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# Effect of the use of indole-3-acetic acid on somatic embryogenesis of sunflower (*Heliantus annuus*) grown in the southern region

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**Abstract**

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops worldwide. However, one of the early uses of this species was as an ornamental plant due to the color and variety of its flowers. Today, there is increasing interest in tissue culture techniques in this species using growth regulators for callus formation. We evaluated three concentrations of indole-3-acetic acid, 0.1, 0.5 and 1 mg/L, on embryogenic callus formation using cotyledon, hypocotyl and root of sunflower cultivated in Southern Mexico. Results indicate that the three concentrations promoted callus formation, however, the best development was observed at 0.1 mg/L indole-3-acetic acid. Thus, indole-3-acetic acid is an efficient regulator for H. *annuus* plant growth and organogenesis.

**Keywords:** Heliantus annuus; indole-3-acetic acid; Organogenesis; Plant tissue culture; Sunflower

Abbreviations: IAA: Indole-3-acetic acid; H. *annuus: Helianthus annuus*

## Introduction

Sunflower (*Helianthus annuus*) belongs to the considerable amount of Mexican domesticates. There is evidence of its domestication by 2,600 B.C. *H. annuus* cultivation was widespread in Mexico and extended as far south as El Salvador by the first millennium B.C. This crop was well-known by the Aztecs, and nowadays is still being used in traditional tributes by Mesoamerican cultures. The sunflower's association with indigenous solar religion and warfare in Mexico may have led to its suppression after the Spanish Conquest Lentz [1]. Nowadays, this crop is the 4th most important oilseed crop in the world, after soybean, rapeseed, and peanut, contributing to the 12% of the edible oil produced at this level Cakmak [2]. Sunflower seeds are particularly intriguing among plant proteins since they are widely available. They comprise high lipids and protein content, which contribute to their nutritive value and nutraceutical properties. The quantity of anti-nutritional components in sunflower is very low as compared to other plant sources Kaur & Ghoshal [3]. Sunflower seeds are an excellent source of healthy fats, fiber, vitamins and minerals, as well as protein (20%). Hence, the intake of these seeds may improve human health and prevent several metabolic disorders, thus, they must be included in the daily diet Alagawany [4]; González-Pérez & Vereijken [5].

Extracts from sunflower are known to be a potential source of antimicrobial, anti-inflammatory, antitumor, and antioxidants agents, in order to protect human cells against reactive oxygen molecules and pathogenic microorganisms. Also, pharmacological studies on *H. annuus* seeds have revealed its capacity to treat different diseases. Some of the most relevant health benefits of these seeds include hypertension and hyperglycemia control, skin protection, hypocholesterolemic effect, and anti-obesity capacity Adeleke & Balalola [6]; Leverrier [7]. Additionally, they have shown bactericidal and fungicidal properties Maria-Neto [8]; Ribeiro [9]. Additionally, the use of sunflower seed byproduct and its fractions are promising ingredients for the development of healthier and less expensive foods as well as the alternative to decrease the environmental problems caused by the sunflower oil industry de Oliveira Filho & Egea [10]. It is worth mentioning that in vitro plant cell and tissue culture techniques are the basis of scientific micropropagation and breeding programs, and as an alternative to produce secondary metabolites.

## Materials and Methods

Sunflower seeds were provided by Rancho Los Molinos from Tuxtla Gutiérrez, Chiapas, Mexico. Samples were disinfected as described by Elaleem [11]. Seeds were placed on sterile liquid culture jars with 25 ml of supplemented Murashige and Skoog medium with 0.1, 0.5 and 1 mg/L indole-3-acetic acid (IAA) as growth regulator and incubated in a bioclimatic chamber (25 2°C, 16 h photoperiod, 14 weeks). Statistical analysis was performed by multifactorial ANOVA.

## Results and Discussion

Callus formation was observed in the three types of *H. annuus* explants at 7 days of growth; results were recorded at 4 weeks of development. Concentration of 0.1 mg/L promoted consistent development in all explants. Besides, callus formation was achieved in all explants under the three different IAA concentrations. Root explants at 0.1 mg/L IAA developed adventitious roots (Figure 1A). Cotyledon extracts at 0.5 mg/L IAA showed root formation (Figure 1B). Explants from roots and hypocotyls added with 0.1 mg/L showed 100% callogenesis (Figure 1C & Figure D). Hypocotyl explants at 0.5 mg/L IAA did not develop callus (Table 1). Nowadays, there is no data on callogenesis formation using IAA as unique growth regulator. In general, root explants showed the best callogenic induction. Elaleem [11] obtained sunflower callus using concentrations of 1.5 mg/L 2,4-dichlorophenoxyacetic acid, as well as only with 2 mg/L α-naphthalene acetic acid as growth regulator. Interestingly, this is the first report of callogenesis induction of *H. annuus* explants by using IAA as unique plant growth regulator.

