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EFFECTS OF SODIUM NITROPRUSSIDE ON INDIRECT SHOOT ORGANOGENESIS AND IN VITRO ROOT FORMATION OF TAGETES ERECTA:AN IMPORTANT MEDICINAL PLANT

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Abstract

Tagetes erecta is known as a medicinal, industrial, and ornamental plant that has been used to treatvarious diseases due to its broad range of secondary metabolites. Considering the importance and increasingdemand for this plant, low viability and poor germination as well asgenetic manipulation, tissue culture could be proposed as a solution. Therefore, the aim of this study was to investigate the effect of sodium nitroprusside (SNP) on in vitro shoot regeneration from callus that derived from hypocotyl segments as well asroot formation of this valuable plant. Based on our results, Murashige and Skoog (MS) medium supplemented with containing 1.0 mg/l 6–benzylaminopurine (BAP) plus 0.1 mg/l 3–indolebutyric acid (IBA) along with 30 μM SNP had the highest shoot organogenesis frequency (86.66%) and shoot number (10.33) from callus. Moreover, themaximum root formation (100%) and root numbers (14.86) were achieved in MS medium containing 60 μM SNP. It seems that the current protocol can be considered as a rapid and reliable protocol for mass production of Marigolds.

Key words: Nitric oxide; Callus; Plant growth regulator; Marigold.

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Introduction

Tagetes erecta belongs to Asteraceae family and is known as Aztec Marigold, Mexican Marigold, American Marigold, etc., which is native to Central America and Mexico and naturalized in the rest of Central America and the western Andes of South America [1]. Looking at the history of the consumption of the plant reveals its importance in traditional life as a medicinal and ornamental plant [2, 3]. Nowadays, many of the therapeutic properties ofTagetes erecta have been determinedsuch as blood coagulating, antiproliferative/Cytotoxicity, antidiabetic, hypolipidemic, anti–wrinkle/wound healing, hepatoprotective, keratolytic, anticataract, anthelminthic, antiaging, nematocidal, fungicidal, antioxidant, anti–mutagenicity, larvicidal, antimicrobial and insecticidal activities [1, 4]. The marigold flower (Tagetes erecta L.) represents a rich source of lutein, and it forms up to 80% of the dry matter. Lutein is a yellow plant pigment that belongs to the carotenoid family which is extracted from marigolds which is used as an additive to poultry feed as it improves bird's fat, skin and egg yolk pigmentation, and is also used as a food colouring agent and a nutrient supplement in a wide range of baked food [1, 5].

Because of low seed viability and poor germination it is hard to satisfy the increasing demand. So, tissue culture was selected as an solution for large–scale commercial propagation of this plant [6]. Also, in vitro culture of this plant is necessary for producing high quality / price ratio flowers. Moreover, genetic engineering by using biolistic or Agrobacterium methods could be consideredas a viable alternative in traditional breeding methods [7, 8] for developing distinguishedTagetes erecta cultivarsin order to satisfy market demands [5]. Adjusting the culture medium with suitable plant growth regulators (PGRs) in various combinations and concentrations could enhance the propagation potential of various genotypes and explants [9–18]. Thus, it is necessary to improve the propagation protocols by using suitable PGRs in order to overcome the difficulties associated with clonal regeneration and gene transformation strategies to satisfy the increasing demand for *Tagetes erecta* [5, 6, 19, 20].

In recent years, nitric oxide (NO) has been used for developing in vitro plant propagation [21]. Nitric oxide is

known as a ubiquitous bioactive molecule that mainly contributed to various plant developmental processes such as fruit ripening, flowering, organ senescence, and germination [22]. The exterior usage of nitric oxide might improve the tolerance of plants under various stresses such as temperature, heavy metals, ultraviolet radiation, drought, and salinity [23–26]. Although there has been little research about the effect of nitric oxide on improving in vitro shoot organogenesis, there is no research evidence on the effect of this molecule on the shoot organogenesis of *Tagetes erecta*. Thus, the aim of this study was to evaluate the effect of sodium nitroprusside (SNP) on indirect shoot organogenesis as well as root formation that derived from hypocotyl explants of *Tagetes erecta*.

Materials and Methods

The seeds were washed under running water for 30 minutes and five to six times rinsed with tap water and then with liquid soap solution followed by washing with tap water. Further surface sterilization treatment was conducted in a laminar air flow chamber. The seeds were surface sterilized with 70% aqueous ethanol for 50 seconds and for 10 minutes dipped in 10% (v/v) NaOCl with 5.25% active chlorine solution, and then washed 3 times in sterilized distilled water. The sterilized seeds were incubated on MS medium. After 8–10 days, the seeds were germinated, and the hypocotyl segment from *in vitro* seedling was employed as a source of explant for the latter experiment.

