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Effect of Different Concentrations of Phytohormones on Callus Induction in Saffron (*Crocus sativus* L.)

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ABSTRACT

Herbal secondary metabolites possess high economic value, and the chemical synthesis of these metabolites is typically complex and costly. In light of this, the production of metabolites through various biotechnological methods, including plant cell and tissue culture, can offer a beneficial alternative. In this experiment, corm explants of saffron were collected from the Torbat-e Heydarieh, a native Iranian ecotype. After sterilization, they were cultured in ½ MS medium supplemented with different concentrations of NAA, IBA, and/or 2,4-D in combination with the BAP hormone at 21°C under dark conditions. The experiment followed a completely randomized design with three repetitions for hormones and their levels. The findings revealed that, among the various callus induction media tested, the ½ MS medium, when supplemented with 0.5 mg/L of 2,4-D and 0.1 mg/L of BAP, demonstrated the highest percentage of callus formation. Meanwhile, the ½ MS medium with hormone combinations of 0.5 mg/L of BAP and 2 mg/L of IBA resulted in the lowest callusing. On the other hand, although NAA was effective for callus induction, the rooting percentage of calli is higher and is not suitable for callus induction or the establishment of suspension culture in saffron.

Keyword: Callus induction, Phytohormone, Saffron

Abbreviations: 2,4-D; 2,4-Dichlorophenoxyacetic acid, NAA; 1-Naphthaleneacetic acid, IBA; Indole-3-butyric acid, BAP; 6-Benzylaminopurine, MS; Murashige and Skoog medium

INTRODUCTION

Medicinal plants are considered one of the most important medicinal sources and have been used since thousands of years ago. According to the World Health Organization, more than 80% of people make use of medicinal plants, either in modern or traditional ways. In addition, some chemical drugs are also modeled from herbal materials [1]. Saffron (Crocus sativus L.) belonging to the Liliaceae family, with a chromosomal count of 2n = 3x = 24, is a tiny plant, typically reaching a maximum length of 10–30 cm, propagated through corms. This species has several applications, both as a medicinal and nutritional herb. Among the 85 species in the *Crocus* genus, *C. sativus* is considered the most important. Some scholars believe that this plant originated in Iran [2]. Tissue culture is a type of nonsexual propagation whose advantage over traditional methods includes the production of a large number of plants with uniform genetic characteristics in a shorter time and a smaller area. Moreover, many secondary metabolites produced by whole plants are also produced by their callus and cell cultures and are used as a crucial source of economically valued compounds [3]. The first report of saffron successful propagation included the application of 2,4-D and IAA hormones to its root explants [4]. The other successful reports include production of small corms and regeneration by 2,4-D hormone [5]; production of callus through corms by using 2,4-D hormone [6]; shoot development through corms by using cytokinin hormones and 2,4-D [7]; optimized callus production from flower and ovary explants [8]; callus production in different light conditions [9]; and direct regeneration through ovaries and by making use of BA and NAA hormones under continuous darkness and light conditions [10].

The present investigation aims to investigate the impact of different concentrations of NAA, IBA, and 2,4-D hormones in combination with BAP on callus induction in saffron as a first stage for the establishment of a cell suspension culture and the in vitro production of valuable metabolites.

MATERIAL AND METHODS

The corms of the saffron plant, originating from the Torbat-e Heydarieh ecotype in the Khorasan Razavi province, underwent an initial washing process lasting fifteen minutes with flowing water. Subsequently, they were immersed in 70% ethanol for a duration of 30 seconds. In the next step, surface disinfection was performed for 20 minutes with 0.1-0.2% HgCl2 solution. Finally, the corms were subjected to four washes with sterilized distilled water. The surface sterilized saffron corm sprouts were cultured on ½ MS medium supplemented with NAA, IBA, and 2,4-D and BAP hormones (Table 1) at 21°C under dark conditions. Hormones and their levels were conducted in a completely randomized design with three repetitions. Callusing percentage, rooting percentage, percentage of abortive explants, and lack of growth were measured 46 days after the culture of the explants, with 15 replications for each culture media. The obtained results were analyzed using SPSS software.

Table 1 Different combinations of phytohormone in ½ MS medium used for callus induction in saffron.

NO.	1/2MS medium supplemented with
1	0.1 mg/L BAP + 0.5 mg/L 2-4-D
2	0.2 mg/L BAP + 1 mg/L 2-4-D
3	0.2 mg/L BAP + 0.5 mg/L 2-4-D
4	0.2 mg/L BAP + 1 mg/L IBA
5	0.2 mg/L BAP + 1 mg/L NAA
6	0.5 mg/L BAP + 2 mg/L 2-4-D
7	0.5 mg/L BAP + 2 mg/L IBA

RESULTS

The results of the analysis of different concentrations of growth regulators indicate that 2,4-D and BAP have been effective in callus induction in corm explants of saffron. Furthermore, calluse depends on the concentration and ratios of 2,4-D and BAP hormones. As shown in Table 2, among the different callus induction mediums, the ½ MS medium supplemented with 0.1 mg/L BAP + 0.5 mg/L 2-4-D with a callusing percentage of 61.66% and the ½ MS medium supplemented with 0.2 mg/L BAP + 1 mg/L NAA with a callusing percentage of 60.67% possess the highest callusing percentage. Meanwhile, the lowest callusing (6.45%) was observed in ½ MS medium supplemented with 0.5 mg/L BAP and 2 mg/L IBA (Figs. 1 and 2). It indicates a difference between the effects of IBA and 2,4-D or NAA hormones on callus induction in saffron. In this regard, the IBA hormone is ineffective on callusing, while the 2,4-D and NAA hormones are the most effective on callusing in saffron corm explants. Several studies have shown that the combination of auxin and cytokinin hormones has significant roles in callus induction and has diverse effects in different varieties and species depending on their type and concentration [11–14]. Most studies indicate that auxin hormones, especially 2,4-D, have a major role in the induction of callus. In addition, these hormones have better performance in terms of the production and growth of calluses if cytokines such as BAP are also present in culture media [15, 16].

