STUDIES REGARDING OPTIMIZATION PROTOCOL OF REGENERATION TO IN VITRO AT *RUBUS IDAEUS* AND *RIBES NIGRUM*

CERCETĂRI PRIVIND OPTIMIZAREA PROTOCOLULUI DE REGENERARE IN VITRO LA *RUBUS IDAEUS* ŞI *RIBES NIGRUM*

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Abstract. *Selection of biological material from grown plants for use in micropropagation often to be infected, therefore it is necessary to establish the optimal experimental protocol for preparing explantes. This requires developing a protocol for regeneration of a particular variety on their experiments, using existing knowledge as guidelines. This study aimed to identify the most effective method of sterilization by studing the number of „clean"and live explants for each sterilisation method. Were used axillary buds explaints of black currant, axillary buds explaints of raspberry and leaves explaints of raspberry red. Three sterilization methods were tested using 55 bleach solution with three time on exposure after prior treatment with Topsin 70 PU 0,1% or 70% ethanol. In the third method of sterilization used only 5% sodium bleach. The lowest percentage of 'clean'explants was provided by the method involving a preliminary treatment of biological material with ethanol, but the procentage of live explants was lowwer than the method with Topsin, so that there is the possible use of higher concentrations of fungicide, ultimately resulting in better sterilization.*

Key words: raspberry, blackcurrant, micropropagation, sterilization

Rezumat*. Materialului biologic recoltat din cultura mare este adeseori infectat, de aceea este necesar a se stabili prin experimentare protocolul optim de pregătire a explantelor. Astfel se impune elaborarea unui protocol de regenerare pentru fiecare soi pe baza propriilor experimente, folosind cunoştinţele existente ca linii directoare. Acest studiu a urmărit identificarea modului optim de sterilizare a explantelor de coacăz negru şi zmeur prin stabilirea numărului de plante contaminate şi a numărului de plante viabile pentru fiecare variantă de sterilizare. S-au utilizat ca explante muguri axilari de coacăz negru, muguri axilari şi limbul foliar de zmeur roşu. Au fost testate trei metode de sterilizare folosind hipocloritui de sodiu 5% cu trei graduări ale timpului de expunere (15', 20' şi 30') după un tratament prealabil cu Topsin 70 PU 0.1% sau cu alcool etilic 70%. În cea de-a treia variantă de sterilizare s-a folosit doar hipocloritul de sodiu 5% . Rezultatele obţinute demonstrează că decontaminarea explantelor se realizează mai bine prin spălare cu etanol, dar viabilitatea explantelor sterile este superioară în cazul utilizării Topsin-ului, astfel încât există posibilitatea utilizării unor concentraţii mai mari de fungicid, în final rezultând o sterilizare mai bună.*

Cuvinte cheie: zmeur, coacăz negru, micropropagare, sterilizare.

INTRODUCTION

Sterilization of explants is a crucial step for induction and maintenance of the cultures *in vitro*. The loss caused by contamination in vitro is between 3 and 15% for every subculture and in most cases is likely bacterial or fungal (Leifert, et al 1994). The major risk factor for micropropagation is the use of the explants harvested directly from the plantations of fruit bushes and not in glasshouses or greenhouses, where is possible the phytosanitary control of the mother plant (also called donor plants). Therefore it is necessary for sterilization of biological material used to find the most effective and easiest to use for each type of species and explant.

In techniques of micropropagation all the disinfectants used are surface disinfectants substances, capable of destroying in short time the micro organisms adherents to the surface, but not into too much depth to them and destroy them.

MATERIAL AND METHOD

The researches were carried in Laboratory *in vitro* culture of the discipline of Genetics and Plant Breeding from U.S.A.M.V. Iassy. Biological material, collected in September 2008, was axilary buds (Tsema variety), axilary buds of raspberry and leaf of raspberry (Willamete variety). In conducting research aimed to respect the general protocol making cultures meristems (Reed WB et al, 2004).

For to establish the most effective alternatives to sterilize of the explants, have used the 4 experimental variants in rehearsals by 3:

The first variant: treatment with antifungal Topsin PU 70 (0.1%) and sodium hypochlorite 5%

The second variant: washing with 70% ethanol and sodium hypochlorite 5%

The third variant: treatment with sodium hypochlorite 5% (control variant).

In all variants, were tested 3 times for the exposure of explants to the sodium chloride: 15 minutes (T 15), 20 minutes (T20), 30 min (T30).

Organization experience: 3 rehearsals [x 3 variants (5 x 4 explants)]. The explants after sterilization were introduced in 5 balloons Erlenmayer with 40 ml culture medium of Murashige & Skoog (MS). The explants were inoculated aseptically. The cultures were maintained at 25 ± 2°C under 16 h photoperiod. Subsequently, the cultures were maintained by regular subculture at four week intervals on fresh medium with the same composition.

At 7 and 14 days was determined the percentage of contamination identified visible reporting the number of infected to explant the initial explants. Also at 14 days was determined and the percentage of viable explants.

