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Viability of Explants Rhododendrons (Rhododendron L.) Depending on Sterilizing Compounds

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Abstract: Results are presented on the influence of sterilizing compounds upon the yield of viable explants of Rhododendron in sterilized culture. Yield of viable explants is dependent upon type of sterilizing compound, species belonging of plant and type of explant. Results show, that 0,1% solution of silver nitrate is a most effective compound for sterilization of seeds of 8 Rhododendron species (sterilization for 5 minutes) and 0,1% solution of sublimate and diacid (sterilization for 8 minutes) are most effective for sterilization of buds of 4 Rhododendron species.

Keywords: sterilizing compounds, seeds, buds, rhododendrons

INTRODUCTION

The process of clonal micropropagation consists of explant isolation, sterilization and planting on nutritious medium. Cacas and Lasa (1986) investigated efficiency of 5 sterilizing compounds on yield of explants of sugar beet. Chloride of mercury is a most effective in concentration 1% for 1 min. Kudina and Dovbysh (1990) for sterilization of buds of rose sorts offered to use 70% solution of ethanol for 2 min and 10% solution of hydrogen peroxide for 15 min. Ruskauskas et al. (1989) note, that the best mean for sterilization of orchid are next compounds: 70% solution of ethanol (sterilization for 2 min) and 0,1% solution of diacid (sterilization for 5 min) and 10% solution of chloramine (sterilization for 10 min). Sudhadevi and Nataraja (1987) for sterilization of explants *Dalbergia latifilia* Roxb offered to use chloride of mercury. More effective compounds for sterilization of explants of tea (Camellia sinensis (L.) Kuntze) are 1-2% solution of hydrogen peroxide and 50-60% soluton of ethanol (the first sterilization – 10-15 sec) (Tvartkiladze and Mezentzev 1987).

At the second sterilization is applicated 0.05-0.2% solution of diacid for 5-10 min. Balakrishnamurthy and Rangasamy (1988) offer to sterilize floral apex of banana by 70% solution of ethanol for 30 sec, after that with 0.1% solution of sublimate for 5 min with the next washing in steriled water.

We divide sterilizing compounds into some groups:
1. Compounds, possessed by strong disinfecting action
2. Compounds, possessed by middle disinfecting action
3. Compounds, possessed by weak action

Compounds, which contain mercury (sublimate, diacid, nitric acid mercury), nitric acid silver, belong to the first group. Compounds, which contain active chlorine, sodium and potassium hypochloride, chloramine, chloride of lime, belong to the second group. Hydrogen peroxide, potassium permanganate with their oxidizing properties belong to the third group. Chloramine and hydrogen peroxide possess by weak toxic action owing to their fast decomposition. We use these substances for sterilization of tender tissues. Combinations, which contain mercury are used in the case of uneffective action of solutions with chlorine.

Chlorine active combinations (chloride of lime, chloramine) are traditional means for sterilization. Mechanism of destruction of microorganisms with the help of free chlorine is not cleared. Probable ways of chlorine affection are related to suppression of some important ferment reactions in microbe cell, denaturation of proteins and nucleic acids (Dychdala 1983). Preparations, which contain oxygen (for example hydrogen peroxide) are strong oxidants, base of action of which is formation of free radicals, which injure lipid of cell membranes, DNA and another important components of microbe cell.

In spite of synthesis of catalase by microorganisms, which protect cells from affection of hydrogen peroxide by the way of decomposition it into water and oxygen, used in sterilization concentrations H_2O_2 allow to overcome present mechanism of resistance

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(Turner 1983). However, hydrogen peroxide has positive and negative properties.

Hydrogen peroxide in high concentrations has a wide spectrum of activity, ability to dissolve many biological combinations, has no an adour, a fast decomposition into untoxic products in environment. And hydrogen peroxide has such negative properties: high tissue toxicity, which is developed in destruction of plant pigments, that leads to colourless of tissues. So, it is necessary to use it with care.

From group of spirits, ethyl alcohol and isopropyl alcohol are widely used in disinfection. Mechanism of their action consists of denaturation of microbe proteins (Larson 1991).

It is necessary to note, that for every species of plants optimum regime of sterilization, which promotes high yield of viable explants, is determined by experimental way. Thus, according to data of Achmedova (1999), from tested concentrations of various sterilizing compounds (nitrate of silver, chloramine, hydrogen peroxide) only 0.1% solution of nitrate of silver ensured the high yield of viable explants of sugar-beet. Analogous investigations were carried out with the plants of black currants (Atroschenko at al. 1990), aconite (Melnichuk at al. 2004), dahlia (Shumichin 2004), barley (Rokityanskaya 2005) and other plants.

It should be concentrate attention on data of japanese scientists Kiyosue and Kamada (1989) about investigations of affection of various sterilizing compounds and their concentrations under conditions of disinfection treatment of carrot seeds.

It was interesting fact, that use of potassium hypochloride in 5% concentration, calcium hypochloride in 6% concentration, sodium hypochloride in 10% concentration subsequently stimulated differentiation of somatical germs of carrot. In a case of application of calcium hypochloride solution we can reveal positive correlation between duration of treatment and frequency of formation of somatical germs.

Unfortunately in literature there were not revealed data of investigations, accoding to influance of sterilizing compounds on the yield of viable explants of introduced plants of rhododendron species. For every plant optimum regime of sterilization, promoting to high yield of viable explants, is determined by experimental way. In this connection we were carried out experimental investigations as for this question.

