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RESEARCH ARTICLE

Effect of some components on micropropagation of garden crops

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Micropropagation of garden crops (steppe cherry "Pamjati Lewandovskogo", remontant raspberry "Hercules", chrysanthemum korean (multiflora group) "Harmoniya" and "Daphna") was performed on Murashige and Skoog (MS) medium supplemented with different auxin and cytokinin growth regulators. Agar and potato starch at different concentrations were used as a medium amendment. The combination of 4.0 gr/l agar and 20.0 gr/l starch has been found to be very effective. The addition of antibiotic (amoxicillin) to the culture medium at the micropropagation stage prevented contamination of regenerants and improved their growth.
Keywords: cherry; raspberry; chrysanthemum Korean; tissue culture; potato starch; antibiotic; multiplication

Introduction

In vitro micropropagation of plants has been widely used throughout the world for more than 50 years, not for solving fundamental issues only. First of all, it is an auxiliary tool in plant breeding, as well as the technique to create genetic collections and to preserve a wide diversity of plant species. This method is widely used for commercial purposes for rapid replication of new hybrids, selected forms and varieties of flowers, ornamental plants, fruits, berries and agricultural crops.
In vitro cultivation of plants (organs/parts) is carried out using solid or liquid media containing micro- and macroelements, vitamins, growth regulators, sucrose and agar. The most common is MS medium (1962). This medium is used for propagation of sweet cherry (Sedlak et al., 2008; Paprstein et al., 2017), peach (Rerez-Jimenez et al., 2012), strawberry (Ayub et al., 2016), raspberry (Georgieva et al., 2016), and chrysanthemums (Jerzy et al., 2015). An essential amendment component of the solid medium is agar used at concentration of 7.0–8.0 gr/l. Gelrite is added to culture medium along with agar (Sriskandarajah et al., 2009). The laboratories engaged in investigation of tissue culture *in vitro*, face the problem of growing the explants which are free from bacterial and fungal contamination at every cultivation stage. Often a latent infection, which does not manifest itself immediately, leads to the loss of plant material and reduces the yield of the plants grown. This is especially important when it comes to commercial laboratories (Reed et al., 1998). Studies related to modernization of culture media are relevant, since on the one hand, they maximize regenerant yield, and on the other hand, they can reduce the costs of *in vitro* micropropagation of plants.
The study aims to investigate the effect of media composition on garden crops regeneration in clonal micropropagation.

Materials and methods

Explants. The material used in the study was micro-shoots isolated from *in vitro* cultivated regenerants of steppe cherries "Pamjati Lewandovskogo", remontant raspberry "Hercules", and chrysanthemum korean (multiflora group) "Harmoniya" and "Daphna" from the collection of the Research Institute of Horticulture in Siberia.
Culture media for micropropagation. An agar medium (bacteriological agar (Pronadisa ™) in combination with potato starch (20.0 and 40.0 gr/l)) was used to cultivate the explants. The culture medium was composed of Murashige and Skoog (MS) salts (Murashige and Skoog, 1962). The culture medium was also supplemented with various concentration of plant growth regulators (PGRs) including kinetin (Kn) 1.0 μM, 6-benzylaminopurine (BAP) 0.6–4.43 μM, thidiazuron (TDZ) 0.2–0.6 μM, and indolyl-3-butyric acid (IBA) 0.2–0.89 μM. The pH of the culture medium was adjusted to 5.8 before autoclaving. To prevent bacterial contamination during cultivation, various antibiotics – levomycetin, erythromycin and amoxicillin – were added to the culture medium at concentration of 50.0 or 100.0 mg/l. In all experiments, 15 to 18 shoot tips were used. Each experiment

was repeated four times. Clonal micropropagation was performed in accordance with the generally accepted techniques (Metodicheskie rekomendacii.., 2005).

Cultivation conditions. Explant cultivation was carried out for 35–40 days. The photoperiod lasted 16/8 h (light/darkness), the temperature maintained was 25 ± 2° C, and the light intensity attained 3–4 klx. The following data was recorded: the average length of the shoots (mm); total propagation rate, including the number of adventitious, lateral shoots and internodes (for chrysanthemum). Statistical data was processed using the software package Microsoft Office Excel 2007. Treatment means were compared with the confidence interval (CI) of the mean, α = 0.05.

