Journal of Saffron Research (semi-annual)
Vol.10, No.2, Fall & winter, 2022-2023
p. 276-284
http://dx.doi.org/10.22077/JSR.2022.5718.1198

University of Birjand

Original Article:

Optimization of Callogenesis and Cell Suspension Culture in Saffron

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Received 22 October 2022; Accepted 05 December 2022

Extended Abstract

Introduction: Despite the economic importance of saffron, significant research has not been done in the field of molecular biology and genetics of this plant. Sterility and triploid genome, along with lack of genetic diversity are main reasons for this lack of research. Optimization of saffron cell suspension is a great step forward. It will provide the possibility of studying the production of saffron secondary metabolites, gene transformation, and mutagenesis cellular level. There have been few reports of cell suspension culture in saffron, mainly for the production of secondary metabolites (Yoon et al., 2015; Moradi et al., 2020; Taherkhani et al., 2019). In order to optimize the production of crocin and phenolic compounds, Moradi and co-workers produced calli from saffron stigma and then created a suspension culture from these calli (Moradi et al., 2020). However, the production of embryogenic cell suspension in saffron has not been reported. The aim of the current research was to achieve a cell suspension system with acceptable growth in saffron. For this purpose, different explants were subjected to different hormonal treatments to determine the optimal callus formation and suspension culture media.

Materials and Methods: Saffron corms were used to prepare explants. The lateral and terminal buds and the base of the corms were removed and the central part of the corms was used to prepare explants. Then, the explants were sterilized with 2.5% Sodium hypochlorite for 15 minutes. Explants were grown on CIM1 to CIM5 media (Table 1). After 5 weeks, the percentage of callus formation and the fresh and dry weight of calli were measured. The calli obtained from CIM2c treatment were used to prepare suspension culture with three different types of culture media (Table 1). To compare the speed of cell growth in different cell suspension treatments, the optical density (OD) measurement method was used. For all the assays, data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey Honestly Significant Differences (HSD) Post-Hoc test using SPSS® statistical software, version 22 (IBM Corp., USA). The graphs were created using GraphPad Prism version 9 for Windows (GraphPad Software, California USA).

Results and Discussion: The results showed that the concentration of 2 mgL^{-1} of 2,4-D and 1 mgL^{-1} of BAP had the best performance in callus formation and callus fresh weight. The calli of this treatment were used for cell suspension culture. SM3 medium had the best growth rate in cell suspension culture, but it did not have cells with appropriate shape and form. SM2 medium had a suitable growth rate and cells with small size and dry color, which had the best performance in terms of quality. In order to increase the quality, phenol production control materials were used. Adding PVP increased the growth rate of cell suspension in the SM2 medium. As we have shown, among the auxins 2, 4-D has a greater effect than NAA, contrary to other studies reported (Ahmad et al., 2013; Verma et al., 2016; Safarnejad et al., 2016; Georgiev et al., 2009; Sharma et al., 2008).

Conclusion: The current research showed the effect of treatment of 1 mgL^{-1} of BAP and 2 mgL^{-1} of 2,4-D on increasing the percentage of callus formation and the weight of calli taken from the saffron corms. SM2 and SM3 treatments had the best cell suspension growth rate. However, the SM3 treatment had cells with high starch vacuoles. Nevertheless, the SM2 treatment had smaller cells containing pigments.

Conflict of Interest: The authors declare no potential conflict of interest related to the work.

Keywords: Callus formation, 2,4-D, BAP, growth regulators, phenolic substances.

Five Important References:

Ahmad, I., Hussain, T., Ashraf, I., Nafees, M., Maryam, R. M., & Iqbal, M. (2013). Lethal effects of secondary metabolites on plant tissue culture. *Am Eurasian J Agric Environ Sci*, *13*(4), 539-547.

Moradi, A., Zarinkamar, F., De Domenico, S., Mita, G., Di Sansebastiano, G. P., Caretto, S. (2020). Salicylic Acid Induces Exudation of Crocin and Phenolics in Saffron Suspension-Cultured Cells. *Plants*, *9*(8), 949.

