Propagation in the Conditions of In Vitro of Fruit and Berry Cultures for Conservation and Restoration Ancient Forms of Plants

Bekkuzhina S.S., Mukhtubaeva S.K., Bugubay G.Zh., Sitpayeva G.T.

Abstract. Micropropagation of ancient plant forms and their accelerated replication for the purpose of creation of a basis of selection of fruit and berry cultures is a relevant task. For development of selection of fruit and berry cultures in the conditions of northern Kazakhstan, and for gardening improvement, local forms of plants are entered into the culture of in vitro. Test tube plants of cherry, currant, apricot are received. Various options of the MS environment with addition of the gibberellic acid (GA) in combination with the ascorbic acid (AA) are optimized. It is revealed that addition of gibberellic acid and ascorbic acid in concentration of 3 mg/l is more effective for propagation of blackcurrant.

Key words: in vitro propagation, BAP-6 Benzylaminopurine, GA-gibberellic acid, commercial culture cherry, currant, apricot.

I. INTRODUCTION

The resolution of the government of the Republic of Kazakhstan, of February 27, 2015 No. 4-1/168, defines the strategy of our Republic and relevance of problems, and search of new decisions on cultivation of commercial cultures [1].

Cultivation of domestic grades of fruit and berry cultures is generally carried out in the south of Kazakhstan. Use of biotechnological methods allows to advance cultivation of fruit and berry cultures and to the north Kazakhstan.

Now one of effective instruments of biotechnology of plants is micropropagation. Micropropagation is widely used for industrial production of fruit and berry [2, 3, 4, 5], decorative cultures and herbs around the world. Especially this method is important at propagation of rare endangered endemic species and also for the accelerated propagation and conservation ancient forms of plants.

One of the strategic objectives of fruit breeding is the revival and conservation of the unique properties of the famous aport.

At the Institute of Biology and Biotechnology, a "new plant" of aport in vitro was obtained by microclonal propagation. In 2012–2014, the Institute of Fruit Growing and Viticulture (KazNIIPIV) carried out molecular genetic studies on the genotyping of 11 forms of the Sivers wild apple tree and aport.

In KazNIIZIKR in the mountains of the Dzungar and Zailiysky Alatau, as well as Tarbagatai, about 30 forms of the Sivers apple tree were selected [6].

According to the statement of the famous selector A.I. Vavilov, one of important points of development of selection of plants is creation of a gene pool of local forms of plants [7].

Cornerstone for creation of bases of selection of fruit and berry cultures in the conditions of northern Kazakhstan is the accelerated propagation ancient forms of plants, their replication by means of micropropagation.

The purpose of this work introduction to the culture of in vitro of local forms of fruit and berry plants and creation of a collection in the conditions of the minimum growth and also receiving the microcrops exempted from endophytic and other types of an infection.

II. MATERIALS AND METHODS

Theoretical and applied aspects of micropropagation are described in many in many works of research character [8, 9, 10] and also monographs and textbooks [11,12].

Now successful protocols and methodical features of cultivation of different types of vascular plants are detailed and described.

It is necessary to distinguish from a set of advantages of micropropagation especially improvement the test tube of plants from a viral, mushroom and bacterial infection [13].

As objects of researches used local forms of fruit and berry cultures: currants, cherries, raspberries, the apricot growing in the city of Nur-Sultan and nearby regions.

Collecting plant material for introduction to the culture of explant was carried out in 2 steps in the spring and in the fall. The beginning of collecting in the fall - the end of September and October, buds in a condition of deep rest, and in the spring when buds did not waken from winter rest and also the dismissed first leaves.

At a stage of introduction to the culture of in vitro the sterilizing agents used: Na hypochlorite, antibiotics (ampicillin, etc.), potassium permanganate and also antioxidant substances.
Sterilization of plant material was carried out according to generally accepted methods [14]. Buds separated from wood and washed out in flowing water 3 times; washed out \( KMnO_4 \) within 5 minutes; washed 70% with solution sodium hypochlorite within 20 minutes. When using of the sprouted buds used 50% sodium hypochlorite solution within 15 minutes.

Cultivation of microshoots carried out in conditions – the photoperiod 16/8 illumination the 5000th luxury, temperature 23±3 ºC and humidity of 70%. The analysis of culture was carried out every 2 week and in 3-4 weeks culture was replaced to fresh nutrient medium.

For increase in efficiency of micropropagation added to the Murasige-Skuga environment (MS): cytokines: BAP-6 Benzylaminopurine – 0.5 and Indole acetic acid (IAA)-0.5. GA used in combination with AA, options of nutrient mediums: - GA 0.5 mg; GA 1 mg + AA- 1 mg; GA 3 mg + 3 mg of AA. As control option of a bud and a meristem cultivated on the MS environment without hormones.

III. RESULTS OF RESEARCHES

It is known that micropropagation, with use of a meristem as the explant, is effective and the frequency of an exit of sterile culture is higher for many cultures, apical sites of buds and sheet explant give vent the low test tube of plants.