Table 2 Callusing, rooting and callus browning percentages of saffron under different phytohormone treatments with a completely randomized design and Duncan's multiple range test.

½ MS medium supplemented with	Callusing percentage	Rooting percentage	Callus browning percentage
0.1 mg/L BAP + 0.5 mg/L 2-4-D	61.66a*	2.88 d	35.46 e
0.2 mg/L BAP + 1 mg/L 2-4-D	29.99 b	6.67 c	63.34 d
0.2 mg/L BAP + 0.5 mg/L 2-4-D	28.57 b	7.14 bc	64.29 d
0.2 mg/L BAP + 1 mg/L IBA	15.2c	8.3 b	76.5 c
0.2 mg/L BAP + 1 mg/L NAA	60.67 a	26.67 a*	12.66 f
0.5 mg/L BAP + 2 mg/L 2-4-D	13.33 d	0 e	86.67 b
0.5 mg/L BAP + 2 mg/L IBA	6.45e	0 e	93.55 a*

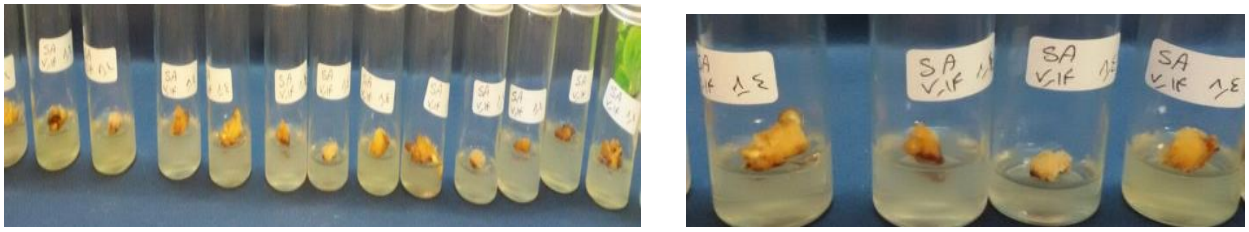

Fig. 1 Suitable callus induction in ½MS medium supplemented with 0.1 mg/L BAP and 0.5 mg/L 2-4-D

Fig. 2 Rooting induction in ½ MS medium supplemented with 0.2 mg/L BAP and 1 mg/L NAA

DISCUSSION

On the other hand, in the ½ MS medium supplemented with 0.2 mg/L BAP + 1 mg/L NAA, the highest level of rooting (26.67%) was observed, while the presence of 0.1 mg/L BAP and 0.5 mg/L 2,4-D led to the lowest level of rooting from explants (2.88%). Besides, calluses generated by this hormone predominantly exhibited deformities. It indicates that although NAA is a suitable hormone for initiating callus formation in saffron, the primary consequence is the development of roots in the resulting calli. It renders them unsuitable for callus culture or the establishment of a viable suspension culture intended for the production of valuable secondary metabolites in this plant. Hence, it induced that 2,4-D exhibits superior efficacy compared to NAA in the context of callus production. However, most of the saffron explants and produced calluses were aborted and got browning and necrosis at high concentrations of 2,4-D and IBA (2 mg/L) with 93.55% and 86.67%, respectively. The findings by Ramandi *et al*. in 2023, focusing on the optimization of callus formation and cell suspension culture in saffron, indicated that the concentrations of 1 mg/L of BAP and 2 mg/L of 2,4-D demonstrated optimal performance in terms of callus formation and callus fresh weight. In other words, the effect of BAP and 2,4-D treatments increases the percentage of callus formation and the weight of callus taken from saffron stem. Also, these treatments had cells with high starch vacuoles and the best cell suspension growth rate [17]. Safarnejad *et al.* (2016) concluded that the most corm formation was observed in 2 mg/l BAP. Maximum callus induction was achieved on MS supplemented with 1 mg/l 2,4-D + 2 mg/l BAP. Also, this medium was suitable for the germination of embryos. Corm formation was only observed in MS supplemented with 1 mg/l 2, 4-D, + 2 mg/l BAP in the indirect method [18].

In the research of Parray and colleagues, the TDZ hormone, which is a type of cytokinin for fertility, has been introduced. The use of high concentrations of BAP for the production of tubers has been proven in the past [19].

CONCLUSION

It is concluded that the optimal hormone combination in the ½ MS medium for saffron utilization is 0.5 mg/L of 2,4-D and 0.1 mg/L of BAP. While NAA is effective for callus induction, the elevated rooting percentage of calli renders it unsuitable for callus induction or the establishment of suspension culture in saffron.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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