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USING CULTURE *IN VITRO* IN VITICULTURE

Summary

The influence of various jelling components, the composition of the nutrient medium on the survival, growth and development of initial grape explants in the primary stages of clonal micropropagation of grapes was investigated. The optimal nutrient medium Murasige and Skoog with the addition of a modified corn starch was developed and implemented to accelerate the breeding of grape variety Kobzar in vitro culture. The cost of microclon on the primary stages of clonal micro propagation has been reduced. The developed methods are tested in practice.

Keywords: in vitro culture, initial explants, clonal micropropagation, microclones, nutrient medium.

The main idea of biotechnology in agriculture is using biotechnology processes, systems and organisms in agriculture. All these make agriculture highly and competitive culture. The main goal is the improving old and make new highly various of grapes. Biotechnology in agriculture makes easier the new technology that improving efficiency agriculture [1]. There is the method of culture or genetic engineering the most popular in another countries. They make highly and invulnerable (for pests and hubrids) various of grapes. They make the technique for plants with more healthy plants excluding infections. It is important for plants which propagation vegetative.

The plant's cells are using isolation cells culture is propagation and recovery planting mathematical. This method name is clonal micro propagation. If we using this method, we will get able from one plants (donor) thousand explants. The propagation is microclonal only then microclones are the identical donor's plant. This process will be good if we will use axillary buds. The axillary bugs is genetically stable without bacterial.

The clonal propagation advantaged is:

- 1- the high rate of plants propagation
- 2- the works possibility throughout all year.



- 3 - the saving greenhouse area.
- 4 - we have genetically uniform planting material.
- 5 - easier plant's propagation.
- 6 - propagation plants which have a long life cycle (the trees).
- 7 - the distriktion of viral infection.

The rate of clonal micro propagation depends on genotype donor plants, physical condition, size of initial explants and nutrient medium. The plant's quality depends on nutrient medium, cultivation conditionally and rooting. We can confidently say that culture in vitro the most productive and actuality. But this culture has some problems and questions. For general use in practice we must upgrade something stage.

The purpose of the work - the perfection culture in vitro for accelerate grape's propagation.

To achieve we must:

1 - determine the optimal consist of nutrient medium and cultivation's conditions.

2 - search a new jelly components and increase efficiency clonal micropropagation the various of grape «Kobzar» to culture in vitro.

Research materials and methods

For solving problems we carrying research «using cornstarch and some various of nutrient medium for accelerate and cost-effective plants propagation in vitro. For research we used the various of grape « Kobzar ». Harvesting vines vas conduct in grapes plants (we checked their absence of disease). The vines derminated to stage «green sprout» after then we selected axillary buds and using their in culture in vitro. For cultivation we using nutrient medium: Gamborga, Nitsch and Nitsch, MC (modified)[2]. For jelly property we used: potato and corn starch, agar. For culture in vitro nutrient medium must be optimally thick, provides vertical position explants. Experimentally was determined an optimal concentration of starch- 70g/l. This nutrient medium was milky-white color, has flat, thick surface, jelly consistency. We sterilized nutrient medium 20 min(autoclave). After that we entered explans in culture in vitro in laminar box. Cultivation initial explants conducted in the cultural box at 25-27 corners C. We determine the survival rate in 10-20th day from the starch of cultivation, after that we can see a begins proliferation and roots formation. After that we will transplant explants in medium 2 phase. We conduct this propagation by technology of Tairov NRC for viticulture and vine [3, 4]. After 2cond place plants transplant in greenhouse.



Results

Using new jelly substances in micropropagation grape's plants in vitro.

The basic of method culture «in vitro» is induction organogenesis from initial buds to condition cultural box. Good effect due to correct selection nutrient medium. Quantitative and qualitative composition affect the environments to survival and begins proliferation and rizogenesis. Usually in culture in vitro we using nutrient medium with agar. Made in abroad (USA, Russia). It is so expensive. The Ukrainian agaroid differed from abroad agar. Be can't use agaroid in culture in vitro. We conducted search a new jelly components. Was evaluated replament agar to effective process clonal micropropagation [4]. In results we have approbation some various of starch in different concentration. As first, we investigated influence different jelly substances in nutrient medium(MC). When we comparing the survival rate we can see, that the best results was in nutrient medium when was modified agar. When we have some conditions of cultivation survival initial explants in this nutrient medium was on 12-20% more than with agar.