Results

At the stage of micropropagation, four variants culture media were tested for steppe cherry "Pamjati Lewandovskogo". The culture medium, which we usually use for micropropagation of cherries, was used as control. The highest regeneration capacity was observed for the variant with a combination of agar 4.0 gr/l + starch 20.0 gr/l: the number of shoots/explants 2.5-fold exceeded the control value and was 20.1±0.9 pcs. (Fig. 1).

The analysis of the shoot length showed that in most cases the height of the regenerants was not lower than the reference level and attained 14–21 mm.

Addition of amoxicillin at concentration of 50.0 or 100 mg/l to the culture medium had a positive effect on the growth and development of cherry and raspberry plants. It is a broad-spectrum antibiotic. Due to its presence in the medium, the plants were free from bacterial microflora, grew vigorously and had green leaves in contrast to the action of other antibiotics being studied, which caused chlorosis and yellowing of leaves, inhibited growth and caused shoot death.

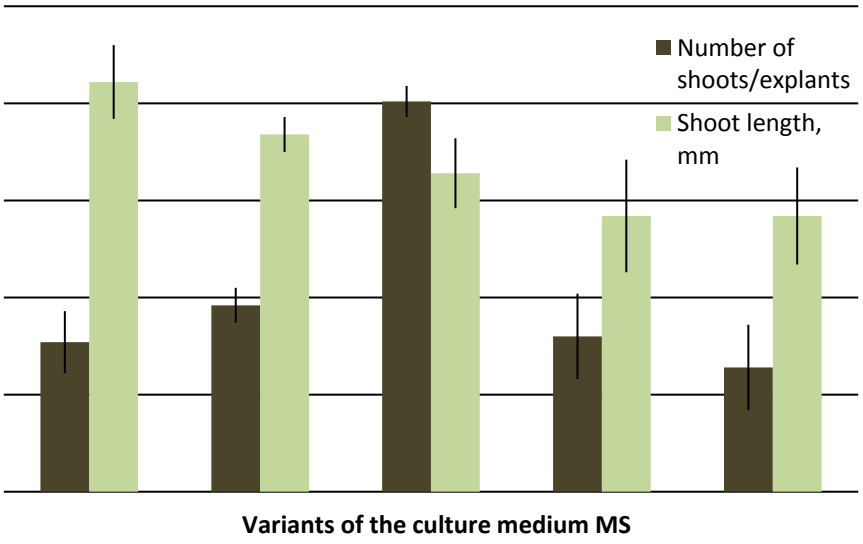

Figure1. Micropropogation of steppe cherry "Pamjati Lewandovskogo" (n=17)

Note: control – BAP 2.2μM + IBA0.89 μM + agar 7.0 gr/l; 1 – BAP 2.2 μM + IBA 0.89 μM + agar 4.0 gr/l + starch 20.0 gr/l; 2 – BAP 2.2 μM + IBA 0.89 μM + agar 2.0 gr/l + starch 40.0 gr/l; 3 – BAP 2.2 μM + IBA 0.89 μM + amoxicillin 50.0 mg/l + agar 4.0 gr/l + starch 20.0 gr/l; 4 – BAP 2.2 μM + IBA 0.89 μM + amoxicillin 100.0 mg/l + agar 4.0 gr/l + starch 20.0 gr/l. Mean ± CI

The mericlones grown are shown in Fig. 2. The morphology of the regenerated cherries was normal. Shoots on media with starch had large green leaves, and the shoot length was longer than that in the control by 2–5 mm. These shoots could be immediately transferred to the rooting medium.

