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Influence of sterilization methods on the yield of viable explants of introduced varieties of Surfinia and Calibrachoa

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Abstract

It is shown that, of the four methods of sterilization tested by us (No. 1-No. 4), a high yield (75-90%) of viable explants of introduced varieties of surfinia and calibrachoa, was obtained using method No. 1, which includes treatment of explants with a 3% solution of the fungicide "Topaz" and a 5% solution of sodium hypochloride "Whiteness" at an exposure of 15 minutes for both compounds.

Keywords: methods of sterilization, viability, varieties, surfinia, calibrachoa

Introduction

It is well known that the process of clonal micropropagation begins with the selection of a plant, isolation of the explant, its sterilization and planting on a nutrient medium.

One of the fundamental roles in this process belongs to the selection of sterilization methods, including various sterilizing compounds, the effectiveness of their concentrations, the duration of processing time in order to free the material from infection and obtain a high yield of viable explants.

Our own research, as well as the research of numerous authors, show that obtaining a sterile plant material is a complex task, the successful solution of which depends on the correct choice of the sterilizing agent, its concentration, exposure time, type and size of the initial explant, as well as the timing of introduction into culture.

Based on experimental studies by Tavartkiladze and Mezentsev(1987), it was concluded that the optimal sterilizing agents for tea explants should be considered an aqueous solution containing 1.5 - 2% hydrogen peroxide and 50 - 60% ethyl alcohol, at the first stage of sterilization for 10-15

seconds; at the second stage of sterilization − 0.05 - 0.20%-an aqueous solution of diacid for 5-10 minutes .

According to Ogurtsov (1988), it is advisable to use a 10% chloramine solution for sterilization of mulberry anthers (exposure time is 8-15 minutes). For sterilization of date palm explants Das et al. (1989) a 0.1% solution of sulema was used.

Studies conducted by Shornikov (2008) have shown high efficiency of using non-woody green shoots of honeysuckle, actinidia, lemongrass and eleutherococcus as explants of nodes. Explantation during the period of active growth of shoots made it possible to obtain 50-91% sterile viable explants. Nodes of lignified growths were characterized by low viability (0-20%), high infection (up to 88.6%) and were unsuitable for obtaining a sterile culture. A high level of asepsis was ensured by the use of a commercial preparation of sodium hypochloride "Whiteness", diluted with sterile water in a volume ratio of 1:1 at an exposure of 5-7 minutes.

As a result of studying Liu Liu-Kang et al. (1988) surface sterilization of Xanthosoma spp explants. the authors came to the conclusion about the greatest effectiveness of the chlorox preparation in concentrations from 1 to 10%. Treatment exposure 1-10 min.

According to literature sources, various sterilizing compounds with different concentrations and exposure times are used for sterilization (Raškauskas et al., 1989; Trukhanov et al., 1990; Kozhakhmetov and Alimgazinova, 1990; Atroschenko et al., 1990; Lukicheva and Migrinova, 2001; Averyanova et al., 2002; Melnichuk et al., 2004; Shumikhin, 2004; Rokityanskaya, 2005; Neskorodov et al., 2007; Bulatova et al., 2009; Badoni and Chauhan, 2010; Mihaljević et al., 2013; Zhumagulova and Frolov, 2014; Wegayehu, 2015; Karule et al., 2016; Zhatko et al., 2017; Lebedev et al., 2019; Nikitina et al., 2020).

It should be noted that for each type of plant or variety, the optimal mode (method) of sterilization, which contributes to a high yield of viable explants, is determined experimentally. The introduced varieties of surfinia and calibrachoa are no exception.

Materials and Methods

The objects of the study were six introduced varieties of surfinia: Surfinia x hybrida hort "Purple", Surfinia x hybrida hort "Black prince", Surfinia x hybrida hort"Blue Vein", Surfinia x hybrida hort "Double Red", Surfinia x hybrida hort "Star Yellow", Surfinia x hybrida hort "Blue" and two varieties of calibrahoa: Calibrachoa x hybrida hort "Kabloom deep blue", Calibrachoa x hybrida hort "Kabloom white".

