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Original Article

CALLUS INDUCTION AND DIRECT ORGANOGENESIS IN SAFFRON CALLUS BY USING PLANT EXTRACT AS AN ALTERNATIVE TO PHYTOHORMONES

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ABSTRACT

Objectives: Saffron (*Crocus sativus*) is mostly propagated vegetatively. Therefore, cost-effective methods need to be developed, in order to multiply and increase the yield of saffron. The aim of present study was to use plant extract as an alternative to phytohormones for regeneration of saffron corms in in-vitro conditions.

Methods: Tiny slices or fragments of saffron corms were used as explants and cultured on Murashige and Skoog (MS) media supplemented with plant extract to obtain the callus.

Results: Callus initiation was observed in all concentrations of plant extract. However, the callus grown in 20% plant extract resulted in direct organogenesis. But callus growth was slow at 40% concentration of plant extract.

Conclusion: Our results showed that plant extract can be used as an efficient alternative source to phyto- hormones.

Key words: *Crocus sativus,* Callus, Organogenesis, MS media, Phytohormone, Plant extract.

INTRODUCTION

Saffron (*Crocus sativus*) is a genus in the family Iridacae. Among the 85 species belonging to genus crocus, saffron is the most fascinating and intriguing species. Saffron was first cultivated in Greece. Crocus grows to 20-30 cm and bears upto 4 flowers, each with three vivid crimson stigmas which are the distal ends of a carpel. The saffron crocus is a triploid, that is self-compatible and male sterile. It undergoes aberrant meiosis and is hence incapable of independent sexual reproduction. All propagation is by vegetative multiplication. Iran now accounts for approximately 90% of the world production of saffron. Reproduction hinges on human assistance, clusters of corms underground bulb like starch storing organs must be dug up divided and replanted. It can survive cold winters, tolerating frosts as low as 10 °C and short periods of snow cover rain immediately preceding flowering boosts saffron yield. Rainy or cold weather during flowering promotes disease and reduce yields. Persistently damp and hot conditions harm the crops.

Saffron has three main secondary metabolites including water soluble carotenoid pigments mainly Crocin, bitter taste glycoside - Picrocrocin and spicy aroma –Safranal [1-3]. Planting is mostly done in June in the northern hemisphere where corms are lodged 7-15 cm. Roots, stems and leaves can develop between October and February. Mother corms planted deeper, yield higher quality saffron. Italian growers optimize thread yield by planting 15 cm deep and in rows 2-3 cm apart. Depths of 8-10 cm optimize flower and corm production. *Crocus sativus* prefer friable loose low density, well-watered and well-drained clay, and calcareous soils with high organic content. Saffron contains more than 150 volatile and non-volatile and aroma yielding compounds.

Saffron is most commonly used in medicine as well as dye and spice in food industry. It has been also used as a drug to treat various human health conditions such as stomach disorders, colic, coughs, insomnia, chronic uterine hemorrhage, famine disorder, scarlet fever, small pox, cold, asthma and cardiovascular disorders [4, 5]. It has been shown that saffron is protective agent against chromosomal damage [6]. It has been reported that saffron has anti-inflamatory, anti-seizure [7] and blood pressure reducing [8] effects in animals. Saffron extract or its active constituents, crocetin and Crocin could be useful as treatment for neuro-degenerative disorders accompanying memory impairmen [9]. Crocin extracts have been used for the treatment of nervous, cardiovascular and respiratory systems [10]. Crocin is also unique antioxidant that struggle with oxidative stress in neurons [11]. The antidepressant effect of saffron petals and stigma in mice was also reported [12]. Saffron extracts were used against different kind of tumors and cancers in ancient times [13].

Given that the saffron and its products have multiple uses but its yield is highly affected by various environmental factors all over the world. The production of saffron is decreasing day by day. Tissue culture is one of the best methods for the *in vitro* regeneration of saffron corms, which in turn would help in enhancing the yield. But, the problem with tissue culture is that it requires expensive phytohormones. Thus, the corms regenerated by using *in-vitro* technique would be costly, therefore, not affordable to a common farmer. Hence, it is important to look for alternative and cost effective ways in order to enhance the yield. The main focus of this study was to find an alternative for phytohormones in order to generate corms in a cost-effective method. In this paper, we have studied the effect of various concentrations of plant extract for *in vitro* regeneration of saffron plantlets from callus.

MATERIALS AND METHODS

Plant materials

The healthy and disease free saffron corms were collected from Pampore town of the Kashmir valley (fig. 1). Buds were separated and used as a source of explant.