The basic culture medium consists of Murashige and Skoog (MS) medium was reinforced with 30 g/l sucrose and gelled with 0.6% agar, and the pH of the medium was regulated to 5.8 ± 0.2 with 0.1 N KOH or 0.1 N HCl after adding plant growth regulators. The medium was distributed in a culture tube and autoclaved at 121 °C, for 30 minutes. All growth regulators except sodium nitroprusside (SNP) were added before autoclaving. SNP was added after autoclaving by filtering. All the cultures were kept in a sterilized culture room at 26 ± 2 °C, under 16 h photoperiods that provided by cool white fluorescent light (65 μmol m–2s–1) with 55–60% relative humidity.

Explants with 0.5–1.0 cm length, from hypocotyl of 1–week–old seedlings (Fig. 1a) were cultured on a basal MS medium containing different concentrations (0.0, 0.5, 1.0 and 1.5 mg/l) of 2,4–D (Duchefa biochemie, Netherlands) for callus induction. All of the cultures were incubated at 25 ± 2°C in the absence of light. Data of callus formation frequency (%) and callus weight (g)were measured after 4 weeks of culture.

Calli were cultured in the regeneration medium containing 1 mg/l BAP plus 0.1 mg/l IBA supplemented with different SNP concentrations (0, 10, 20, 30, 40, and 50 μM). The shoots regeneration frequency and the number of shoots per callus were determined after 5 weeks of treatment.

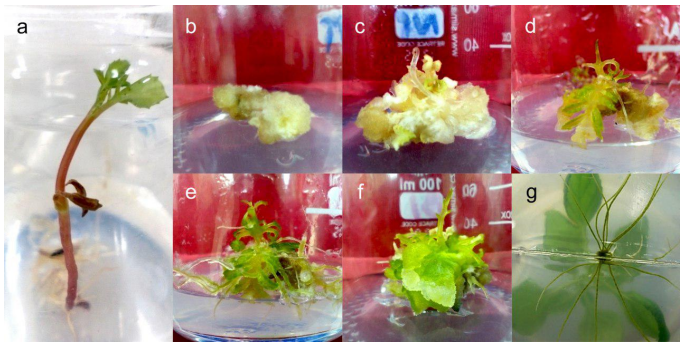

Fig. 1: *In vitro* shoot regeneration through indirect organogenesis from seedling derived *hypocotyl* segments of *Tagetes erecta* L. (a) Seedling from *in vitro* seed germination; (b) Yellow–brownish and friable callus induction on MS medium containing 1.0 mg/l 2,4–D; (c–e) Shoot regeneration from callus on MS medium containing 1 mg/l BAP plus 0.1 mg/l IBA along with 30 μM SNP after 1, 2 and 4 weeks, respectively; (f) Callus proliferation on MS medium containing 1 mg/l BAP plus 0.1 mg/l IBA along with 50 μM SNP; (g) *In vitro* root formation on MS medium along with 60 μM SNP.

The shoots with 0.5–1.5 cm in long were transferred to MS medium supplemented with 1 mg/l GA_3(elongation medium) for 4 weeks.The elongated shoots 2–3 cm long chosen from elongation medium were harvested and transferred to the half strength MS medium containingdifferent concentrations (0, 20, 40, 60, 80, and 100 μM) of SNP. Rooting percentage (%) and root number were evaluated after 30 days.

The experiments were set out in completely randomized design (CRD) with 10 replicates per treatment and each treatment was repeated three times. The data were analysed by ANOVA followed by Duncan's multiple range test ($P<0.05$). The data analysis was conducted by using SAS version 9.3.

Results and discussion

This experiment was conducted in order to figure out the most suitable and efficient concentration of 2,4–D for callus induction. The result of this study indicated that the maximum percent of callus induction (90%) (Fig. 2) and callus weight (1.56 g) (Fig. 3)were obtained on MS medium supplement with 1.0 mg/l 2,4–D (Fig. 1b) which is similar to the results of Godoy–Hernández and Miranda–Ham [4] who have reported that acceptable callus formation from stem explants of Tagetes erecta was observed in MS basal medium supplemented with 1.0 mg/l 2,4–D. There are various studies indicated 2,4–D as one of the most applicable auxins for callus formation [9–11]. However, in another study [27], the callus formation from leaf explant of Tagetes erecta was achieved on MS medium supplemented with 2.0 mg/l NAA and 1.0 mg/l BAP plus 0.5 mg/l GA_3.