Shokouhi, D., Seifi, A. (2021). Growth daynamics and cell viability in tomato suspension cultures derived from different types of calli. *International Journal of Horticulture Science and Technology*, 8, 25-35.

Safarnejad, A., Alamdari, S. B. L., Darroudi, H. & Dalir, M. (2016). The effect of different hormoneson callus induction, regeneration, and multiplication of saffron (*Crocus sativus* L.) corms. *SaffronAgronomy & Technology*, *4*(2), 143-154.

Taherkhani, T., Asghari Zakaria, R., Omidi, M., & Zare, N. (2019). Effect of ultrasonic waves on crocin and safranal content and expression of their controlling genes in suspension culture of saffron (Crocus sativus L.). *Natural product research*, *33*(4), 486-493.

Table 1. The compounds of growth regulators of callus formation media (CIM) and cell suspension culture (SM).

Media	The combinations of growth regulators				
	BAP (Sigma, B3408)	2,4-D (Sigma, D6679)	IAA (Sigma, I3750)	Zeatin (Sigma, Z0164)	NAA (Sigma, N0640)
CIM1	2	1	0	0	0
CIM2	1	2	0	0	0
CIM3	4	2	0	0	0
CIM4	2	4	0	0	0
CIM5	0.5	0.1	0.5	0	0
CIM6	1	0.1	0	0	0
SM1	0.5	0.1	0	0	0
SM2	1	2	0	0	0
SM3	0	0.2	0	0.2	2

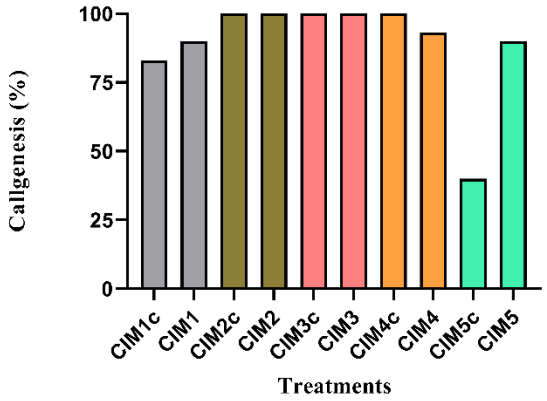

Fig 1. Callogenesis percentage of five treatments CIM1, CIM2, CIM3, CIM4 and CIM5 on MS and MS with activated charcoal.