Sample sterilization

Corms were sterilized using three step sterilization process involving: i) Surface sterilization of corms by first scrubbing them gently under running tap water for 10 min to remove coating layer of microorganisms ubiquitously found on them, followed by dipping in antiseptic detergent in a flask for 5 mins. and shaking it vigorously. This was followed by rinsing corms with water till the froth was completely removed ii) The corms were now sterilized with 70% ethanol for 1 min and iii) then they were treated with Mercuric chloride solution (0.2%) for 10 mins. and rinsed thoroughly about five to six times with sterile distilled water. This step was specifically carried out under the laminar airflow cabin.

Plant extract preparation

10 gm of willow plant material was taken and cut into small pieces. It was boiled in 100 ml of water for1 hour. The Plant extract was filtered through many layers of cheese cloth and later on through a normal filter paper.

***In-vitro* culture conditions**

Murashige and Skoog (MS) media was supplemented with 0.01mg/l 2,4-Dichlorophenoxy acetic acid (2,4-D) and different concentrations of plant extract. The 2, 4-D concentration was kept constant throughout in all the combinations of media (table 1). The sterile explants were inoculated and grown in test tubes in five replicates for each concentration of plant extract and the pH of the medium was adjusted to 5.8. Different concentrations of plant extracts were used as shown in table 1. The cultures were incubated in the growth room at 22 °C and 16hours light period.

Fig. 1: *Crocus sativus* (Saffron) A) Corm with flower B) Flower

RESULTS AND DISCUSSION

RESULTS

After 20 days of incubation of crocus cultures in controlled condition, we observed the callus initiation in all sets of cultures (fig. 2) particularly in presence of 10%, 20% and 30% plant extract (fig. 2A, B, C). However, the amount of callus was high in the presence of 20% plant extract (fig. 3). Also the organogenesis was observed in cultures grown in presence of 20% concentration of plant extract only (fig. 2B), which was apparent in terms of development and growth of shoots from callus. At high concentration (40%) of plant extract used, slow or no growth of callus was observed (fig. 3) which resulted in the absence of organogenesis (fig. 2D). Our results also indicate that plant extract has the promising potential for the induction of callus and direct organogenesis, thus could be used as an alternative to phytohormones such as 2, 4 D, BAP or kinetin.

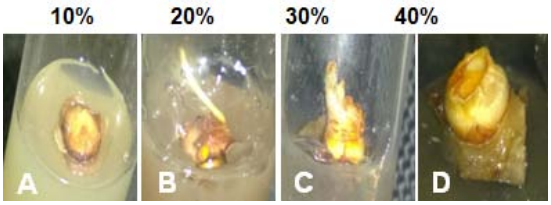

Fig. 2: *In vitro* callus initiation from saffron explants using different concentration (%) of Plant extract in MorashigeSkoog (MS) agar media. A) Callus B) Direct organogenesis from callus C) Dormant organogenesis and D) Arrested callus formation and organogenesis

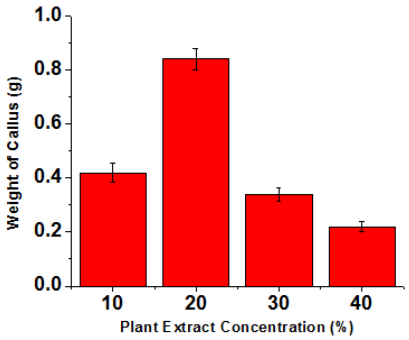

Fig. 3: Weight of saffron callus grown *In vitro* using different concentration of Plant extract in full strength Morashige Skoog (MS) agar media

Conventional propagation methods are very slow and propagation by tissue culture represents an important potential to effectively propagate it. Phytohormones are the costly chemicals in plant tissue culture. Our main idea behind the study was to use plant extract as alternative to phytohormones. Our results indicate that plant extract has the promising potential for the induction of callus and director ganogenesis. So far, many scientists have tried to achieve the *in vitro* culture of saffron corms.

Table 1: Results of different concentrations of plant extract

Plant extract conc. (%)	hyphen 2,4-D concentration (mg/l) of MS media	Results
10%		Callus initiation
20%	0.01	Callus initiation, Direct organogenesis
30%		Callus initiation, Dormant organogenesis
40%		Arrested callus formation and Organogenesis

Most of them succeeded in obtaining good results by producing microcorm on corm explants and leaves were regenerated by subculturing of microcorms [14, 15]. Tissue culture of *C. sativus* has been reported earlier [16] and bud development has also been obtained from the cut surface of corms. Different concentrations of Plant growth regulator (PGRs) ensuring initial bud sprouting, direct shoot regeneration from the base of the sprouted bud and cormlet production from multiple shoots have also been reported [17]. Our work was similar in terms of tissue culture techniques employed from many years. However, the main difference and idea of this study were to understand whether plant extract could be used as analternative to phyto-hormones or not. And our results suggested that plant extract indeed can be used as an effective alternative to phytohormones in tissue culture.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests

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CONFLICT OF INTERESTS

Declared None

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