RESULTS AND DISCUSSIONS

The disinfection of plant material prior to sterilization effect increased in both species studied, but it was not possible a full decontamination of the explants. The efficiency was higher only at the disinfection with hypochlorite for 30 minutes. At the same time this version has a very pronounced phytotoxic effect, the number of viable explants was below 10%, leading to a reduced applicability of this method of sterilization. The use of Topsin in sterilization of biological material ensured the highest proportion for currant explants (table 1) and the raspberry explants (figure 1).

Table 1

Effect of the different sterilization methods on the blackcurrant explants

Sterilization method		Explants	Percentage of contamination (%)	Percentage of viable explants (%)
1. Topsin	**T 15**	axillary buds	57	69
	T 20	axillary buds	22	53
	T 30	axillary buds	2	12
2. Ethanol	**T 15**	axillary buds	34	56
	T 20	axillary buds	11	51
	T 30	axillary buds	0	8
3. Control variant	**T 15**	axillary buds	59	81
	T 20	axillary buds	38	62
	T 30	axillary buds	2	12

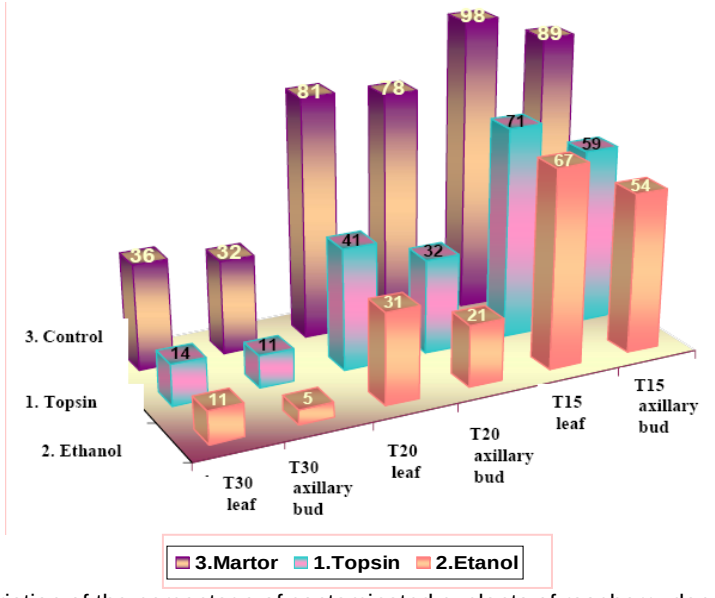

Fig.1. Variation of the percentage of contaminated explants of raspberry depending on sterilization

Decontamination of explants is performed better by washing with ethanol, but the percentage of viable explants in this case is small. The percentage of viable explants increased when used Topsin (fig 2) so that there is a possibility to use higher concentrations of fungicide, ultimately resulting in better sterilization.

The changes of time sterilization with sodium hypochlorite showed an increase in the degree of decontamination with increasing time of exposure, but also phytotoxicity inversely proportional to it. The reaction of explants at the action of sodium chloride depended on the type of the explant. The leaf blade presents an increased sensitivity to the sodium chloride so that the sterilization of these explants can't be achieved only by using pre-treatment with Topsin which allows for the use of a shorter exposure action of sodium chloride.

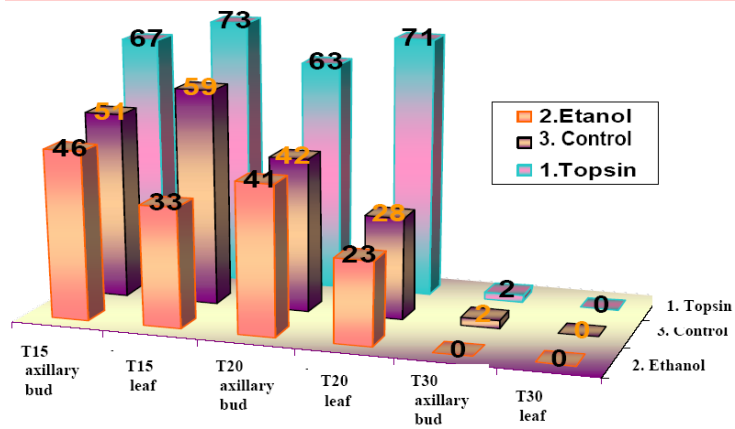

Fig.2. Changes in the percentage of viable explants of raspberry depending on sterilization

Decrease of the percentage of the viable explants in the experimental variants II and III can be caused by a number of endogenous pathogens that can not be removing used of the ordinary disinfectants.

CONCLUSIONS

The degree of contamination of the explants harvested directly from the field of culture was higher than that encountered in the literature for the explants collected from glasshouses or greenhouses.

Increasing exposure time of the explants to hypochlorite sodium from 15 to 30 minutes to ensure a greater number of uninfected explants, but decreases the number of viable explant.

The sterilization with the best results was when to use the disinfection prior Topsin 1% and treated with sodium hypochlorite 5% for 20 minutes.

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