As explants, buds with a piece of a stem 5-6 mm long, isolated from the shoots of the above-listed varieties, were used. To free explants from infection, we used four methods of sterilization developed by us (No. 1-No. 4), including the following stages of treatment:

Method No. 1

- washing the shoots with soap solution followed by rinsing them with running tap water for 10 minutes;
- treatment of shoots with a 3% fungicide solution ("Topaz") for 15 minutes with six times rinsing with tap water for 12 minutes;

- sterilization of explants with a 5% commercial preparation of sodium hypochloride "Whiteness" with the addition of 2-3 drops of detergent "Tween 80" at exposure for 15 minutes, followed by washing them in three shifts of sterile bidistilled water for 10-15 minutes each.

Method No. 2

- washing the shoots with soap solution followed by rinsing them with running tap water for 10 minutes;
- treatment of explants with 70% ethyl alcohol (30 seconds exposure) followed by their washing in

three shifts of sterile bidistilled water for 10-15 minutes each;

- sterilization of explants with a 3% commercial preparation of sodium hypochloride "Whiteness" with the addition of 2-3 drops of detergent "Tween 80" at an exposure of 15 minutes, followed by washing them in three shifts of sterile bidistilled water for 10-15 minutes each.

Method No. 3

- washing the shoots with soap solution followed by rinsing them with running tap water for 10 minutes;

- treatment of explants with 70% ethyl alcohol (exposure of 15 seconds) followed by their washing in three shifts of sterile bidistilled water for 10-15 minutes each;

- sterilization of explants with a 5% commercial preparation of sodium hypochloride "Whiteness" with the addition of 2-3 drops of detergent "Tween 80" at an exposure of 30 minutes, followed by washing them in three shifts of sterile bidistilled water for 10-15 minutes each.

Method No. 4

- washing the shoots with soap solution followed by rinsing them with running tap water for 10 minutes;

- treatment of explants with 70% ethyl alcohol (exposure of 10 seconds) followed by their washing in three shifts of sterile bidistilled water for 10-15 minutes each;

- sterilization of explants with a 1% solution of silver nitrate at an exposure of 10 minutes, followed by their washing in six shifts of sterile bidistilled water for 15-20 minutes each.

After sterilization, the material was planted on a modified agarized medium MS. Test tubes with planted explants were placed on racks where the air temperature was 24 ° C, illumination – 4000 lux, relative humidity – 70%, photoperiod – 16 hours. Infected, oxidized and viable explants were counted daily for 2 weeks. The experimental data are given in the Table.

Results and Discussion

The figures in the Table indicate the dependence of the yield of viable buds of introduced varieties of surfinia and calibrachoa on the method of sterilization, the concentration of the sterilizing compound, and the exposure time.

A high yield (80-90%) of viable buds was noted in the introduced varieties of surfinia and calibrachoa when using the sterization method No.1, which includes treating the buds with a soap solution for 10 minutes, a 3% solution of fungicide ("Topaz") for 15 minutes, a 5% solution of sodium hypochloride "Whiteness" during exposure 15 minutes.

An almost similar yield (70-90%) of viable explants of introduced surfinia varieties was observed during sterilization with a 5% solution of "Whiteness" with an exposure of 30 minutes using method No. 3. This indicator was slightly lower (60-65%) in introduced Calibrachoa x hybrida hort "Kabloom white", Calibrachoa x hybrida hort "Kabloom deep blue" with the same method of sterilization.

The low yield (20-40%) of viable explants is characteristic of all the studied introduced varieties of surfinia and calibrachoa without exception, using sterilization method No. 4, consisting of treating the buds with soap solution for 10 minutes, treating explants with 70% ethyl alcohol (exposure 10 seconds), sterilizing explants with 1% solution of silver nitrate at an exposure of 10 minutes.

When using the sterilization method No. 2, the studied varieties of calibrachoa and surfinia occupied an intermediate position in terms of the yield of viable explants, which amounted to 50-60%, respectively.

