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A New Approach to Biotechnology of Saffron (Crocus Sativus L.)

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I. Introduction

Saffron (*Crocus sativus L.*) as the most valuable medicinal plant known since ancient times. In recent years the area of cultivation of saffron in the world tends to increase, although in a number of European countries the production of its flower production was reduced due to the high cost and the urbanization of rural areas. Presently, demand for saffron is much higher than the norm of its reproduction. The increasing worldwide demand for floral products saffron stimulate research related to its reproduction, including with the use of biotechnological methods.

At the first international symposium on saffron biotechnology has been included in the list of priorities for the next hundred years [1]. Currently conducted biotechnology research towards the development of micro propagation techniques of different cultures, including of saffron.

Prospects for biotechnology *in vitro* considered as the basis for implementing future advances in molecular genetic improvement of saffron, as well as obtaining planting material free of pathogens.

Although research in cellular biotechnology *Crocus sativus L.* already have their own history [2-4], but so far the results achieved do not give grounds for approval of availability methodical basis, to effectively and consistently get the results that ensure the production of planting material with a sufficiently high rate of reproduction.

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Analysis of the available information in the press really shows that one of the reasons for the current situation in this area is the lack of a common approach in researches on biotechnology in vitro.The first phase of research on the induction of callus formation, morphogenesis, organogenesis in vitro, which was characterized by the establishment of optimal concentrations of hormonal inductors, and combinations thereof, has been successfully implemented. Despite the large number and variety of options for phytohormones and received on that basis the effects did not contributed to the development of a single methodological approach. The next stage in the development of methodological approaches based on the use of low positive temperature (5°C) for the induction of organogenesis in the single-stage circuit producing microcorns from callus cells, which is common at this time.

However, the ability to maximize the use of the temperature factor is not fully implemented.

In this respect, the classic position on the impact of state initial explant on the progress in the implementation of morpho -physiological potency *in vitro* relative geophytes such as the crocus is the most illustrative.Numerous observations suggest that the winter months with negative temperatures lead to increased formation of floral organs of *Crocussativus L.*

The aim of this work was to improve the method of clonal propagation applied to saffron.

II. Material and Methods

The starting material in our research was corms of saffron. To study the effect gibberellin on embryogenesis and organogenesis corms incubated ingibberellic acid solutions of 20-50 mg / l for 12 and 24 hours. As starting explant were used discs cormssliced in the transverse direction.

Corms sterilized in 70-80% ethanol followed by 2-3 times washing with sterile water. For further sterilization used a solution of sodium hypochlorite containing 5% of the basic substance, at various dilutions with the addition of TWEEN 20. Then were washed 3-fold with sterile water for 10 -15 minutes. Sterilized corns planted on an agar medium M-S [5].

Embodiments of the medium contained BAP (benzylaminopurine) 2.4 D (2, 4 -dichlorphenoxyacetic acid), kinetin (6-furfurylaminopurine), NAA (1-naphthylacetic acid), at various concentrations (mg /

l),agar - 0.7-0.8%; sucrose - 30, 60, 90 g / l; pH - 5.6-5.8. Planted material was cultivated in the dark at 21^0 C and relative humidity of 70-80%.

III. Results and Discussion

The experiments with the use of a temperature gradient revealed variants of medium, ensuring the most effective organogenesis. In conditions of incubation at a constant temperature did not obtained organogenesis of explants *in vitro*. When had used the temperature gradient, depending on the ratios of phytohormones and initial stages of embryogenesis before temperature gradient, on medium, containing BAP (4 mg / l) and NAA (1 mg / L) had formed of 10 to 25 micro corms in a flask (Fig.1).

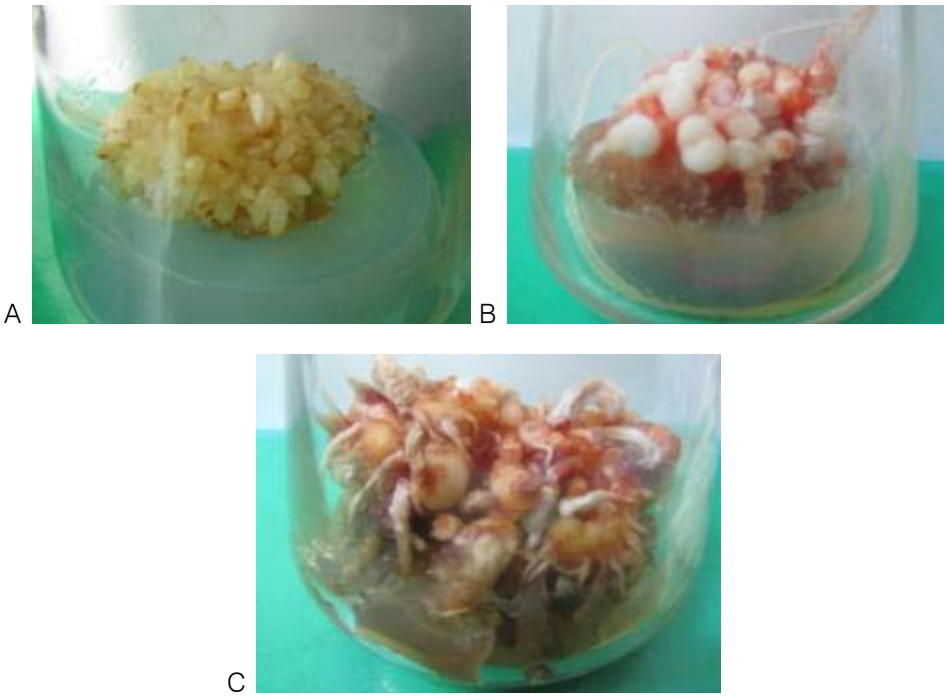

Fig.1 : Consecutive stages of embryogenesis (A), morphogenesis (B) and organogenesis (C) from Crocus sativusL. in culture *in vitro*

In the process of the temperature gradient, depending upon the composition of medium,was observed increase number of formed embryoids.

A similar effect has not presented in the literature. The applied temperature gradient could potentially allow reduce the time of incubation, which is currently equal to 5 weeks at a stable temperature.

Effect of temperature on themorpho - physiological processes may be associated with its impact on the level of free gibberellin when temperatures will contribute to their release from the bound state.Influence of exogenous gibberellin on activity of replacement meristems in corms saffron flowers and increasing the number of floral shoots had shown in the experiments [6].

Increasing the concentration of sucrose in a medium to 60 -90 g/l resulted in an increase of embryoids, but in this case significantly increased infection and it appeared relatively late. Apparently, it was associated with infection of internal tissues of corms.

Using gibberellin in our experiments was effective only in cases of environments in which were present BAP, 2,4-D and kinetin. In the absence of BAP, 2,4-D and kinetinuse of gibberellin has not very effective, and in some cases adversely affect the processes of organogenesis. This may reflect the complex interactions gibberellic acid with other phytohormones and perhaps may be particularly characteristic of corms *in vitro*. Regarding the callus cells from leaf explants *Crocus sativus L.* is evidence of a positive effect of exogenous gibberellin on organogenesis at various hormonal background[7,8].

IV. Conclusions

Thus, consideration of the above factors that could have some impact on the efficiency of organogenesis in conditions *in vitro*, as well as the use of a temperature gradient that was not applied early, allowed realize morphogenesis on the stage of organogenesis and *de novo* get corms of saffron *in vitro.*

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