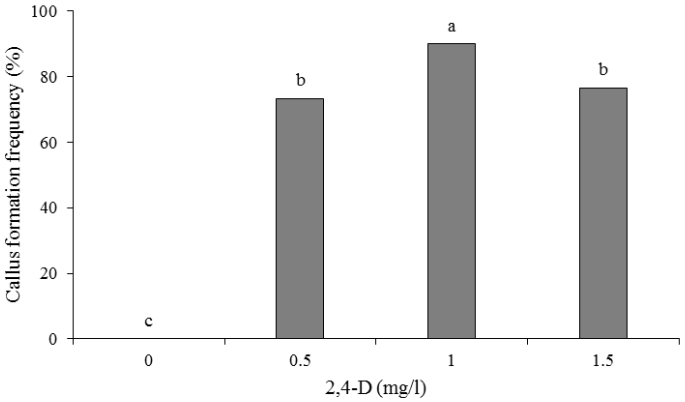

Fig. 2: Effect of different concentrations of 2,4–D in MS medium on callus formation frequency of *Tagetes erecta*.

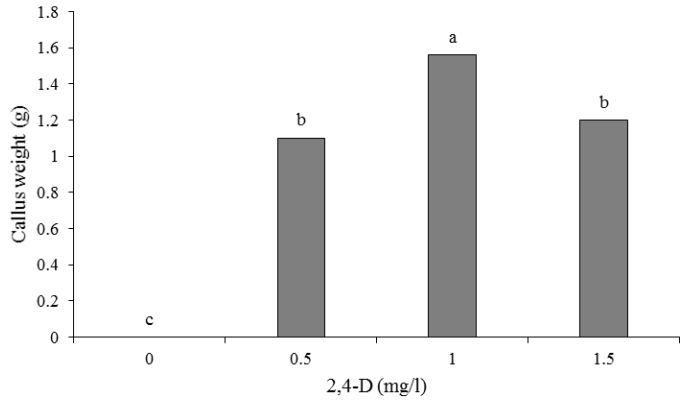

Fig. 3: Effect of different concentrations of 2,4–D in MS medium on callus weight of *Tagetes erecta*.

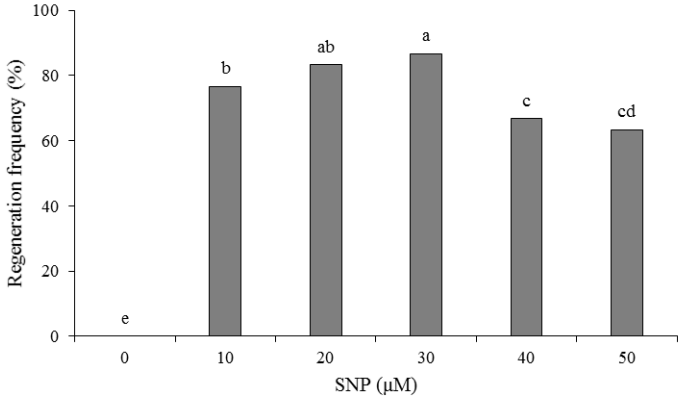

Fig. 4: Effect of different concentrations of SNP in MS medium containing 1 mg/l BAP plus 0.1 mg/l IBA on regeneration frequency of *Tagetes erecta*.

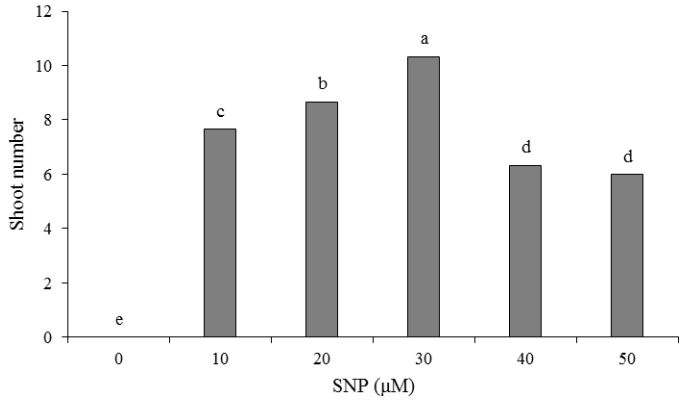

Fig. 5: Effect of different concentrations of SNP in MS medium containing 1 mg/l BAP plus 0.1 mg/l IBA on shoot number of *Tagetes erecta*.

