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Influence of sterilizing compounds on the yield of viable explants of introduced varieties of *Lonicera edulis* Turcz.ex Freyn, *Chrysanthemum coreanum* (H. Levl. et Vaniot) Nakai ex T. Mori

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Abstract

The paper presents the results of experimental studies on the effect of various sterilizing compounds on the yield of viable explants in introduced varieties of chrysanthemumkorean and honeysuckle edible. It was shown that the yield of viable explants depends on the type of sterilizing compound, variety and species affiliation of plant.

Keywords: sterilizing compounds, explants, honeysuckle edible, chrysanthemum Korean

Introduction

The process of clonal micropropagation begins with the isolation of the explant, its sterilization and sowing on a nutrient medium. One of the fundamental roles in this process belongs to the selection of sterilizing compounds, the effectiveness of their concentrations and the duration of the treatment time with the aim of exemptionof material from infection and reception a high yield of viable explants.

An analysis of literature data and our own studies, regarding sterilization of plant material, which was introduced into culture in vitro showed, that various sterilizing compounds with different concentrations and exposure times are used for sterilization (Zuraida et al., 2014; Abou Dahab et al., 2010; Kiyosue et al., 1989; Mohammed and Butenko, 1998; Raškauskaset al., 1989; Akhmedova, 1999; De Lange et al., 1987; Balakrishnamurthy and Rangasamy 1988; Tvartkiladze and Mezentsev 1987; Goncharuk and Kalashnikova, 1998).

Sterilizing compounds according to the degree of their disinfecting effect can conditionally be divided into several groups:

1. Compounds with a strong disinfectant effect.
2. Compounds with a medium disinfectant effect.
3. Compounds with a mild disinfectant effect.

The first group includes compounds, containing mercury (mercuric chloride, diacid, mercurynitrate), silver nitrate. The second group includes hypochlorides of sodium, potassium, chloramine, bleach, that is, compounds containing active chlorine in their composition. The third group includes hydrogen peroxide and potassium permanganate with their inherent oxidizing properties.

Chloramine and hydrogen peroxide have the least pronounced toxic effect due to their rapid decomposition. They are usedfor sterilization of delicate, easily damaged tissues. Compounds, containing mercury are used in case of an ineffective action of solutions containing chlorine.

Chloroactive compounds (bleaching powder, chloramine) are traditional sterilization means. The mechanism of destruction of microorganisms by free chlorine is not fully cleared. Among the possible ways of influence ofchlorinerefer the suppression of some of the most important enzymatic reactions in the microbial cell, the denaturation of proteins and nucleic acids (Dychdala, 1983). Oxygen containing preparations, in particular hydrogen peroxide, are strong oxidizing agents, the basis of action of which is the formation of free radicals, that damage the lipids of the cell membrane, DNA (deoxyribonucleic acid) and other important components of the microbial cell.

Despite the synthesis by many microorganisms of catalase, which protects cells from influence of hydrogen peroxide by decomposing it into water and oxygen, used under sterilization of concentration H_2O_2, allow in most cases to overcome this mechanism of resistance (Turner, 1983). However, in its high concentrations, on the background of such positive qualities as a wide spectrum of activity, including bacterial spores, the ability to dissolve many biological compounds, lack of smell, rapid decomposition in the environment into non-toxic products, negative qualities are expressed - high tissue toxicity, manifested in the destruction of existing in the plant pigments, that leads to discoloration of the tissues. Therefore, use it with caution.

From the group of alcohols, ethyl and isopropyl alcohols are most widely used for disinfection. Their mechanism of action is in the denaturation of microbial proteins (Larson, 1991). Compounds, containing mercury, silver, are used in case of uneffective action of solutions, containing chlorine.