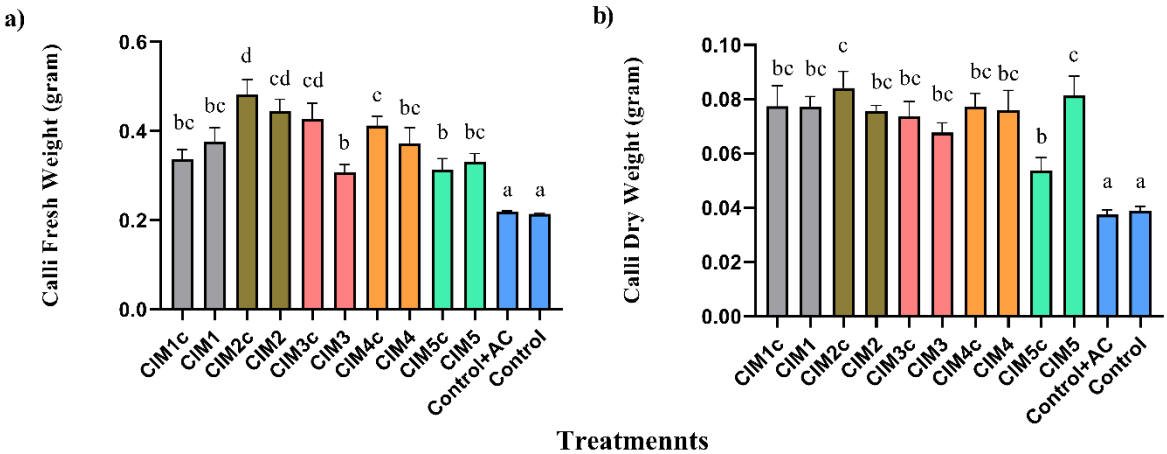

Fig 2. a) The effects of different treatments on fresh callus weight. CIM2 and CIM2c treatments had the highest and CIM3 and CIM5c had the lowest callus fresh weight. b) The effects of different treatments on callus dry weight. CIM5 and CIM1 treatments had the highest and CIM5c treatment had the lowest callus dry weight.

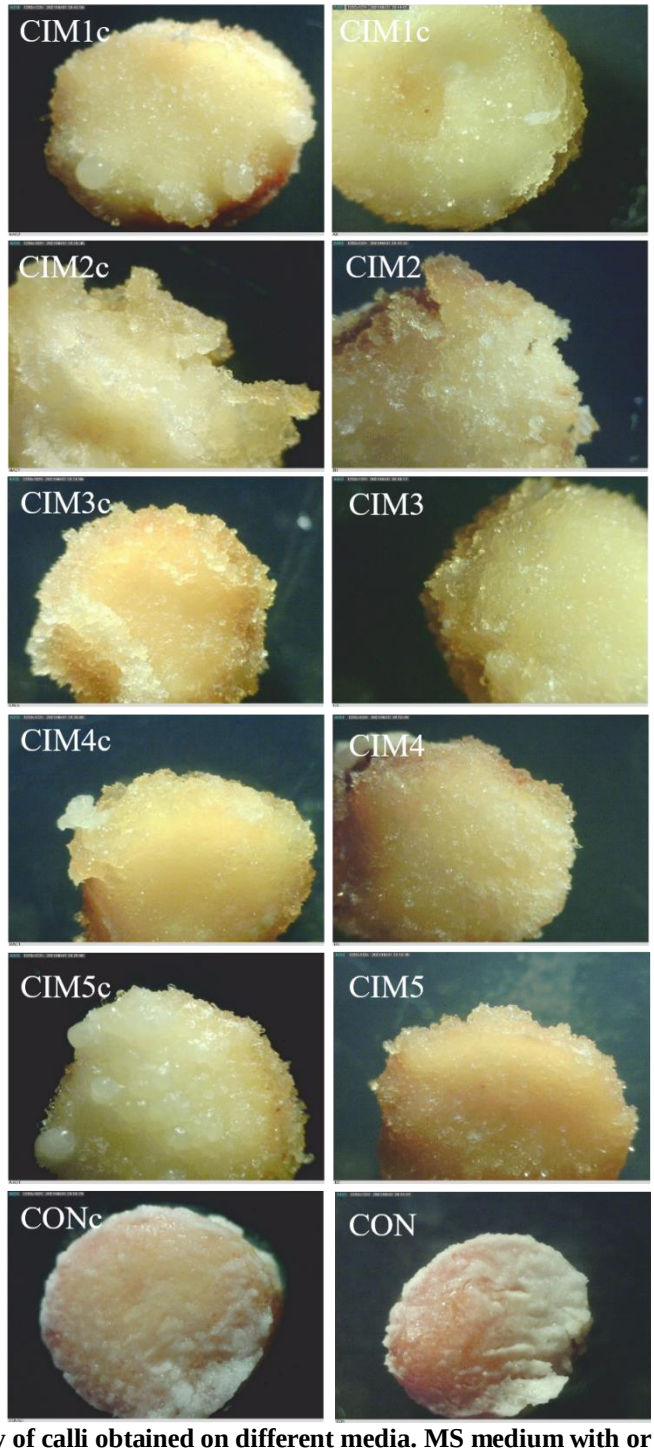

Fig 3. Morphology of calli obtained on different media. MS medium with or without activated charcoalm supplemented with five different combinations of growth regulators were used. CIM2 and CIM2c calli had fragile structure. Treatments CIM1, CIM2c and CIM5c had light yellow calluses, CIM1c, CIM3, CIM3c, CIM4c had amber calluses, and CIM2, CIM4 and CIM5 had dark amber calli.

