Comparative Studies of *In Vitro* Regeneration Capacity in Some Breeding Forms of *Prunus persica* (L.) Batsch

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Abstract. Peach [*Prunus persica* (L.) Batsch] is one of the most important stone fruit crops in the world. Preservation of valuable genotypes and creation of new breeding forms need the effective methods for plant propagation. Biotechnological method makes it possible to multiply valuable genotypes *in vitro* and produce high-quality plant material. Plantlets were obtained from hybrid peach embryos in five cross combinations. The induction of morphogenesis and the studies of regenerative capacity were carried out on culture media Murashige, Skoog (MS) and Gamborg, Eveleigh (B5) with vitamins and plant growth regulators. The segments of plantlets with 2-3 internodes were placed on MS and B5 media. Use of B5 medium with 0.75-1.0 mg L^{-1} BAP and 0.1 mg L^{-1} IBA induced organogenesis in the studied hybrid forms. The microshoots of the hybrid form 'Summerglo' × 'Nikitskiy Podarok' had a high regeneration capacity. In the forms 'Persey' × 'Nikitskiy Podarok' and 'KAT 92-2210' × 'Nikitskiy Podarok' low regeneration capacity was noted. An increase in BAP concentration resulted in formation of hydrated microshoots and non-morphogenic callus. It was determined that to obtain normal peach microshoots, the optimal culture parameters were a temperature of 24 ± 1°C, 16-hour photoperiod, and 37.5 μM $m^{-2}s^{-1}$ light intensity.

1 Introduction

Peach [*Prunus persica* (L.) Batsch], genus *Prunus* L., family Rosaceae Juss. is one of the main commercial stone fruit crops in the world. It is grown in more than 60 countries. The most important peach growing regions are the countries of Asia (China, Iran), Europe (Italy, Spain, Greece and Turkey) and the USA [1, 2]. In Russia, peach trees are grown in the southern regions of the country [3, 4]. Due to their high nutritive value and the presence of biologically active substances peach fruits have become an essential food product. The increased demand for fresh peach fruits reveals the promise of increasing their production in the Russian Federation. To solve this problem, it is necessary to create new peach cultivars adapted for cultivation in the south of Russia. Preservation of valuable genotypes

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and creation of new breeding forms requires effective methods for their propagation. Micropropagation method makes it possible to multiplicate valuable genotypes and produce high-quality planting material [5, 6]. In this regard, an effective and reliable system of peach microshoots regeneration from somatic tissues under *in vitro* conditions is necessary [7, 8]. It is known that peach is one of the crops to most difficult propagate under *in vitro* conditions [6, 9, 10]. The aim of the presented study was to identify morphogenetic capacity of organs and tissues in peach hybrid forms under *in vitro* conditions for further studies and use in the breeding process.

2 Materials and methods

Studies were carried out in the Plant Biotechnology and Virology Laboratory of the Plant Developmental Biology, Biotechnology and Biosafety Department, Federal State Funded Institution of Science "The Labor Red Banner Order Nikita Botanical Gardens - National Scientific Center of the RAS" (NBG-NSC). The starting material was segments with 2-3 internodes of seedlings grown *in vitro* from the embryos obtained in 5 cross combinations of peach cultivars 'Jerseyglo' × 'Nikitskiy Podarok', 'KAT 92-2210' × 'Nikitskiy Podarok', 'Loadel' × 'Nikitskiy Podarok', 'Persey' × 'Nikitskiy Podarok', 'Summerglo' × 'Nikitskiy Podarok' and cotyledons of cross combination of peach cultivars 'Jerseyglo' × 'Nikitskiy Podarok', 'Summerglo' × 'Nikitskiy Podarok', 'KAT 92-2210' × 'Nikitskiy Podarok". Maternal plants were 'Persey' cultivar and hybrid form 'KAT 92-2210' originated in Nikita Botanical Gardens, american cultivars 'Jerseyglo', 'Summerglo' and Hungarian cultivar 'Loadel'. As paternal plants, the peach cultivar 'Nikitskiy Podarok', originated in NBG and zoned in the Russian Federation, was used.