The axillary buds proliferation with starch begins in 3-4 day, then usually it's 3.7-5 day. In nutrient medium with agar proliferation begins in 5-7 day, then usually 6.3-7 days. After long observation we can say that plants grow and develop so good. After observing we can say that plants who lives in potato starch was died in 7-8 day. We understand that next observation potato starch is mining-less. The nutrient medium with potato starch split apart and it's so bad. In next observation we can't use potato starch.

Survival initial explants

For testing and implementation results We conducted a series of studies using corn starch and different nutrient mediums for accelerate propagation implants various of grape «Kobzar» in vitro. We using nutrient mediums: MC, Nisch and Nisch, Gamborga (table 1).

Table 1

Survival initial explants various of grape «Kobzar» in different nutrient mediums

| Nutrient mediums | Average value |
|--|---------------|
| Murasige and Skoog+agar | 73.75 |
| Murasige and Skoog(modified)+corn starch | 91.25 |
| Nisch and Nisch+agar | 65.00 |
| Nisch and Nisch(modified)+corn starch | 73.75 |
| Gamborga+agar | 47.50 |
| Gamborga(modified)+corn starch | 60.00 |



We understand that cornstarch make a positive effect to survival initial explants this variety of grape «Kobzar» (fig 1). Thanks corn starch content we have seen an increase survival initial explants. But when jelly consisted with agar, we observed a bad results. And we have to understand that nutrient medium Murasige and Skoog(modified)+corn starch was the best.



Fig. 1. The development of initial explants on the nutrient medium

Proliferation

Proliferation has the main role in plant's development. After speed proliferation plants development so good. In process research we conducted observation our explants development and learn proliferation process. We understand that propagation developed on variety nutrient medium. When we study nutrient mediums and their proliferation's influence, we must say that proliferation in nutrient medium Gamborga was so bad and slowly (table 2). If we use Nisch and Nisch we will speed proliferation for 1-2 day. But different jelly components give for us different results. The analysis of the impact nutrient medium to beginning proliferation. We can see that MC was the best nutrient medium. In this nutrient medium proliferation begins in 3-4 day. And I must to say that MC(modified)+ corn starch was better than MC+agar.

Rizogenesis microclones in different nutrient mediums

In next part of this work we analyzed root formation process for explants various of grape «Kobzar» in vitro. We know that acceleration rizogenesis has a positive effect for plant's growth and power (table 3). We found that Gamborga isn't conducive to the information of roots.

For max acceleration roots formation we used MC(modified)+corn starch. In nutrient medium MC(with agar) rizogenesis begins in 9-11,



but in MC(modified)+corn starch roots formation begins in 7 day. It accelerate roots formation to 2.5 day.

Table 2

Proliferation initial explants variety of grape «Kobzar»

*average value, less mentioned

| Nutrient mediums | Average value |
|--|----------------------|
| Murasige and Skoog+agar | 5.70 |
| Murasige and Skoog(modified)+corn starch | 2.90 |
| Nisch and Nisch+agar | 7.50 |
| Nisch and Nisch(modified)+corn starch | 5.80 |
| Gamborga+agar | 9.25 |
| Gamborga(modified)+corn starch | 8.30 |
| Average value+agar | 7.48 |
| Average value+corn starch | 5.68 |

Table 3

Begins roots formation initial explants various of grape «Kobzar» in vitro in different nutrient mediums

*average value,less mentioned

| Nutrient mediums | Average value |
|--|----------------------|
| Murasige and Skoog+agar | 9.60 |
| Murasige and Skoog(modified)+corn starch | 7.10 |
| Nisch and Nisch+agar | 10.7 |
| Nisch and Nisch(modified)+corn starch | 9.45 |
| Gamborga+agar | 13.00 |
| Gamborga(modified)+corn starch | 10.90 |
| Average value+agar | 11.10 |
| Average value+corn starch | 9.15 |

Economic efficiency

The main arguments in favor using corn starch is a price. It cost 20-30 grn/kg, but cost agar is 1500-1700 grn/kg. Economic efficiency using Murasige and Skoog(modified)+corn starch was achieved though reducing the cost of using agar. The increase survival initial explants and reduction timing for 5 days. The cost of planting was 11.9 grn, but if we will use our technology, the cost of planting will be 9.0 grn. Additionally income is 2,5 grn.

Conclusions

The influence of various jelling components, the composition of the nutrient medium on the survival, growth and development of initial



grape explants in the primary stages of clonal micropropagation of grapes was investigated. The optimal nutrient medium Murasige and Skoog with the addition of a modified corn starch was developed and implemented to accelerate the breeding of grape variety Kobzar in vitro culture. The cost of microclon on the primary stages of clonal micro propagation has been reduced. The developed methods are tested in practice.

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