MATERIALS AND METHODS

Objects of investigation were 12 introduced species of rhododendron: *R. catawbiense* Michaux, *R. ponticum* L., *R. smirnowii* Trautv., *R. japonicum* (A.Gray) Suring, *R. brachycarpum* D.Don, (syn. *Azalea brachycarpa* D.Don), *R. kotschyi* Simonk, *R. haemaleum* Balf. f. & Forrest, *R. minus* Michaux, *R. discolor* Franch, *R. roseum* (Loisel.) Rehd., *R. fortunei* Lind., *R. schlippenbachii* Maxim.

For these 12 rhododendron species we tested next sterilizing compounds: 0.1% solutions of diacide, sublimate and silver nitrate in combination with the treatment of 70° ethanol. The time of sterilization with ethanol was 5 sec, with diacide and sublimate - 8 min, with silver nitrate - 5 min. We investigated and used as explants buds and seeds of rhododendron species in culture in vitro. For 4 species of rhododendron (*R.japonicum, R. catawbiense, R. smirnowii, R. ponticum*) used top and lateral buds of young shoots as explants; for 8 rhododendron species (*R. fortunei, R. minus, R. kotschyi, R. schlippenbachii, R. discolor, R. brachycarpum, R. roseum, R. haemaleum*) we used seeds as explants.

After sterilization we washed thoroughly plant material three times in steriled bidistillationed water for 15 minutes, after that this plant material was transferred on nutrient agar Andersen"s medium (1975), wich contain inorganic salts , vitamins , 3 % (w/v) sucrose and 0.8 % Difco bactoagar. The level of pH of the medium was to 4.8 before autoclaving at 1.06 kg/cm^2 pressure for 20 min at 121^{o}C. 15 ml of this medium was used in a 25 x 150 mm test tube. Test tubes with transplanted explants put on the shelfs, where temperature of air was 24 ± 2^{o}C, illumination - 4000 lk, relative humidity of air was 70%, photoperiod - 16 hours. Registration of infested, oxidized and viable explants was conducted daily, during 2 weeks. Experimental data are presented in the Table.

RESULTS AND DISCUSSION

Figures in the table testify to high yield (100%) of viable seeds of investigated species of rhododendron independently of type of sterilizing compound, with the exception of two species *R. minus* (85%) and *R. kotschyi* (80%), which had small sizes of seeds (0.4× 0.1 mm).

Yield of viable buds depends on type of sterilizing compound and species belonging of plants. We marked highest yield of viable buds of *R. japonicum*. This index is lower as for *R. catawbiense* (85%), *R. ponticum* (90%) and *R. smirnowii* (95%). It is connected with belonging of *R. japonicum* to deciduous shrubs and of another species of rhododendron to evergreen shrubs. Buds of deciduous *R. japonicum* are less infected, because they were isolated from shoots, grown under conditions of glasshouse. Unfortunately, shoots of evergreen rhododendrons are incapable of growth

under these conditions, so their buds are more infected.

At the base of our investigations we drew a conclusion, that yield of viable explants depends on type of sterilizing compound, species belonging of plant and also depends on type of explant. 0,1% solution of silver nitrate is a most effective compound for sterilization of seeds of 8 Rhododendron species (sterilization for 5 minutes) and 0,1% solution of sublimate and diacid (sterilization for 8 minutes) are most effective for sterilization of buds of 4 Rhododendron species.

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Table 1. Viability of explants of introduced species of rhododendron depending on sterilizing compounds.

Species	Explant	Concentration of solution of sterilizing compound, % v/v								
		Silver nitrate, 0.1			Diacide, 0.1			Sublimate, 0.1		
		Time of exposition, min								
		5			8			8		
		I	O	V	I	O	V	I	O	V
R. catawbiense	buds	0/0	3/15	17/85	0/0	0/0	20/100	0/0	0/0	20/100
R. ponticum	buds	0/0	2/10	18/90	0/0	3/15	17/85	0/0	0/0	20/100
R. smirnowii	buds	0/0	1/5	19/95	0/0	0/0	20/100	0/0	0/0	20/100
R. japonicum	buds	0/0	0/0	20/100	0/0	0/0	20/100	0/0	0/0	20/100
R. brachycarpum	seeds	0/0	0/0	20/100	0/0	0/0	20/100	0/0	0/0	20/100
R. kotschyi	seeds	0/0	4/20	16/80	0/0	0/0	20/100	0/0	0/0	20/100
R. haemaleum	seeds	0/0	0/0	20/100	0/0	0/0	20/100	0/0	0/0	20/100
R. minus	seeds	0/0	3/15	17/85	0/0	0/0	20/100	0/0	0/0	20/100
R. discolor	seeds	0/0	0/0	20/100	0/0	0/0	20/100	0/0	0/0	20/100
R. roseum	seeds	0/0	0/0	20/100	0/0	0/0	20/100	0/0	0/0	20/100
R. fortunei	seeds	0/0	0/0	20/100	0/0	0/0	20/100	0/0	0/0	20/100
R. schlippenbachii	seeds	0/0	0/0	20/100	0/0	0/0	20/100	0/0	0/0	20/100

Abbreviation: I - infected, O - oxidized, V - viable explants; Quantity of explants is in numerator (pieces); in denominator is %.

Annotation: Calculation was carried out issued from 20 explants for every species.

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