Table.1 Viability of explants, introduced varieties of surfinia and calibrachoa, depending on the method of their sterilization

Variety	Explant	Sterilization method											
		1st			2nd			3rd			4th		
		V	O	I	V	O	I	V	O	I	V	O	I
Surfinia x hybrida hort" Purple"	buds	18/90	0/0	2/10	11/55	7/35	2/10	14/70	4/20	2/10	6/30	14/70	0/0
Surfinia x hybrida hort "Black prince"	buds	17/85	3/15	0/0	12/60	8/40	0/0	18/90	0/0	2/10	4/20	16/80	0/0
Surfinia x hybrida hort "Blue Vein"	buds	14/70	4/20	2/10	11/55	5/25	4/20	14/70	3/15	3/15	6/30	12/60	2/10
Surfinia x hybrida hort "Double Red"	buds	17/85	2/10	1/5	10/50	6/30	4/20	16/80	2/10	2/10	4/20	15/75	1/5
Surfinia x hybrida hort "Star Yellow"	buds	16/80	2/10	2/10	12/60	6/30	2/10	14/70	4/20	2/10	6/30	13/65	1/5
Surfinia x hybrida hort "Blue"	buds	16/80	2/10	2/10	12/60	6/30	2/10	15/75	2/10	3/15	8/40	12/60	0/0
Calibrachoa x hybrida hort "Kabloom deep blue"	buds	16/80	3/15	1/5	10/50	6/30	4/20	13/65	4/20	3/15	4/20	12/60	3/15
Calibrachoa x hybrida hort "Kabloom white"	buds	15/75	2/10	3/15	10/50	7/35	3/15	12/60	3/15	5/25	5/25	12/60	3/15

Abbreviations: V - viable explants, O – oxidized, I - infected; in the numerator the number of explants, pcs., in the denominator – %.
Note. The calculation was made based on 20 explants for each variety.

Conclusion

Based on the analysis of the results of experimental studies obtained to study the effect of four sterilization methods No. 1- No. 4 on the yield of viable explants in introduced varieties of surfinia and calibrachoa, it can be stated that their yield depends both on the sterilization method, including the type of sterilizing compound, its concentration, and on the varietal belonging of the plant.The optimal method of sterilization for the studied introduced varieties of surfinia and calibrachoa should be considered method No. 1 using a 3% solution of the fungicide "Topaz" and a 5% solution of sodium hypochloride "Whiteness" at an exposure of 15 minutes for both compounds. The high viability of the explants of surfinia and calibrachoa was 75-90% when using the 1st method of sterilization; the lowest viability of 20-40% was noted when using the 4th method of sterilization. The intermediate position in terms of viability of 50-60% and 60-90% was occupied by the 2nd and 3rd sterilization methods, respectively.

References

Atroschenko, G.P., Gusev, G.G., Cherepanova, M.A. 1990. Clonal micropropagation of black currant. Tez.dokl. conf. of young scientists and students. LSHI, March-Apr., 1990: 43-44.

Averyanova, V.A., Alexandrova, I.V., Bykov V.A. 2002. Features of the callusogenesis of the May lily of the valley (*Convallaria majalis* L.) depending on the state of the explant.Biotechnology.5: 49-58.

Badoni, A.,Chauhan, J.S.2010. In vitro sterilization protocol for micropropagation of *Solanum tuberosum* cv. 'Kufri Himalini'. Academia Arena.2 (4): 24-27.

Bulatova, A.A. Shapchits, M.P., Yurin, V.M. 2009.Obtaining cell culture *in vitro* and optimizing the composition of the nutrient medium for the active growth of fragrant callisia. Proceedings of BSU.4(1): 1-4.

Dass, H.C., Ramesh, K. Kaul., Joshi, S.P., Bhansali, R. Raj. 1989. *In vitro* regeneration of date palm plantlets. Curr.Sci. (India).58 (1): 22-24.

Karule, P., Dalvi, V., Kadu, A., Chaudhari, R., Subramaniam, V.R., Patil, A.B. 2016. A commercial Micropropagation protocol for virupakshi (AAB) banana via apical meristem. African Journal of Biotechnology.15 (11):401-407.

Kozhakhmetov, M.K., Alimgazinova, B.Sh. 1990. Proliferation of callus from sugar beet explants for microcloning in vitro. Probl.teor. and approx. genet. in Kazakhstan: mater. Rep. conf. Alma-Ata, November 18-22, Alma-Ata:116-117.