MS medium supplemented with sodium nitroprusside were used for shoots organogenesis from callus. The shoot organogenesis and appropriate shoot development occurred under 10–30 μM sodium nitroprusside particularly in the medium supplemented with 30 μM SNP that the frequency of shoot organogenesis (Fig. 4) and the average shoot numbers (Fig. 5) obtained were 86.66% and 10.33, respectively (Fig. 1c,d,e). However, the higher level of sodium nitroprusside might limit the shoot numbers and shoot organogenesis frequency. Calli can grow in MS medium supplemented with 50 μM sodium nitroprusside (Fig. 1f). These obtained results revealedthat sodium nitroprusside can promote shoot organogenesis in proper doses. According to the recent study, Carimi et al. [28] it was reported that nitric oxide can produce in suspension cell cultures that were treated by BA in a proper dose. The effect of NO on plant growth and development was completely associated with plant growth regulators [21].

Also, Tun et al. [29] showed a swift increase of nitric oxide release in cell cultures promoted by cytokinin. Since the use of exterior cytokinin led to release nitric oxide in plant cell culture, nitric oxide might play an important role in cytokinin signal transduction. Cytokinins have various actions in plants such as regulating some of significant processes such as *in vitro* plant regeneration as well as shoots differentiation [30, 31]. Our results indicated that sodium nitroprusside definitely promoted shoot organogenesis from hypocotyl segments in MS medium along with 1 mg/l BAP plus 0.1 mg/l IBA. Similar results were observed in a previous study that conducted byHan et al. [22] in *Malus hupehensis*.

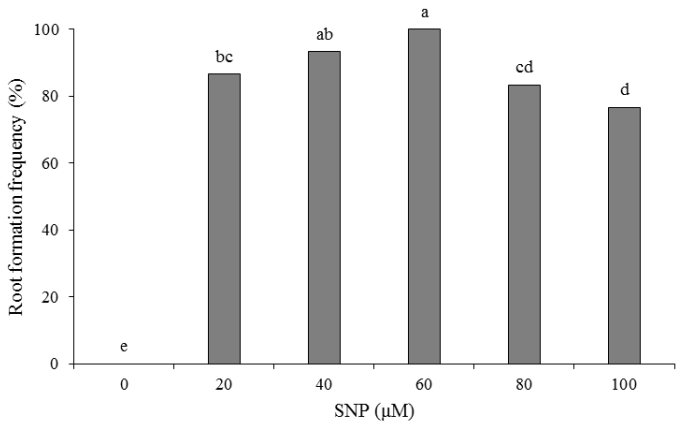

Fig. 6: Effect of different concentrations of SNP in MS medium on root formation of *Tagetes erecta*.

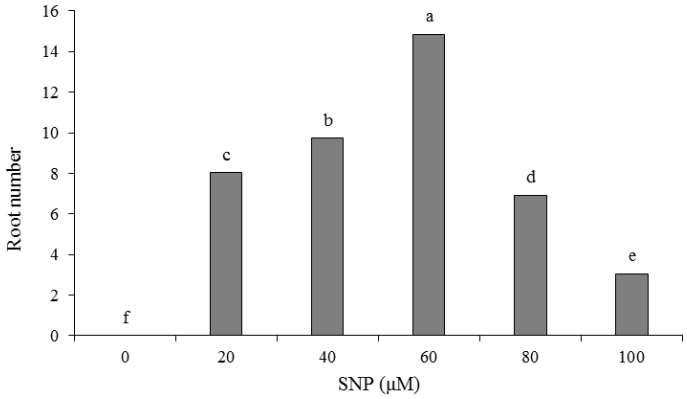

Fig. 7: Effect of different concentrations of SNP in MS medium on root number of *Tagetes erecta*.

There was no root formation existing in the MS medium without sodium nitroprusside while by adding SNP root formation was promoted significantly. By increasing the concentration of SNP from 0 to 60 μM, the root formation frequency (100%) (Fig. 6) and root numbers (14.86) (Fig. 7) were increased significantly (Fig. 1g). However, the root numbers decreased when the SNP level was over 60 μM.The positive effect of nitric oxide on improving root inductionhave beenreported in various species [22, 32, 33]. Sarropoulou et al. [34] recommended that nitric oxide could (a) produce an antioxidant condition that protects auxins from deteriorations as well as oxidation, (b)speed up cell expansion in order to improve rooting in plants, (c) serve as a downstream messenger in the IAA signalling pathway, (d) regulate enzyme activities or cell–cycle genes that are associated with auxin signal transduction, and (e) reduce the lignification of cell walls. Also, Pagnussat et al. [35]] reported that nitric oxide can improve rooting as the same as IAA in cucumbers. In another study, Tewari et al. [36] indicated that nitric oxide can play a significant role in auxin–induced signalling cascade in order to induce root development in various species.By using exterior sodium nitroprusside, the root induction in mung bean was promoted significantly [32].

In conclusion, it seems that the current protocol can be considered as a rapid and reliable protocol for mass production of *Tagetes erecta*.

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