

In this research, using different fungicides with irrigation showed that applying 10% benomyl fungicide + 0.2% mercuric chloride (for 20 minutes) on N explants was very effective in controlling the percentage of fungal contamination (Tables 3). Benomyl is a systemic fungicide from the group of benzimidazole fungicides, which acts against a wide range of microorganisms, such as invertebrates and many fungal pathogens (Dane and Dalgic 2005). Many researchers have reported plant disease control using benomyl fungicide (Dane and Dalgic 2005; Mamza *et al.* 2010; Daud *et al.* 2012; Sawant *et al.* 2017). In many studies, mercuric chloride, ethanol, and sodium hypochlorite, alone or combined, appeared as optimal disinfection treatments (Daud *et al.* 2012; Mihaljević *et al.* 2013; Read and Preece 2014). Sarmast *et al.* (2018) used sodium hypochlorite at different concentrations and effectively reduced the microbial contamination of several perennial plant species. However, in our experiment, regarding different disinfectants in controlling surface contamination of rhizome explants, using oxidine and sodium hypochlorite alone had no effect in controlling the contamination. Applying mercuric chloride 0.2% for 20 minutes + benomyl (10%) on N explants was the best treatment for controlling the surface contamination well (Table 3). It has been reported that the 100 mg/L mercury chloride significantly reduced the barberry fungal contamination under *in vitro* conditions (Nazary Moghaddam *et al.* 2019). Mercury chloride is a stronger disinfectant than sodium hypochlorite, and due to the presence of mercury as a heavy metal, it can penetrate the tissue and alleviate intra-tissue infections. Also, the effect of mercury chloride remains longer (Marinescu *et al.* 2013).

The N explant was an optimal material with lower fungal infections, especially, when the mercuric chloride 0.2% (for 20 minutes) + benomyl (10%) was used on this explant. The percentage of contamination of explants can change by the location of the explant source and the type of tissue,

diameter, and size of explants. Explant N was less contaminated due to its smaller diameter and different texture, compared to the other two explants. The highest burn percentage occurred in the MN explant due to the presence of a part of this explant along with the leaf and the greater sensitivity of the leaf tissue to disinfectants. Explants prepared from underground organs have the highest level of contamination because of being in contact with the soil (Marinescu *et al.* 2013). The majority of the recognized fungi belong to *Phytophthora* genus. All explants in this research were from underground organs. However, they had different intra-tissue contamination levels due to the difference in the diameter and size of the explants, as well as having some explants with terminal meristems and terminal buds (Figure 1).

Conclusion

According to our results, the best solution for preparing rhizome explants in the *Sansevieria* plant is cultivation in a perlite bed. Also, our results showed that the nodal rhizome explants (the N- Type) should be obtained from the mother plant and disinfected with 70% alcohol for 2 minutes, followed by treatment with 0.2% mercury chloride for 20 minutes under a laminar flow hood along with the application of the benomyl (10%) fungicide through irrigation water. Considering the high value of this ornamental plant and the naturally low production rate of seed from the mother plant, having designed an appropriate method of *in vitro* propagation and finding optimal instructions for its tissue culture can significantly contribute to the large-scale production of this ornamental plant.

Authorship Contribution

M.K.B: Performed the experiment and prepared the data. M.K.S: Conceived the research and wrote the original draft. M.N.P.D: Provided the resources, reviewed and revised the manuscript M.A: Provided ideas and guidance to the work. All authors read and approved the final version of the paper.

Funding

This study was funded by Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Data Availability

All data generated or analyzed during this study are either included in this manuscript, or available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest with other people or organizations.

References

Ahmadi MR, Shahhoseini R, Hakimi L. 2022. Micropropagation of Iranian native oregano (*Origanum vulgare* L.) using growth regulators. J Plant Physiol Breed. 12(2): 105-116. https://doi.org/10.22034/JPPB.2022.16376

Altan F, Bürün B, Sahin N. 2010. Fungal contaminants observed during micropropagation of *Lilium candidum* L. and the effect of chemotherapeutic substances applied after sterilization. Afr J Biotechnol. 9(7): 991-995. https://doi.org/10.5897/AJB08.090

Arnold MA. 2004. Landscape plants for Texas and environments, Third Edition. USA: Stipes Publishing.

Bach A, Sochaki D. 2012. Propagation of ornamental geophytes: physiology and management systems. In: Kamenetsky R, Okubo H. (eds.). Ornamental geophytes from basic science to sustainable production. Boca Raton, Florida: CRC Press, pp. 261-287. https://doi.org/10.1201/b12881

Byrom MH. 1950. Progress with long vegetable fibers crops in peace and war. The Year Book of Agriculture. US Department of Agriculture, Washington DC: U.S. Government Printing Office.

Carey DJ, Whipker BE, McCall I, Buhler, W. 2008. Benzyl adenine foliar sprays increase offsets in *Sempervivum* and *Echeveria*. J Hort Sci. 53: 19-21.

Dane F, Dalgiç O. 2005. The effects of fungicide benomyl (benlate) on growth and mitosis in onion (*Allium cepa* L.) root apical meristem. Acta Biol Hung. 56(1- 2): 119-128. https://doi.org/10.1556/ABiol.56.2005.1-2.12

Daud NH, Jayaraman S, Mohamed R. 2012. An improved surface sterilization technique for introducing leaf, nodal and seed explants of *Aquilaria malaccensis* from field sources into tissue culture. Asia Pac J Mol Biol Biotechnol. 20(2): 55-58.