**Table 1**: Physiological responses obtained by evaluating different concentrations of indole-3-acetic acid (IAA) in three types of sunflower explants.

| Physiological effect | | | | |
|---|---|---|---|---|
| **IAA (mg/L)** | **Explant** | **Callus** | **Shoots** | **Roots** |
| 0.1 | Cotyledon | $25.0 \pm 10.9^{a}$ | $41.67 \pm 10.4^{b}$ | $25.0 \pm 12.15^{a}$ |
| | Root | $100.0 \pm 10.9^{b}$ | $0 \pm 0^{a}$ | $93.33 \pm 12.15^{b}$ |
| | Hypocotyl | $100.0 \pm 10.9^{b}$ | $0 \pm 0^{a}$ | $100.0 \pm 12.15^{b}$ |
| 0.5 | Cotyledon | $83.33 \pm 10.9^{b}$ | $8.33 \pm 10.4^{b}$ | $83.33 \pm 12.15^{b}$ |
| | Root | $93.33 \pm 10.9^{b}$ | $0 \pm 0^{a}$ | $73.33 \pm 12.15^{b}$ |
| | Hypocotyl | $0 \pm 0^{a}$ | $0 \pm 0^{a}$ | $0 \pm 0^{a}$ |
| 1 | Cotyledon | $50.0 \pm 10.9^{a}$ | $0 \pm 0^{a}$ | $50.0 \pm 12.15^{b}$ |
| | Root | $83.33 \pm 10.9^{b}$ | $0 \pm 0^{a}$ | $80.0 \pm 12.15^{b}$ |
| | Hypocotyl | $33.33 \pm 10.9^{a}$ | $0 \pm 0^{a}$ | $33.33 \pm 12.15^{a}$ |

IAA: indole-3-acetic acid

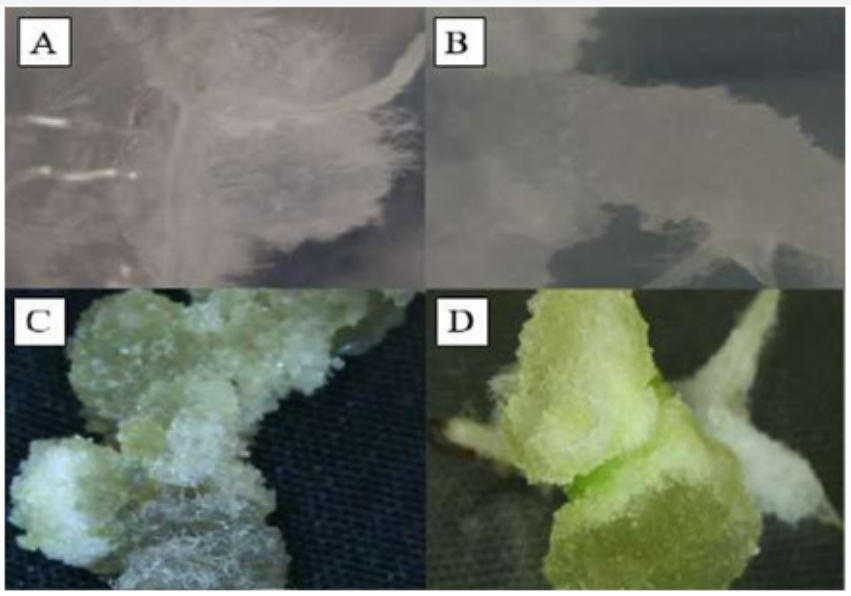


**Figure 1:** Callus formation of *H. annuus* from A) root explants at 0.1 mg/L IAA, B) cotyledon explants at 0.5 mg/L IAA, C) and D) hypocotyl explants at 1 and 0.1 mg/L IAA, respectively.



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## Conclusions

In summary, our results on callogenesis formation of sunflower seeds only using IAA, shows that root explants developed the best callogenic induction. Interestingly, this is the first report of callogenesis induction of Mexican *H. annuus* explants using IAA as unique plant growth regulator. Future studies need to be performed to establish better protocols on somatic embriogenesis in sunflower seeds.

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