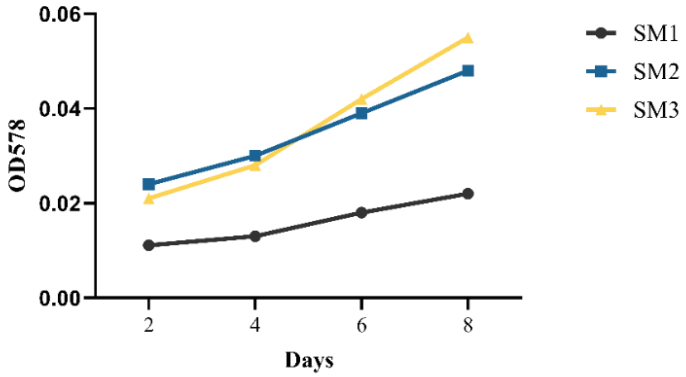

Fig 4. Comparison of cell growth rate in 3 different cell suspension culture media. Investigating the process of cell growth in liquid culture is shown based on the measurement of absorbance at 578 nm in a period of 8 days.

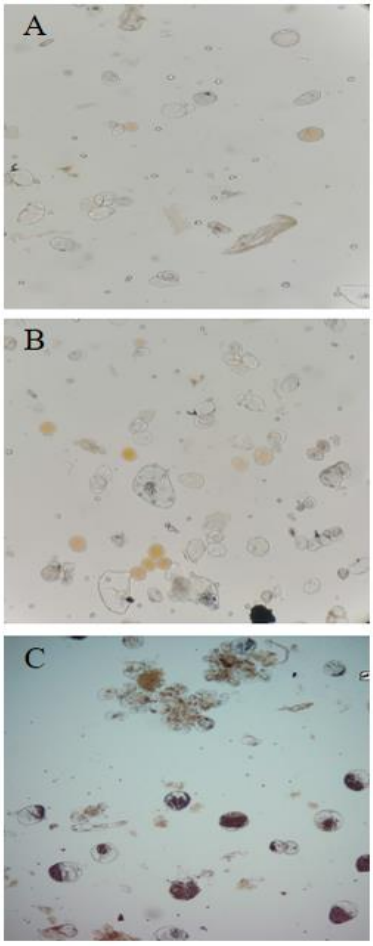

Fig 5. Morphology of cells in different cell suspension culture media. SM1 had low cell density and small spherical cells (A). SM2 and SM3 had large vacuoles that stored a large amount of starch (B and C).

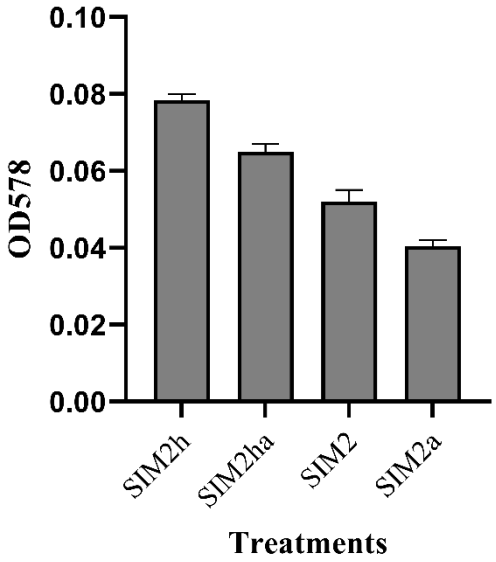

Fig 6. The cell growth rate in different suspension culture media. The growth rate was compared by measuring absorption at 578 nm after 8 days of initiation of cell cultures.