Dolati M, Abbasabad A, Seyfi E, Alizadeh M, Khoshhal Sarmast M. 2023. Appraisal of leaf cutting, soil mixture and leaf explants on production of *Sansevieria trifasciata* under ex/in vitro condition. Flower and Ornamental Plants. 7(2): 261-276 (In Persian with English abstract). https://doi.org/10.61186/flowerjournal.7.2.261

García-Hernández E, Loera-Quezada MM, Morán-Velázquez DC, López MG, Chable-Vega MA, Santillán-Fernández A, Zavaleta-Mancera HA, Tang JZ, Azadi P, Ibarra-Laclette E, et al. 2022. Indirect organogenesis for high frequency shoot regeneration of two cultivars of *Sansevieria*

trifasciata Prain differing in fiber production. Sci Rep. 12: 8507 https://doi.org/10.1038/s41598-022-12640-4

Ma X, Gang DR. 2006. Metabolic profiling of in vitro micropropagated and conventionally greenhouse grown ginger (*Zingiber officinale*). Phytochemistry. 67(20): 2239–2255. https://doi.org/10.1016/j.phytochem.2006.07.012

Maloupa E, Mitsios I, Martinez PF, Bladenopoulou S. 1992. Study of substrates used in gerbera soilless culture grown in plastic greenhouse. Acta Hortic. 323: 139-144. https://doi.org/10.17660/ActaHortic.1993.323.12

Mamza WS, Zarafi AB, Alabi O. 2010. *In vitro* evaluation of six fungicides on radial mycelial growth and regrowth of *Fusarium pallidoroseum* isolated from castor (*Ricinus communis*) in Samaru, Nigeria. Arch Phytopathol Plant Prot. 43(2): 116-122. https://doi.org/10.1080/03235400701806401

Marinescu MV, Teodorescu A, Şuţan NA. 2013. Preliminary results on the *in vitro* propagation by leaf explants and axillary buds of *Iris aphylla* L. J Hortic For Biotehnol. 17: 279-282.

Mihaljević I, Dugalić K, Tomaš V, Viljevac M, Pranjić A, Čmelik Z, Puškar B, Jurković Z. 2013. *In vitro* sterilization procedures for micropropagation of 'Oblačinska' sour cherry. J Agric Sci. 58(2): 117-126. https://doi.org/10.2298/JAS1302117M

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15: 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nazary Moghaddam Aghayeh R, Abedy B, Balandari A, Samiei L, Tehranifar A. 2019. Contamination control in Iranian seedless barberry micropropagation. J Plant Physiol Breed. 9(1): 97-110. https://doi.org/10.22034/jppb.2019.10387

Ngezahayo E, Liu B. 2014. Axillary bud proliferation approach for plant biodiversity conservation and restoration. Int J Biodivers. 2014: 27025. https://doi.org/10.1155/2014/727025

Podwyszyńska M. 2012. The mechanism of *in vitro* storage organ formation in ornamental geophytes. Floriculture Ornamental Biotechn. 1(6): 9-23.

Read PE, Preece JE. 2014. Cloning: Plants – Micropropagation/tissue culture. In: Van Alfen N (ed.). Encyclopedia of Agriculture and Food Systems. 2: 317-336. San Diego, CA, USA: Academic Press. https://doi.org/10.1016/b978-0-444-52512-3.00224-2

Reed BM, Tanprasert P. 1995. Detection and control of bacterial contaminants of plant tissue cultures. A review of recent literature. Plant Tissue Cult Biotechnol. 1(3): 137-142.

Sarmast MK. 2018. In vitro propagation of conifers using mature shoots. J For Res. 29: 565-574. https://doi.org/10.1007/s11676-018-0608-7

Sawant IS, Wadkar PN, Ghule SB, Rajguru YR, Salunkhe VP, Sawant, SD. 2017. Enhanced biological control of powdery mildew in vineyards by integrating a strain of *Trichoderma afroharzianum* with sulphur. Biol Control. 114: 133-143. https://doi.org/10.1016/j.biocontrol.2017.08.011

Tarinejad A, Amiri S. 2019. Influence of plant growth regulators, carbohydrate source and concentration on micropropagation and other physiological traits of grape (*Vitis vinifera* L. cv. Shahroudi) under in vitro conditions. J Plant Physiol Breed. 9(1): 75-82. https://doi.org/10.22034/JPPB.2019.10378

Teixeira da Silva JA, Winarto B, Dobránszki J, Zeng S. 2015. Disinfection procedures for *in vitro* propagation of *Anthurium*. Folia Hortic. 27: 3-14. https://doi.org/10.1515/fhort-2015-0009

Wolverton BC, Johnson A, Bounds K. 1989. Interior landscape plants for indoor air pollution abatement. NASA. ID: 19930073077.

Yang BM, Huang YL, Xu WJ, Bao LX. 2015. Explant selection and cluster buds induction *in vitro* of saffron (*Crocus sativus* L.). Agric Sci Technol Commun. 2: 106-108.

Yasmin S, Nehvi FA, Wani SA. 2013. Tissue culture as an alternative for commercial corm production in saffron: a heritage crop of Kashmir. Afr J Biotechnol. 12(25): 3940-3946. https://doi.org/10.5897/AJB2013.12378