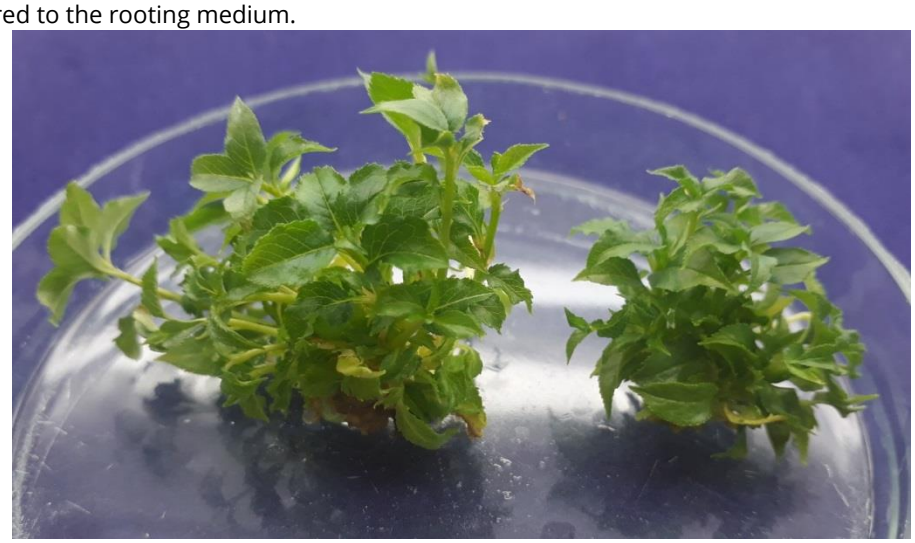

Figure 2. Regenerants of steppe cherry "Pamjati Lewandovskogo": on the left – plants grown on the medium supplemented with BAP 2.2 μM + IBA 0.89 μM + agar 4.0gr/l + starch 20.0 gr/l, on the right – plants from the control: BAP 2.2 μM + IBA 0.89 μM + agar 7.0 gr/l

The analysis of the results obtained in micropropagation of remontant raspberry "Hercules" showed that partial replacement of agar with starch at concentration of 20.0 gr/l in the medium composition did not reduce the propagation rate relative to the control level (5.1±0.8). The length of the induced shoots on these media exceeded the control value by 4–6 mm (Fig. 3). Similar to the case with cherries, addition of antibiotic to prevent bacterial contamination at concentration of 100.0 mg/l stimulated regeneration and development of the regenerants.

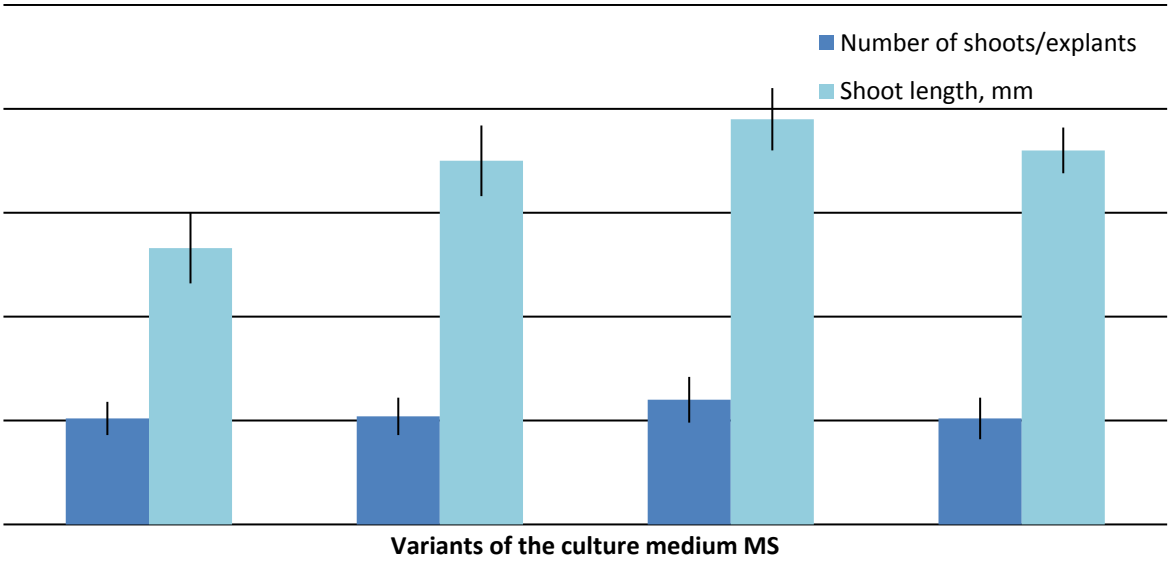

Figure 3. Micropropogation of remontant raspberry "Hercules" (n=18)
Note: control – BAP 4.43 μM + 2Fe + agar 7.0 gr/l; 1 – BAP 4.43 μM + 2Fe + agar 4.0 gr/l + starch 20.0 gr/l; 2 – BAP 4.43 μM + 2Fe + agar 7.0 gr/l + amoxicillin 100.0 mg/l; 3 – BAP 4.43 μM + 2Fe + agar 4.0 gr/l + starch 20.0 gr/l + amoxicillin 100.0 mg/l. Mean ± CI

The combination of agar, starch, Kn and IBA for induction of adventitious shoot formation in chrysanthemum varieties yielded a positive effect (Fig. 4). A combination of TDZ with IBA was observed to inhibit propagation.

