ВЫДЕЛЕНИЕ ГЕНОМА ДНК МЕТОДОМ STAB ЛЕКАРСТВЕННОГО ГРАНАТА

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Аннотация

Биотехнологические методы широко применяются в практике мирового сельскохозяйственного производства, причем наиболее широко из них является метод in vitro, который в последние годы применяется для создания новых плантаций нетрадиционных растений. Одним из преимуществ метода in vitro является то, что он позволяет выращивать множество микрорастений в небольших лабораторных условиях независимо от времени года. Поэтому важным научно-практическим и актуальным вопросом является использование метода микроклонального размножения in vitro ценного растения стевии, выбор оптимальной питательной среды для культуры тканей, правильное проведение процесса стерилизации, выращивание корней, перенос микро рассаду в нестерильные условия и доставить ее в открытый грунт.

ISOLATION OF DNA GENOME BY STAB METHOD OF MEDICINAL POMEGRANATE

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Annotation

Biotechnological methods are widely used in the practice of world agricultural production, and the most widespread of them is the in vitro method, which in recent years has been used to create new plantations of non-traditional plants. One of the advantages of the in vitro method is that it allows the cultivation of many microplants in small laboratory conditions, regardless of the time of year. Therefore, an important scientific, practical and topical issue is the use of the method of microclonal propagation in vitro of a valuable stevia plant, the selection of the optimal nutrient medium for tissue culture, the correct implementation of the sterilization process, growing roots, transferring micro seedlings to non-sterile conditions and delivering

them to open ground.

Key words. *In vitro,* medicinal pomegranate, microcloning, dnk passportization.

Relevance of the problem. In order to increase the volume of pomegranate fruits in the republic, it is necessary not only to expand the areas, but also to introduce new innovative technologies and scientifically based methods into the pomegranate plantation network. The development of the pomegranate fruit crop, the quality of the fruit and its yield largely depend on the quality of the planting material. Varietal purity of pomegranate orchards, especially for export, is of great importance. *In vitro* conditions apical meristems are used in plant propagation because viruses penetrate apical meristems more slowly than other parts of plants. Therefore, parts of the plant's apical and lateral buds (growth points) are used. [4].

However, it is worth noting that it takes a long time to grow pomegranate seedlings in the traditional way. Therefore, nowadays *in vitro* microcloning technology is one of the most promising methods of growing pomegranate seedlings and maintaining variety purity [1] .

, it is possible to increase the required amount of high-quality material, free of virus and bacterial infection , and it is possible to obtain seedlings with high purity [2, 3].

Purpose of the study. Medicinal pomegranate (*punica granatum* l.) study the process of selection and sterilization of explants.

Methods and techniques. . Medicinal pomegranate peel (*punica granatum* l. Uzbekistan, Tashkent) as plant raw material, 70% ethyl alcohol as extractant, Murasiga Scooga was used to grow nutrient medium.

Experience part. In order to ensure the genetic integrity of local pomegranate varieties, samples were taken to extract DNA from pomegranate varieties. DNA was isolated from the samples according to the STAV method.

Isolation of genomic DNA from plant tissue by STAB method

Required reagents :	**Necessary equipment :**
• Liquid nitrogen • 2xCTAB • 10xCTAB • Chloroform:isoamyl (24:1) • CTAB Precipitation • High Salt RNA buffer (50 ml/200 µl l) • Isoproponol • 70% alcohol	• Mortar or test tube (1500/2000 µl) • Postavka (96 pieces) • Centrifuge • Refrigerator • 65 ^{0}C Autoblot and Vortex • Concentrator • Rubber gloves and gloves (100, 200 and 1000 µl)

• TE buffer	

1. The leaf is frozen and homogenized in **liquid nitrogen** .
2. 600 µl **2xCTAB** and placed in 60 ^{0} C **AUTOBLOT for** 20 minutes (mixed every 5 minutes).
3. On top of 600 µl x **lorof o rm: iso a mil** (24:1) is added and mixed vigorously for 2 minutes.
4. 5 minutes /10,000 rpm **centrifuged** _ and another 600 µl from the top of the supernatant is taken into a test tube .
5. On top of 60 µl **10xCTAB** put and mix .
6. On top of 600 µl **Add chloroform : isopropyl alcohol (** 24:1) and mix vigorously **for** 2 **minutes** .
7. 5 minutes /10,000 rpm **centrifuged** _ and another 600 µl from the top of the supernatant is taken into a test tube .
8. On top of it in a 1:1 ratio **NaAc+Ethone** is placed and 40 minutes **to** 25 ^{0} C **AUTOBLOT** put in incubation .
9. 15-20 minutes / 14,000 rpm **centrifuged** _ and the supernatant is poured .
10. 500 µl on the precipitate **High Salt** and vortexed for 5 minutes and then 15 minutes stored at room temperature .
11. On top of 300 µl isoproponol put and mix slowly for 2 minutes .
12. 15 minutes /10,000 rpm **centrifuged** _ and the supernatant is poured .
13. 600 µl on the precipitate Add **70% alcohol and** mix for 5 minutes .
14. 3 minutes / 14,000 rpm **centrifuged** _ and alcohol is poured . (The precipitate is washed twice with alcohol).
15. The precipitate is DNA **in the concentrator** dry for 10-15 minutes
16. 100 µl on the precipitate **TE buffer** put is **vortexed** and shorts **centrifuge** and -20 ^{0} C is placed **in the refrigerator** .

Conclusions . Explants collected from existing cultivars in the experimental field as a result of sterilization by different methods, their effectiveness has been proven to be in different indicators. The survival rate of the explants in the period of 15 days from the day of the experiment was different. Thus, in the first and second variants of the experiment *in vitro* , the yield of sterile explants was 10-30 percent. In the third version of the experiment, the yield of sterile explants of these varieties was 92% and was considered acceptable. Especially the use of sterilization components in combination with fungicides and antibiotics has been shown to give good results in pomegranate *in vitro culture.*

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