Biotechnological methods were used in the presented research [8, 11]. Morphogenesis induction and regenerative capacity of peach explants were studied on Murashige, Skoog (MS) [12] and Gamborg, Eveleigh (B5) [13] culture media supplemented with vitamins and plant growth regulators. Such plant growth regulators (PGR) as BAP (6-benzylaminopurine, Sigma, USA) at the concentration 0.5, 0.75, 1.0, 1.5, 2.0 or 2.5 mg L^{-1} and 0.1-0.2 mg L^{-1} IBA (indole-3-butyric acid, DuchefaBiochemie, Holland) were used. In the experiments on cotyledons organogenesis induction medium based on MS medium with 0.11 mg L^{-1} BAP in combination with 1.11 mg L^{-1} 2,4-D (2,4-dichlorophenoxyacetic acid, DuchefaBiochemie, Holland) were tested. Medium pH was 5.7-5.8. The culture media were autoclaved in LAC 5060S sterilizer (DAINAN LABTECH, South Korea) at 120°C for 8-12 minutes. Plant growth regulators and vitamins were sterilized by cold filtration through MILLEX® GP filters (0.22 μm) and added to the media after autoclaving. Explants were subcultured every 3-4 weeks. The explants were maintained in BIOTRON's phytocapsules and in the plant growths chamber MLR-352-PE (Panasonic, Japan) at a temperature of 24±1°C, 16-hour photoperiod, light intensity 37.5 μmol $m^{-2}s^{-1}$ provided with cool white fluorescent lamps (Philips TL, Japan). Micrographs were made with Nikon SM2745T binocular microscope (Japan). The experiments were repeated for three times in 20 replications. Data statistical analyzes was made with the software STATISTICA for Windows 10.0 (StatSoft, Inc.) and the Duncan`s multiple range test ($p \leq 0.05$).

3 Results and discussions

In the process of studying regenerative capacity *in vitro* in 5 peach cross combinations, it was revealed that a number of factors affected plant regeneration, such as genotype, culture medium composition, plant growth regulators, and physical conditions of culture. At the

induction stage of the shoot segments development, the main characteristic of regenerative processes was the number of new axillary, adventitious buds and microshoots under *in vitro* conditions. The cultivation of seedling segments with 2-3 internodes on MS and B5 culture media demonstrated the best development of explants on B5 medium. Use of the combination BAP at a concentration of 0.75-1.0 mg L^{-1} and 0.1 mg L^{-1} IBA in B5 medium induced organogenesis in the studied hybrid forms (Table).

Table. The effect of plant growth regulators and their concentrations in B5 culture medium on the microshoot regeneration in 5 peach hybrid forms (after 2 subcultures)

Concentration of PGR, mg L^{-1}		Genotype*				
BAP	IBA	1	2	3	4	5
		Number microshoots per explant				
0.0**	0.0	0.25 ef***	0.20 de	0.20 ef	0.10 ef	0.05 ef
0.5	0.1	4.91bc	2.0bc	2.24bc	2.12 b	2.50 b
0.5	0.2	4.25 cd	2.67 b	2.13 cd	2.07 cd	2.25 cd
0.75	0.1	6.43 a	4.21 a	3.12 a	3.05 a	3.0 a
0.75	0.2	5.17ab	3.74ab	2.47ab	2.50ab	2.49 b
1.0	0.1	6.19 a	3.96 a	2.93 a	2.75 a	2.97 a
1.0	0.2	5.33 ab	3.48 ab	2.75 a	2.56 ab	2.54 ab

* Hybrid forms: 1 - 'Summerglo' × 'Nikitskiy Podarok', 2 - 'Persey' × 'Nikitskiy Podarok', 3 - 'KAT 92-2210' × 'Nikitskiy Podarok', 4 - 'Jerseyglo' × 'Nikitskiy Podarok', 5 - 'Loadel '×' Nikitskiy Podarok'.
** Control.
*** Means followed by the same letters in the columns do not differ significantly at $P<0.05$ according to the Duncan's multiple range test.

At the same time, microshoots of the hybrid form 'Summerglo' × 'Nikitskiy Podarok' had a high regenerative capacity (Table). After the second subculture, the number of newly formed adventitious buds increased and was up to 7.82 ± 0.15 per explant, number of microshoots was 6.43 ± 0.22 per explant. In experiments with hybrid forms 'Persey' × 'Nikitskiy Podarok' and 'KAT 92-2210' × 'Nikitskiy Podarok' the regenerative capacity was lower: 4.21 ± 0.84 and 3.12 ± 0.97 per explant, respectively (Fig. 1).

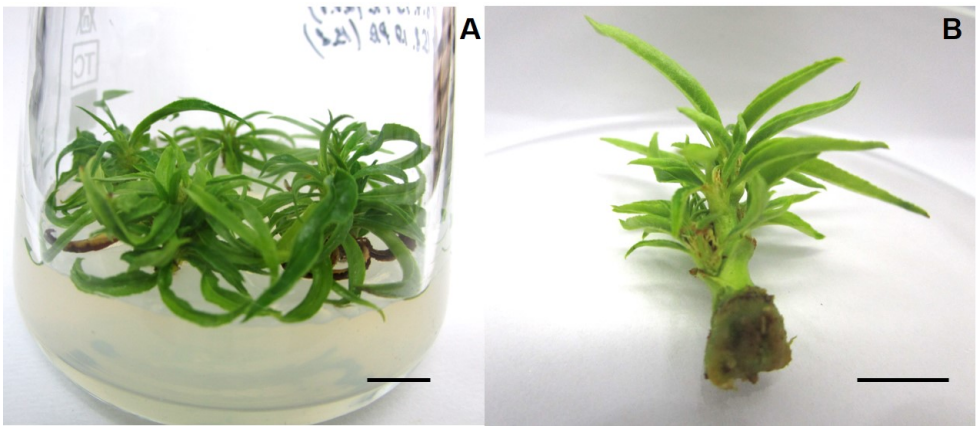

Fig. 1. Multiple shoot formation on B5 culture medium (A) and adventitious shoots formation (B) in a peach cross combination 'Persey' × 'Nikitskiy Podarok'. Scale bar 1 cm.

