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TDZ And 4-PU Are Effective Cytokinins for Clonal Micropropagation In Vitro of Different Genotypes of Everbearing Raspberry.

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ABSTRACT

In this investigation we studied different cytokinins for clonal micropropagation of the hybrids of everbearing raspberries in vitro and for stimulation of the new varieties development. The use of cytokinins of diphenylurea type [tidiazuron (TDZ) and N-(2-chloro-4-pyridyl)-N-phenylurea (4-PU)] allowed cultivating in vitro explants from the flower's buds of everbearing raspberry of various genotypes. At the stage of formation of adventive shoots 4-PU and TDZ gave 9.57 – 14 adventive shoots compared with 1.75- 4.4 on the medium with 6-BAP. Thus, cytokinins of TDZ and 4-PU allowed to accelerate multiplication of valuable breeding hybrids.

Keywords: everbearing raspberry, clonal micropropagation in vitro, cytokinins of diphenylurea type TDZ and 4-PU.

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INTRODUCTION

Modern plant breeding involves biotechnological methods and approaches [1, 2]. Rapid multiplication of new hybrids and varieties is required for their further breeding evaluation or distribution [3, 4].

The present work was carried out on everbearing hybrids and varieties of raspberry, obtained by interspecific hybridization [5]. Modern everbearing varieties have high yield, large berries, high resistance to diseases and pests due to the technology of their cultivation. However, hybrid plants have a low coefficient of vegetative propagation because of their physiological characteristics. These plants give early fruits on the first year shoots and form the fruiting zone up to 80% of the shoot length. Therefore, the main pool of assimilates are consumed by young growing shoots and developing fruit elements, resulting in the fact that the formation of root suckers is dramatically reduced [6]. Development of a method of clonal micropropagation for elite forms of everbearing raspberries allows to remove these restrictions and significantly reduce the time for micropropagation of new varieties.

The everbearing forms of raspberry are characterized by earlier differentiation of axillary buds in flower type [7, 8]. Flower buds explants cannot be cultivated in vitro on MS medium with 6-benzyladenin 6-BAP [9, 10], because they die after 1-2 weeks. The successful development of explants require their dedifferentiation [11]. Therefore, successful cultivation of meristems is a key stage in clonal micropropagation of everbearing raspberry.

MATERIALS AND METHODS

In this work we used five hybrids and ten cultivars of everbearing raspberry, which were provided by breeders of Kokinski department of breeding fruit and berry crops. We used medium MS for clonal micropropagation [6]. The explants were isolated from buds of replacement shoots.

At the stage of introduction of explants in vitro there were used benzyladenin 6-BAP and two diphenylurea cytokinins - tidiazuron (TDZ) and N-(2 chlore-4-pyridyl)-N-phenylurea (4-PU). We counted survived explants and number of shoots on explants after 1.5 months. For the induction of adventive shoot formation, we applied medium MS with cytokinins 6-BAP, TDZ and 4-PU in different concentrations. Cuttings of plants were carried out by separating the axillary and adventitious shoots.

RESULTS AND DISCUSSION

In the development of clonal everbearing raspberry micropropagation the main difficulties are associated with early fruiting and formation of berries on first year shoots. A differentiation of vegetative buds to the flowering type occurs very early, 2-3 weeks after the beginning of regrowth of shoots resumption.

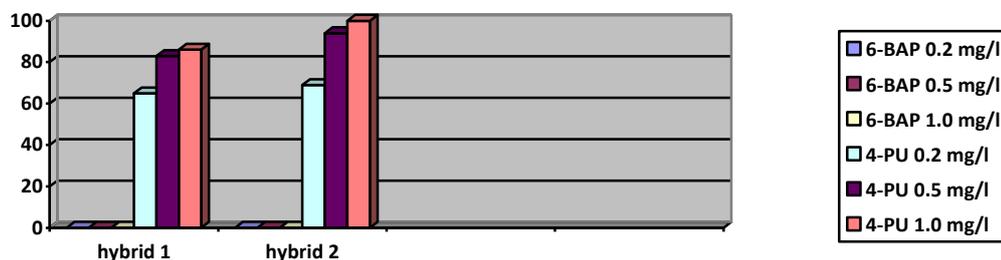


Fig. 1 – Influence of 6-BAP and 4-PU on the development of explants from floral buds two hybrids of everbearing raspberry in vitro

At the first stage we isolated explants from vegetative and flower buds. It was shown that vegetative explants developed successfully in presence of 6-BAP 0.5 - 1.0 mg/l. However, about 50% of everbearing vegetative explants or forms on the medium with 6-BAP have been lost. Flower bud’s explants were lost completely on the media with 6-BAP. We proposed cytokinins of diphenylurea type might stimulate

development of flower's buds explants in vitro. We added 4-PU and TDZ to MS medium for cultivation of flower's explants from two hybrids, that perished completely in presence of 6-BAP.

As shown on Fig. 1, soot regeneration from flower's buds happened on the media with 4-PU in concentration 0,2, 0,5 и 1,0 mg/l, the optimal concentration of 4-PU is 1.0 mg/l. However, on media with 6-BAP there was observed a total loss of explants.

High efficiency of another synthetic cytokinin TDZ at the stage of introduction of the explants in vitro was also demonstrated. We compared the effectiveness of three cytokinins 6-BAP, TDZ and 4-PU on everbearing hybride 3. In this experiment explants from differentiated floral buds were used (Fig. 2).