Silver is associated with the nitrogenous bases of deoxyribonucleic acid, as a result of which DNA stability is violated and, accordingly and the viability of bacteria, fungi and viruses. In addition, the rapid penetration of silver ions into the cell, into the cytoplasmic membrane, violation of function of the cell membrane (bacteriostatic effect) and blockade of many bacterial enzymes (bacteriolytic effect) leads to the inevitable death of microorganisms (Kulsky, 1955).

It should be said, that for each type of plant, the optimal regime of sterilization, which promotes to a high yield of viable explants, is established experimentally. So, for sugar beets, according to Akhmedova (1999) of the tested concentrations of various sterilizing compounds (silver nitrate, chloramine, hydrogen peroxide), only 0,1% silver nitrate solution provided a high yield of viable explants (80-100%).

Based on experimental studies, Tavartkiladze and Mezentsev (1987) concluded, that 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 s; at the second stage of sterilization – 0,05 – 0,20% aqueous solution of diacide for 5-10 minutes.

According to Ogurtsov (1988), it is advisable to use a 10% solution of chloramine for sterilization of anthers of mulberry (exposure time 15 min). For sterilization of date palm explants, Dass et al. (1989) used a 0,1% mercuric chloride solution.

As a result of the study of Liu Lii-Jang et al. (1988) of surface sterilization of explants Xanthosoma spp. the authors concluded, that chlorox was most effective in a concentration of 1 to 10%. Processing exposition 1-10 minutes. Similar studies were conducted for explants of potato (Trukhanov et al., 1990), sugar beets (Kozhakhmetov and Alimgazinova, 1990), blackcurrant (Atroshchenko et al., 1990) and other crops (Neskorodov et al., 2007; Averyanova etal., 2002; Bulatova et al., 2009). Analogous studies were conducted for aconite (Lukicheva and Migrinova 2001), poplar hybrids (Melnichuk et al., 2004), dahlia (Shumikhin, 2004), barley (Rokityanskaya, 2005), and other plants (Badoni and Chauhan, 2010; Mihaljević et al., 2013; Wegayehu et al., 2015; Karule et al., 2016; Buyanov, 2017).

Attention should be paid to investigations, conducted by Japanese scientists (Kiyosue et al., 1989) to study the effect of various sterilizing compounds and their concentrations on the sterilization of carrot seeds. In our opinion, the fact that their use of potassium hypochlorides at 5% concentration, calcium at 6%, and sodium at 10% concentration further stimulated the differentiation of somatic embryos of carrots turned out to be quite interesting. In the case of using a solution of calcium hypochloride, a positive correlation was found between the duration of treatment and the frequency of formation of somatic embryos.

It should be noted that for each type of plant, the optimal sterilization mode, which contributes to a high yield of viable explants, is determined experimentally.

Unfortunately, no information was found in the literature available on studies regarding the influence of sterilizing compounds on the yield of viable explants in introduced varieties of honeysuckle edible, chrysanthemumkorean. In this regard, we conducted experimental studies affecting this issue.

Materials and Methods

The objects of study were five introduced varieties of chrysanthemumkorean: 'Natalie', 'Interval', 'Garnet bracelet', 'Mishal', 'Gold of Scythians' and two varieties of honeysuckle edible: 'Zoyka' and 'Woytek'. As sterilizing compounds for the above varieties, 0,1% solutions of mercuric chloride, silver nitrate, and diacid were tested in combination with treatment with 70° ethanol. The exposition time for ethanol was 5 seconds, diacide, mercuric chloride and silver nitrate – 6 minutes. Taking into account that varieties of chrysanthemum koreanand honeysuckle edible were introduced into a sterile culture, and not species in quality of explants of which, were used buds of young shoots (Table 1). After sterilization, the material was washed in three changes of sterile double-distilled water for 15 minutes in each, and then planted on a modified agar medium MS. Tubes with planted explants were placed on racks where the air temperature was 24 ° C, illumination – 4000 lux, relative humidity – 70%, photoperiod – 16 hours. The registration of infected, oxidized and viable explants was carried out daily for 2 weeks. The experimental data are shown in Table 1.