In general, all tested BAP concentrations in combination with 0.1 and 0.2 mg L^{-1} IBA promoted morphogenesis and regeneration frequency compared to the control. The greatest

indicates of shoot length and leaf number were also noted on B5 medium with 1.0 mgL^{-1} BAP and 0.1 mgL^{-1} IBA. Increasing of regeneration frequency was determined due to the physiological role of BAP, which is the most effective cytokinin for micropropagation of Rosaceae family species [6, 14, 15]. At a concentration of 1.5-2.0 mg L^{-1} BAP and 0.1-0.2 mg L^{-1} IBA in the culture medium, morphogenic callus formation was induced on the cotyledons of the hybrid forms 'Jerseyglo' × 'Nikitskiy Podarok', 'Summerglo' × 'Nikitskiy Podarok' and 'KAT 92-2210' × 'Nikitskiy Podarok'. In the experiments, included the combination of 2,4-D and BAP as an inducer of callusogenesis effectively acting and previously used by other scientists on the cotyledons of peach and nectarine [16]. We used this combination for peach explants. Callus development occurred on MS medium with 0.11 mg L^{-1} BAP and 1.11 mg L^{-1} 2,4-D and followed by adventitious buds formation (Fig. 2).

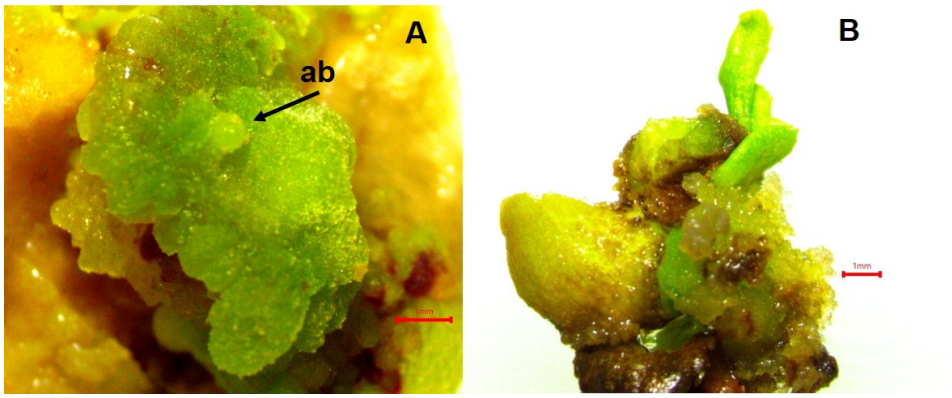

Fig. 2. Morphogenic callus formation on peach cotyledons of the cross combination 'Summerglo'× 'Nikitskiy Podarok': A — adventitious buds formation (ab — adventitious bud); B - adventitious buds development. Scale bar 1 mm.

An increase in BAP concentration up to 2.5 mg L^{-1} and more resulted in the formation of hydrated microshoots and non-morphogenic callus at the basal part of explants on culture media B5 and MS. It was revealed that for obtaining mature peach microshoots, the optimal culture conditions were a temperature of 24±1°C, 16-hour photoperiod and light intensity 37.5 µmol m^{-2} s^{-1}.

4 Conclusions

Simple, reliable and efficient regeneration of microshoots from peach seedling segments was obtained in 5 peach hybrid forms on B5 culture medium. The dependence of the regenerative capacity in peach explants on their genotype, culture medium composition, type and concentration of plant growth regulators and physical factors of culture has been demonstrated. The inducing role of B5 culture medium and such plant growth regulators as BAP (0.5–1.0 mg L^{-1}) and IBA (0.1–0.2 mg L^{-1}) in the microshoot regeneration from seedling segments and micropropagation of peach was revealed. The number of microshoots in the hybrid form 'Summerglo' × 'Nikitskiy Podarok' after the second subculture was 6.43 ± 0.22 ones. Morphogenic callus formation and the development of adventitious buds were induced on peach cotyledons in the hybrid forms 'Jerseyglo' × 'Nikitskiy Podarok', 'Summerglo' × 'Nikitskiy Podarok' and 'KAT 92-2210' × 'Nikitskiy Podarok' on MS culture medium with plant growth regulators BAP (0.11 mg L^{-1}) and 2,4-D (1.11 mg L^{-1}). The results of our studies contribute to the development of protocols for clonal micropropagation of peach hybrid forms and cultivars under *in vitro* conditions.

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