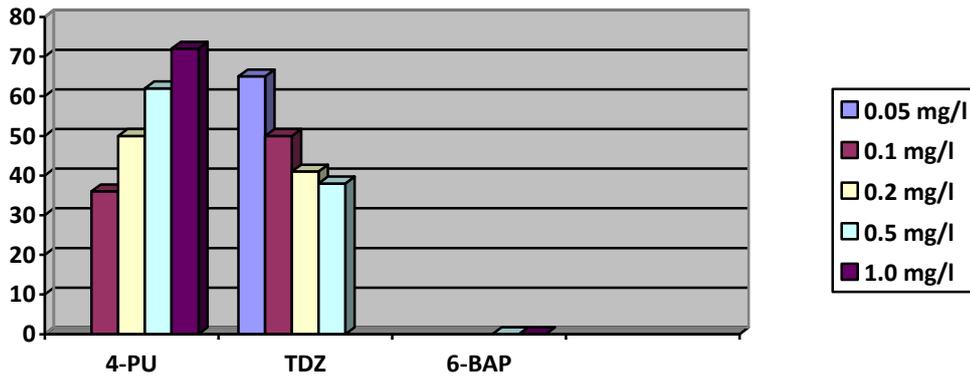


Fig. 2 – The influence of 6-BAP, TDZ and 4-PU on the development in vitro of explants from flower buds of hybrid 3 of everbearing raspberry

Results evidenced that TDZ stimulated effectively the development of everbearing raspberry explants, which were isolated from flower buds. The optimal concentration of 4-PU was 1 mg/l and TDZ - 0.05 mg/l, indicating high activity of TDZ. The larger TDZ concentration was superoptimal, and it had a negative effect on regeneration of explants.

Thus, the use of cytokinins TDZ and 4-PU allowed to introduce into the culture in vitro explants from the bud of raspberry everbearing forms regardless of genotypic characteristics and type of their differentiation.

The effect of cytokinins on induction of micropropagation

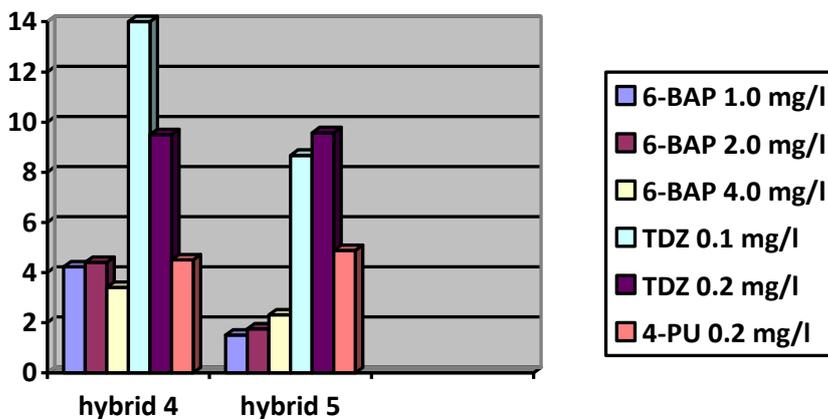


Fig. 3 – The influence of 6-BAP, TDZ and 4-PU by a factor of clonal micropropagation in vitro of two hybrids everbearing raspberries

The large differences between genotypes in growth rate and development of transplant, generation and development of adventitious shoots were observed while everbearing raspberry hybrids were propagated on the MS medium with 6-BAP, 2 mg/l. This eventually determined the rate of reproduction. Most of the genotypes were multiplied (from 3 to 5 times) on the MS medium with 6-BAP, 2 mg/l. However, some valuable breeding samples (hybrids 4, 5) formed few or no adventitious shoots, therefore it was necessary to select other hormones for micropropagation.

TDZ and 4-PU effectively stimulated the development of vegetative shoots from floral buds of everbearing forms, and we assumed that they could activate adventitious shoot formation and micropropagation of plants. For the experiment we used TDZ at a concentration of 0.1 and 0.2 mg/l 4-PU - 0.2 mg/l and 6-BAP - 1,2, 4 mg/L. The experiment was conducted on two hybrids of everbearing raspberry which were promising for breeding (Fig. 3).

TDZ and 4-PU effectively stimulated the formation of adventitious shoots on two hybrids in vitro (Fig. 3). On the medium with 6-BAP hybrid 4 had a reproduction coefficient 3,4-4.4 and replacement of hormone on a TDZ increased the number of adventitious shoots up to 14. For the hybrid 5 the coefficient of reproduction was 1.75 - 2.31 in presence of 6-BAP and it increased to 9.57 on the medium with TDZ and to 4.88 on the medium with 4-PU. Thus, we could conclude that 4 - PU and TDZ allowed to multiply breeding hybrid more effectively than 6-BAP at the stage of formation of adventitious shoots.

At the same time, little shoots, which were formed on TDZ medium had thin stems. This effect caused further problems due to poor rhizogenesis. Therefore, it was preferable to have the formation of 4-6 large well-developed shoots, which rooted easily later. Vitrified shoots were formed after cultivation of plants on media with high concentration of TDZ and they hardly developed and often died. Therefore, we reduced the concentration of TDZ to 0.1 mg/l.

CONCLUSION

The use of cytokinins diphenylurea type TDZ and 4-PU solves the problem of micropropagation of valuable hybrid forms and cultivars of everbearing raspberry in vitro. TDZ and 4-PU cause dedifferentiation of flower tissues that allowed us to use explants from flower buds of raspberry everbearing forms regardless of genotypic characteristics in vitro culture. Nearly 100% of hybrid forms can be introduced in culture in vitro.

There is a significant specificity in development of explants of different hybrids and cultivars of everbearing raspberry on the media with different hormones. Applying of 4-PU and TDZ at the stage of formation of adventitious shoots is very prospective; the coefficient of micropropagation of some cultivars of everbearing raspberry on media with TDZ and 4-PU reaches value of 9-14.

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