Our pilot studies will be coordinated with data of references, induction of shoots of currant and cherry, shown in table 1 shows that forth putting is higher when as the explant used meristems (79.2%).

Unfortunately, in our pilot studies when using sheet explant, their further development, did not yield significant results. For example, for the culture of currant growth was suspended completely, and for cherry sprout-formation left 17.5%.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Explant types</th>
<th>Number of explants, pc</th>
<th>Seedlings, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Currant</td>
<td>Meristem</td>
<td>120</td>
<td>79,2</td>
</tr>
<tr>
<td></td>
<td>Buds</td>
<td>83</td>
<td>48,2</td>
</tr>
<tr>
<td></td>
<td>leaves</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>The coefficient of variation</td>
<td>94,3±0,19</td>
<td>42,46±0,76</td>
<td></td>
</tr>
<tr>
<td>Cherry</td>
<td>Meristem</td>
<td>158</td>
<td>62,1</td>
</tr>
<tr>
<td></td>
<td>Buds</td>
<td>65</td>
<td>46,2</td>
</tr>
<tr>
<td></td>
<td>leaves</td>
<td>57</td>
<td>17,5</td>
</tr>
<tr>
<td>The coefficient of variation</td>
<td>93,3±0,48</td>
<td>41,9±0,15</td>
<td></td>
</tr>
</tbody>
</table>

From explant of buds an exit of sterile culture is lower, than in meristemic culture and made 48.2 46.2 for cherry and currant respectively. From sheet explant shoots did not develop further.

From the sprouted buds of an apricot sterile culture, as shown in fig. 1 is also received, education pass bushes with leaf-bearing shoots.

In numerous researches the key role of phytohormones is noted, at a stage of obtaining sterile culture considerable influence is rendered by exogenous auxins. For example, from cowberry ordinary the greatest number of actively growing shoots is received at cultivation on the WPM environment containing 4 mg/l of IAA[15].

In our pilot studies without addition of GA on net propagation of culture were located as follows: apricot-1.25. cherry - 1.5 currant 1.9 of fig. 2.

Especially the importance, at cultivation of fruit and berry, has use of gibberellic acid for example, for currant. It is expedient to carry out cultivation of primary explant of blackcurrant on the medium containing in addition to cytokinin and auxin and gibberelic acid (concentration of 0.5 mg/l is most effective) [16].

At modification of a medium with different concentration of gibberelic acid it is revealed that increase in concentration of substance positively influences growth of shoots.

As shown in figure 3 obtaining sterile culture of shoots in meristematic culture was more effective on versions of the MS GA 2 – 3 mg/l. Gradual increase in concentration, maybe, would also lead to further increase in germination of shoots, from the landed explant and to emergence of plants with several shoots, but growth of cells stretching it is dangerous by development of thin and fragile shoots.
According to these fig. 3 it is possible to observe that at GA addition primary sprout-formation for the culture of currant is higher, than for cherry. In all pilot studies further development of plants was better for currant in comparison with other fruit crops of fig. 4.

Thus, cultivation on the Wednesday with GA is effective, but in order to avoid emergence the veterified of shoots can result further increase in concentration of GA in negative effect.

IV. CONCLUSION

So, for creation of a basis of selection of fruit and berry cultures it is necessary to have a collection of local forms of the plants long since burgeoning in this region i.e. it is not obligatory to have the zoned grades, and then on their basis to create the zoned grades.

At initial stages it is necessary to microtype, and then to store in conditions of the minimum growth and further to carry out genetic certification. All this is an important condition for creation of effective selection of commercial cultures.
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Brief introduction

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Publications: 6 monographs, more than 170 articles and theses, including in USA, France, Japan, Czech Republic, Bulgaria, Hungary, Greece, Germany, China, Korea, Turkey.
Research experience: Participation in 6 international grants:
- National geographic society Washington, D.C. under the project “Modern and fossil flora of Turgai” (1995-1997);
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- “The study of the flora of steppes of the Ukraine, Kazakhstan and Mongolia” in cooperation with colleagues from the University of Tsukuba /Japan, 2004-2005;
- ISTC K-719P “Detection and identification of natural products from the flora of Kazakhstan for the agrobiological purposes” (2002-2007);
- University of Washington “To create new demonstration plots in the Main Botanical garden” (2010-2011);
- “Profile (zonal) study on climate change and its influence on ecosystems of arid lands in Central Asia” (Xinjiang Institute of Ecology and Geography, CAS, 2010-2012);
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- Development and creation of the exposition of the flora of Kazakhstan in the Botanical garden "Bagatelle " (France), as a prototype of the introduction of plants of Kazakhstan in the green building of Europe (2011-2013).
- Ensuring the preservation and development of collections of plants, animals, microorganisms, viruses and unique genetic banks as the basis of fundamental research (2012-2014).