Results and Discussion

The numbers in Table 1 indicate the dependence of the yield of viable buds on the type of sterilizing compound, variety and species affiliation of the plant.

A high yield (100%) of viable buds was observed in two introduced varieties of chrysanthemumkorean: 'Natalie' and 'Gold of Scythians', regardless of the type of sterilizing compound. This indicator is somewhat lower for the variety 'Interval' (85%), 'Garnet bracelet' (90%), 'Mishal' (95%).

For introduced varieties of honeysuckle edible, a high yield of viable explants was noted when sterilization was conducted with 0,1% diacid solution. For the 'Zoyka' variety, this indicator was 60%, for 'Woytek'– 70%.

Based on the analysis of the results of experimental studies obtained in study of the influence of sterilizing compounds on the yield of viable explants in introduced varieties of chrysanthemum koreanand honeysuckle edible, it can be stated that the yield of viable explants depends on both the type of sterilizing compound and the variety and species affiliation of the plant.An optimal sterilizing compound for the buds of two introduced varieties of chrysanthemumkorean: 'Natalie' and 'Gold' of 'Scythians should' be considered a 0,1% solution of silver nitrate, mercuric chloride and diacide at an exposure of 6 minutes, for the buds of the other two varieties – 'Interval', 'Granate bracelet'– 0,1% solution of mercuric chloride at the same exposure, for buds of varieties 'Mishal' – 0,1% solution of mercuric chloride and diacide; for the kidneys of the introduced varieties of honeysuckle edible: 'Zoyka' and 'Voytek' – 0,1% solution of diacide.

Conclusion

1. In order to prevent the infection of introduced varieties of chrysanthemum korean at the introduction into an in vitro culture, the explants should be sterilized in a 0,1% solution of mercuric chloride, silver nitrate and diacide. Explants of introduced varieties of honeysuckle edible should be sterilized in a 0,1% diacid solution.

Table 1 - Viability of explants of introduced varieties of chrysanthemum korean and honeysuckle edible depending on sterilizing compounds

Species, variety	Explant	Concentration of solution of sterilizing compound, %								
		Mercuric chloride– 0,1			Silver nitrate– 0,1			Diacid – 0,1		
		The exposition time, min								
		6			6			6		
Chrysanthemum korean:		**I**	**O**	**V**	**I**	**O**	**V**	**I**	**O**	**Ж**
'Natalie'	buds	0/0	0/0	20/100	0/0	0/0	20/100	0/0	0/0	20/100
'Interval'	buds	0/0	0/0	20/100	0/0	3/15	17/85	0/0	0/0	20/100
'Garnet bracelet'	buds	0/0	0/0	20/100	0/0	2/10	18/90	0/0	3/15	17/85
'Mishal'	buds	0/0	0/0	20/100	0/0	1/5	19/95	0/0	0/0	20/100
'Gold of Scythians”	buds	0/0	0/0	20/100	0/0	0/0	20/100	0/0	0/0	20/100
Honeysuckle edible:										
'Zoyka'	buds	10/50	0/0	10/50	13/65	0/0	7/35	8/40	0/0	12/60
'Woytek'	buds	12/60	0/0	8/40	15/75	0/0	5/25	6/30	0/0	14/70

Abbreviation: I - infected, O–oxidized,V - viableexplants;
in thenumerator is the number of explants, pieces, in the denominator – %.
Annotation. The calculation was based on 20 explants for each variety.

2. It was shown that, of all the sterilizing compounds, that we tested, a high yield of viable explants of introduced varieties of chrysanthemum Korean was obtained with using of three types of sterilizing compounds: mercuric chloride, silver nitrate, and diacid at a concentration of 0,1%; for introduced varieties of honeysuckle edible, a high yield of viable explants was obtained with using diacid in a analogous concentration. The exposition time was 6 min for all compounds.

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