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THE INFLUENCE OF GROWTH REGULATORS CONCENTRATIONS ON *IN VITRO* MICROPROPAGATION OF *RIBES RUBRUM* SPECIES

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Abstract

In vitro cultures have found in a short time many practical applications, including genetic improvement of crop species, the multiplication of valuable genotypes, free virus propagation of existent vegetal stocks and conservation of genetic resources. In the present study we aimed to undertake a basic and simple protocol for in vitro micropropagation of Ribes rubrum considering the influence of various concentrations of growth regulators. There were used six variants (M1 ÷ M6) of half-strength MS medium supplemented with growth regulators, as follows: M1 (BA 0.2 mg/l and IBA 0.01 mg/), M2 (BA 0.4 mg/l, IBA 0.02 mg/l and GA_3 0.1 mg/l), M3 (BA 0.4 mg/l, IBA 0.02 mg/ and GA_3 0.2 mg/l), M4 (BA 0.15 mg/l, IBA 0.015 mg/l and GA_3 0.15 mg/l), M5 (BA 0.5 mg/l) and M6 (BA 1 mg/l). The best medium for obtaining micro shoots was half-strength M3 medium. Therefore, the highest rate of in vitro shoot proliferation and elongation was obtained by using combination BA/IBA/GA_3 as growth regulators.

Key words**:** *growth regulators, micro propagation, Ribes rubrum, medium*

INTRODUCTION

Ribes is a genus of about 150 species of flowering plants native throughout the temperate regions of the Northern Hemisphere. *Ribes rubrum* (red currant) is a rare species from the spontaneous flora, valuable for its medicinal properties and commercial uses in pharmaceutical and food industry due to his high antioxidant potential, provided by the high content of ascorbic acid [1]. Besides this, offers the possibility to exploit the entire bush, constituted from leaves, fruits, seeds, buds and flowers.

In vitro multiplication efficiency in various species is widely dependent on composition of culture medium, growth regulators and genotype [2, 3, 4].

Usually, gibberellic acid promotes shoot elongation and reduced the callus and roots formation as well as the development of shoots [5].

Studies on *in vitro* currant culture are limited in comparison to other fruit woody species. One of this studies was represented by red currant cultivar *Detvan*. The objective of this study was to develop a multiplication protocol using two different variants of medium containing various concentrations of BAP, IBA and GA_3 [6]. Other study, describe the obtainig of rudimentary plantlets from fertlized ovules collected from old berries of red currant cultured on Miller's medium [7].

However, they were indicated some successfull data with regard to propagation of *Ribes* genus, but the number of newly formed plantlets usually did not exceed 3–4 shoots per original explant [5].

Application of *in vitro* micro propagation of axillary bud has been recorded in great number of blackberry and black currant cultivars. With regard to black currant micro propagation, fourteen black currant cultivars have been studied from a range of geographical origins, using medium supplemented with BA [7, 8, 9].

In vitro micro propagation requires an extraordinary deal of experiments on optimization of both black and red currant cultivar [10].
This study was conducted to establish an efficient method of micropropagation of red currant plantlets.

MATERIAL AND METHOD

Branches of red currant with dormant buds were cut and placed in distilled water under laboratory conditions, at room temperature. After the activation of buds, standard sterilization procedure was applied of the bud surface. The buds were isolated with size ranging from 0.3 - 0.8 cm and placed on Murashige and Skoog [11] nutrient medium with following hormone concentrations: M1(BA 0.2 mg/l and IBA 0.01 mg/), M2(BA 0.4 mg/l, IBA 0.02 mg/l and GA_3 0.1 mg/l), M3 (BA 0.4 mg/l, IBA 0.02 mg/ and GA_3 0.2 mg/l), M4 (BA 0.15 mg/l, IBA 0.015 mg/l and GA_3 0.15 mg/l), M5 (BA 0.5 mg/l) and M6 (BA 1 mg/l). The medium consisted of half-strength MS basal salts and vitamins supplemented with 3% sucrose, and solidified with 0.7 % agar. The pH of MS medium was adjusted to 5.7 using 0.1 N NaOH.
The study was conducted on 40 samples for each variant of medium, inoculating one explant; the tubes were closed and kept in climate chamber at a temperature between 23-25° C and a photoperiod of 16 hours (fluorescent lights).

RESULTS AND DISCUSSIONS

In this experiment were significant differences noticed on plant height and number of shoots (Table 3).
Culture medium containing different concentrations of growth regulators present different values of multiplication in four weeks after starting experiment. Placing the explants for three weeks led to a high rather percentage incidence of microbial infection, which induced a large increase in highly infected cultures, especially in case of M1and M2 (Table 1).

Table 1. Obtainment of aseptic culture – rosette formation

Variants	% of infected cultures	Induced rosette (%)
M1	60.08	39.08
M2	54.55	45.45
M3	26.42	73.58
M4	32	68
M5	55	45
M6	85	15

Upon establishment of aseptic culture, were collected growth peaks and inoculated on MS multiplication medium with different concentrations of growth regulators listed in Table 2. The explants were placed on the multiplication medium and after four weeks, following parameters were monitored: number of shoots and plant height.