Lebedev, M.B., Berestneva, Yu.V.,Volkov, I.V., Bikmetova, K.R., Lebedev, N.I. 2019. Investigation of the effectiveness of various methods of sterilization of potato explants during microclonal reproduction. Successes of modern natural science. 9: 26-30.

Liu, L.-J., Rosa-Márquez, E., Licha, M., Biascoechea, M.L.1988. Tanier (Xanthosoma spp.) propagation *in vitro.* J.Agr.Univ. P.R. 72 (3): 413-426.

Lukicheva, I.A., Migrinova, I.G. 2001.Callus tissue obtained from mature embryos of Aconitum septentrionale Koelle – a promising source of the alkaloid lappaconitin. Bulletin of Bashkir University. 2 (2): 94-95.

Melnichuk, M.D., Pinchuk, A.P., Maurer, V.M. 2004.Processes of callus formation i organogenesis in cultures in vitro hybrid poplars.Biologiya. Biotechnologiya. 5(1): 25-30.

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. Journal of Agricultural Sciences. 58(2):117-126.

Neskorodov, Ya.B., Mishutkina, Ya. V., Gaponenko, A.K., Scriabin, K.G. 2007. Method of regeneration *in vitro* of sunflower shoots (*Helianthus annuus* L.) from aseptic seeds as explants for genetic transformation. Biotechnology.6: 27-33.

Nikitina, A.V., Lenentkin, A.M., Lekontseva, T. G., Fedorov, A.V. 2020. Influence of the method of sterilization and the time of introduction into culture in vitro on the viability of explants of the clone rootstock of apple trees 54-118. Bulletin of the

Udmurt University. The series "Biology. Earth Sciences".4:411- 416.

Raškauskas, V., Kazlauskiené, R., Gudavičiené, N., Sirvydyté, D. 1989. Sterilinančiu medžiaguir mitybos terpiu parinkimas meristeminiu metodu dauginamoms orchidejoms. Scientific works of universities of the Lithuanian SSR. Ser. Biol. Sci.27:36-42.

Rokityanskaya, L.S. 2005. Search for effective methods of sterilization of mature barley embryos. Biology is the science of the XXI century: materials of the 9th International Pushchina School-Conference of Young Scientists. – Pushchino:190.

Shornikov, D.G. 2008.Improvement of the technology of reproduction of rare garden plants in culture in vitro and assessment of their resistance potential to abiotic stressors //Abstract.dis. ... Candidate of Agricultural Sciences. Michurinsk is a science city of the Russian Federation: 1-23.

Shumikhin, S.A. 2004. Optimization of individual stages of microclonal reproduction of cultural dahlia: sterilization of explants. Bulletin of Perm. University.2:61-63.

Tavartkiladze O.K., Mezentsev A.V. Method of tea propagation in vitro: a.s. 1311673 USSR, MCI A01 N 3/from No. 3868024/30-15; announced 10.01.85; published 12.01.87 // Bulletin of Inventions. – 1987.– No. 19.– p. 15.

Trukhanov V.A., Shalamai A.S., Belika A.M. The role of glycyl in increasing the viability of plant tissues introduced into culture // Probl. teor. and approx. genet. in Kazakhstan: mater. Republ. conf., Alma-Ata, November 18-22, 1990. – Alma-Ata, - 1990.– p. 113.

Wegayehu, F., Firew, M., Belayneh A. 2015. Optimization of explants surface sterilization condition for field grown each (*Prunus persica* L. Batsch. cv. 'Garnem') intended for in vitro culture. African Journal of Biotechnology.14(8): 657-660.

Zhatko, K.I., Vodchits, N.V., Volkova, E.M., Volotovich, A.A. 2017. Method of sterilization of strawberry explants (Fragaria L.) at the stage of introduction into culture in vitro. Collection of materials of the II International Scientific and practical conference "Biotechnology: achievements and prospects of development", Pinsk, 7-8 December: 8-10.

Zhumagulova, Zh. B., Frolov, S.N. 2014. Methods of sterilization of pear explants when introduced into aseptic culture. Research, results.Almaty:114-118.

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