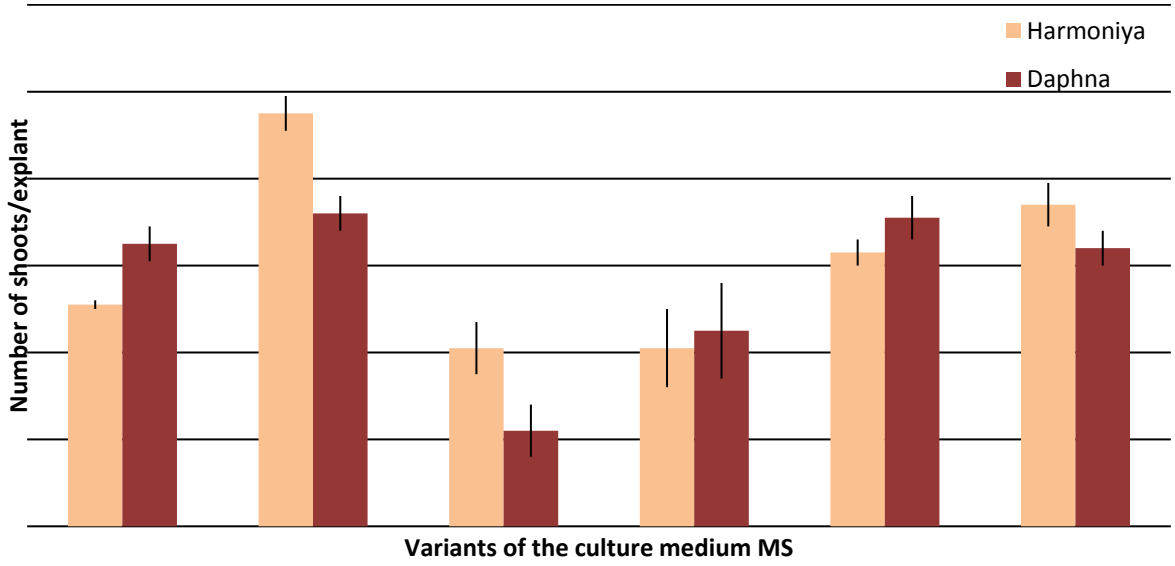

Figure 4. Propagation rate of chrysanthemum korean (multiflora group) during micropropagation (n=15)
Note: control – without PGR_s + agar 7.0 g/l; 1 – Kn 1.0 μM + IBA 0.2 μM; 2 – TDZ 0.6μM + IBA 0.25 μM; 3 – TDZ 0.2 μM + IBA 0.25 μM; 4 – BAP 0.6 μM + IBA 0.25 μM; 5 – Kn 1.0 μM + IBA 0.2 μM + agar 4.0 gr/l + starch 20.0 gr/l. Mean ± CI

Thus, potato starch can partially replace agar in the culture medium (20.0 and 4.0 gr/l, respectively), which reduces the cost of the medium and improves the growth of explants, as evidenced by steppe cherry "Pamjati Lewandovskogo", remontant raspberry "Hercules" and chrysanthemum korean (multiflora group) "Daphna" and "Harmoniya". Addition of the antibiotic amoxicillin at concentration of 50.0–100.0 mg/l had a positive effect on the development of explants of steppe cherry "Pamjati Lewandovskogo" and remontant raspberry "Hercules". This made 100% of plants free from bacterial contamination during the entire period of *in vitro* cultivation.

Discussion

Addition of potato starch to the culture medium stimulated the growth of adventive and lateral cherries. All the studied plants exhibited an increased area of the leaf blade, which was of rich green color. This may be because starch has a more pronounced water holding capacity compared to that of agar, which can be observed after heat treatment. Therefore, the culture medium contains more bound water, and plants grow better on such media. On the other hand, starch molecules are smaller than those of agar are, they are made of glucose monomers, and therefore plants are provided with an additional source of carbohydrates.

Addition of antibiotics to the culture medium is widely used by researchers in plant micropropagation. D. Cornu and M.F. Michel (1987) treated noncontaminated cherry explants with tetracycline and rifampicin antibiotics. The authors noted the efficacy of tetracyclines and high variability of cherry clones in susceptibility to all studied antibiotics. R.A. Ayub and L. Reis (2016) treated leaf explants of strawberry "Festival" with kanamycin, which resulted in complete inhibition of the regeneration process. In our studies, amoxicillin, a broad-spectrum antibiotic, showed its efficacy in micropropagation of garden crops such as cherries and raspberries.

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