Table 2. Hormonal content of medium used in multiplication of *Ribes rubrum* explants

Variants	Macroelements NH_4NO_3 / KNO_3	BA mg/l	IBA mg/l	GA_3 mg/l
M1	MS/2	0.2	0.01	-
M2	MS/2	0.4	0.02	0.1
M3	MS/2	0.4	0.02	0.2
M4	MS/2	0.15	0.02	0.15
M5	MS/2	0.5	-	-
M6	MS/2	1	-	-

There is a significant difference in response of the different explants inoculated on the better selected medium. The best medium for obtaining micro shoots was half-strength M3 medium with regard to plant height. Also, in this case, there were rising many and highest shoots with plantlets having green leaves and firm consistency (Table 3).
Usually, MS medium supplemented with higher concentration of BA stimulates formation of shoots [12]. Therefore, in case of M5 medium, plant height was similar with the results obtaining using the combination BA/IBA/GA_3 (M3). At the opposite pole, we find that a great concentration of BA inhibits the growth of *in vitro* newly plantlets.
According to Figure 1, we can observe a significant influenced shoot proliferation and average number of shoots in case of M5 medium. However, the best results in both plant height and shoot proliferation we obtained in case of M3 medium.

Table 3. Mean values for plant height (PH), assessed at different growth regulators concentrations and number of shoots (NS), after four weeks from cultivation

Treatments	Means	
	PH (cm)	NS
M1	0.51	1.5
M2	0.54	1.24
M3	0.65	1.5
M4	0.62	1.00
M5	0.64	2
M6	0.40	1.6

With regard to M3 and M5 variants of medium, we can say that increasing BA concentration up to 1 mg/l increase and average values of plant height and number of apices.

The difference between mean values for plant height in case of M3 and M5 treatments is 0.01cm, insignificant. However, considering the range of variation in the concentration of BA, 0.1mg/l would induce an increase in height of 0.01 cm.

With regard to the concentration of IBA, we can not say that influenced positively or negatively *in vitro* multiplication of red currant explants. One explanation could be that it was found in much lower concentrations compared with the others growth regulators.

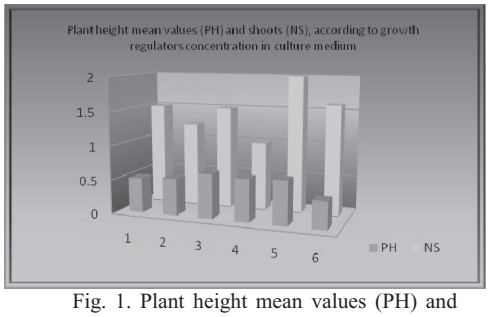

Fig. 1. Plant height mean values (PH) and number of shoots (NS), according to growth regulators concentration in culture medium

To compare the different treatments it was calculated the standard deviation and coefficient of variance within each treatment with regard to plant height and number of shoots (Table 4 and 5).

The calculation formulas used, were:

- The average (μ),

$$\mu = \frac{\sum_{x=i}^{x=n} xi}{n};$$

- Standard deviation (**SD**),

$$SD = \sqrt{\frac{\sum_{x=i}^{n} (xi - \mu)^2}{n}};$$

- Coefficient of variance (**CV**) %,

$$CV = \frac{S}{\bar{x}} \times 100.$$

With regard to CV in Table 4, we can speak about the M6 and M5 variants of medium like a homogenous population of plant height with a high degree of representation. The highest percentage is reached when the M4 medium, being below 45%, having an average with low significance level and a heterogeneous population.

Table 4. Standard deviation and coefficient of variance according to PH, in different variants of medium

The values of standard deviation (SD) and coefficient of variance (CV) according to plant height						
Variants of medium	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**
Average	0.524	0.527	0.634	0.641	0.644	0.408
Standard deviation (SD)	0.13	0.14	0.18	0.25	0.12	0.03
Coefficient of variance (CV) %	25.41	27.18	28.55	39.35	18.87	8.08

According to CV from table 5, we can observe a homogeneous population with a significant degree of representation, present in M6 medium.

At the opposite pole are M3 and M5 variants of medium, having a CV between 43 – 45%. In this case, the average is not representative.

Table 5. Standard deviation and coefficient of variance according to NS, in different variants of medium

The values of standard deviation (SD) and coefficient of variance (CV) according to number of shoots						
Variants of medium	M1	M2	M3	M4	M5	M6
Average	1.533	1.238	1.564	1.06	2	1.62
Standard deviation (SD)	0.52	0.42	0.7	0.23	0.87	0.69
Coefficient of variance (CV) %	34.03	34.4	45.31	22.49	43.64	4.34

The plantlets obtained after one month, were shaped and transferred to a rooting medium: MS (with 1/5 macro) supplemented with 0.01 mg/l BA and kept in darkness for five days. Then, they were transferred to light for three weeks. After this period, the process of rooting was not visible.

CONCLUSIONS

From the results, we can say that plant growth regulators clearly affects shoot development from buds explants cultivated *in vitro* and shoot multiplication.

Growth regulators concentration influenced accumulation of biomass (fresh weight) of *Ribes rubrum* plantlets propagated *in vitro*. The presence of BA 0.4 mg/l, IBA 0.02 mg/ and GA_3 0.2 mg/l in the culture medium was the most efficient treatment for increasing height and fresh weight of plants *in vitro* cultured.

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