

Национальная академия наук Беларуси  
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# **Биологически активные вещества растений – изучение и использование**

Материалы международной научной конференции  
(29–31 мая 2013 г., г. Минск)

Минск  
2013

УДК 58(476-25)(082)  
ББК 28.5(4Бел)я43  
О-81

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О-81 Биологически активные вещества растений — изучение и использование: материалы международной научной конференции 29–31 мая 2013 г., г. Минск. – Минск : ГНУ «Центральный ботанический сад Академии наук Беларуси», 2013. – 356 с.

Изложены материалы Международной научной конференции, посвященной обсуждению актуальных проблем по изучению и использованию биологически активных веществ растений, в том числе биотехнологических аспектов в растениеводстве с участием ученых из Беларуси, России, Украины, Молдовы, Казахстана, Кыргызтана, Венгрии.

На молекулярном, клеточном и организменном уровнях рассмотрены имеющие важное научное и практическое значение вопросы, в числе которых состав, структура, биосинтез и использование веществ вторичного метаболизма растений, антиоксидантная и антирадикальная активность и лечебно-профилактические препараты из растений, сырьевые источники БАВ, биотехнологии в растениеводстве.

**УДК 58(476-25)(082)**  
**ББК 28.5(4Бел)я43**

# OPTIMIZATION OF AGROBACTERIAL TRANSFORMATION METHODS FOR WOODY PLANT CROPS

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Genetic engineering techniques allow to create new forms of plants much faster than classic breeding techniques. However, the *Agrobacterium spp.* mediated transformation frequency of most woody fruit crops remains on a very low level, which significantly inhibits production of the plants with desired characteristics. Long-term generative cycle, genetic, biochemical and physiological particularities, problems with adventive regeneration in vitro and selective virulence of different strains of *Agrobacterium spp.* are the key factors hampering the use of transgenic technologies in agriculture.

When elaborating genetic transformation technologies, it is vital to identify and estimate several factors which affect the transformation frequency and require optimization. These factors include the time of cultivation with *Agrobacterium*, the influence of monosaccharides, antioxidants, vir-inducing compounds.

When choosing and modifying selective protocols, it is also important to take into account the physiology of antibiotic action: namely, the selective agent's effect on the subsequent ability of the transformed cells to regenerate. Therefore, the study of factors having an influence on transfer, integration and subsequent expression of transgenes is basic for the transformation conditions optimization.

Optimization of lingonberry (*Vaccinium vitis idaea* L.), blueberry (*Vaccinium corymbosum* L.) and cranberry (*Vaccinium macrocarpon* Ait.) explants contamination conditions was based on the transient expression analysis of the reporter beta-glucuronidase (GUS) gene. In this work we used the supervirulent *Agrobacterium tumefaciens* strain CBE21, containing binary vector pBI121 with  $\beta$ -glucuronidase gene. The effect of co-cultivation time, vir-gene inducers (acetosyringone, monosaccharides glucose, arabinose and antioxidants) and selective antibiotic kanamycin on GUS transient expression efficiency were investigated.

In order to determine the optimal period of co-cultivation with *Agrobacterium*, an analysis of GUS transient expression level was carried out. The maximum frequency of transformation for lingonberry was observed after 6 days of co-cultivation with *Agrobacterium*. It comprised 13,6% and 47,4% for «red pearl» and «koralle» cultivars respectively. In blueberry explants the maximum level of transient expression (60%) was observed for «concord» variety after 5 days of co-cultivation with *Agrobacterium*, for «atlantic» – after 6 days (30%), in cranberry explants – after 6 days of co-cultivation with *Agrobacterium* (57%).

The adding of acetosyringone into the inoculation medium increased the GUS transient expression level in the leaf explants of lingonberry up to 21,3% for the «red pearl» cultivar, and up to 49,2% for «koralle». The use of acetosyringone during not only the inoculation, but also the co-cultivation, allowed to achieve 45,6% transformation frequency for «red pearl», and 70,3% for the «koralle» cultivar. The GUS transient expression level after 6 days of co-cultivation of cranberry explants in acetosyringone containing medium was 68%. The use of acetosyringone during the co-cultivation stage for high blueberry explants with *Agrobacterium* proved to be ineffective.

The adding of monosaccharides and antioxidants into the co-cultivation medium increased the GUS transient expression frequency.

The analysis of the data obtained through the research made it possible to suggest effective methods for genetic transformation of lingonberry, blueberry and cranberry.