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# Regulation of Callogenesis using Cotyledon and Hypocotyls explants of Cassia senna

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**ABSTRACT:** The present study was carried out to detect the effect of two Auxin namely dichlorophenoxy acetic acid (2, 4-D) and naphthalene acetic acid (NAA) on callus induction from cotyledon and hypocotyledon explants of cassia senna sp. Two explant from in vitro cassia micro plant were used for Callus induction on Murashige and Skoog media (MS) media, as basal media (control) or supplemented with different concentration of 2, 4, - D dichlorophenoxy acetic acid (2,4,D) and naphthalene acetic acid (NAA). On MS basal media (without growth regulators) no callusing obtained. Cotyledon and hypocotyls explants obtained callusing on MS media supplemented with NAA and 2, 4-D at all concentration . Callus growth percentage of cotyledonary leaf segments is varied from 60% to 100%, while the callus growth percentage of hypocotyl segment explant is varied from 75%-to 100%. Highest percentage of callus (100%) was observed in medium supplemented with 2.5 mg/l of the both NAA and 2.4-D. The both explant induce compact callus with all treatment .For callus color , cotyledon explant produced yellowish callus with the both auxin , on the other hand hypocotyls explant produced green to light green callus The highest callus degree (2.55±0.11) was obtained in MS media supplemented with 2.5 mg/l NAA by cotyledon explant , followed by (2.45± 0.15) induced in MS media fortified with 2.5 mg/l NAA(the same treatment) from hypocotyl explant, during same period of time(14 day).

**KEYWORDS:** callus, growth regulators, cotyledon explant, hypocotyl segment, auxin.

## 1 INTRODUCTION

*Cassia senna* is an important medicinal plant, legume , belongs to the family of Caesalpiniaceae [1].

In Sudan *Cassia senna* leaves are mainly used against gonorrhea, for their purgative properties, as a Guinea worm expellant. A poultice of grind leaves is applied to the skin to dermatotherapy such as guinea worm sores. The ash of leaves, are mixed with Shea butter and applied externally to softening arthritis dolores. Pods are used in the medication of skin diseases such as eczema, scabies and ringworm. The disport of the pods is taken as a laxative. The root is laxative as well and in decoction it is used as a stomachic tonic and diuretic , is especially used to remedy edema and gonorrhea. The root contains a dark dye that is used as a body paint( Elojuba, *et al*.,1999). [2]

The present study was carried out to stabilized sterile culture from *cassia* seed using sodium hypochlorite, determine the effect of two different medium namely Murashige and Skoog (MS [3] medium, Gamborg *et al*., medium (B5) of Gamborg et al [4] in full strength in seed germination of *cassia senna* sp. and to detect the effect of two Auxin namely dichlorophenoxy

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acetic acid (2, 4-D) and naphthalene acetic acid (NAA) on callus induction from cotyledon and hypo cotyledon explants of *cassia senna* sp.

## 2 MATERIALS AND METHODS

This study was carried out in the Laboratory of Plant Tissue Culture, department of Biotechnology and Biology, Faculty of Science and Biotechnology, AL Neelain University, Sudan.

### 2.1 SOURCE OF PLANT MATERIAL

The seeds of *cassia senna* used in this study were obtained from the Khartoum state, Alkadarow forest.

### 2.2 MEDIA PREPARATION

Murashige and Skoog (MS) medium [3] and Gamborg medium (B5) of Gamborg *et al* [4] were used in standard component. Media was prepared by adding MS basal medium component + 30 g sucrose and 7.0 g agar , the pH of media was set at 5.8±0.02 for MS media . For B5 media the basal components are used+20 g sucrose ,7.0 g agar, the pH of the medium was set at 5.5 ±0.02 prior to adding the agar . The media dispensed in the tissue culture jar. These jars were then autoclaved at 121ºC for 15 minutes at 15 psi, and stored at incubation room tell use.

### 2.3 GROWTH REGULATORS PREPARATION

Two type of auxin were used for callus induction namely 2, 4, - D dichlorophenoxy acetic acid (2, 4-D) and naphthalene acetic acid (NAA). The powder of the appropriate auxins was weighed (100 mg) and dissolved in drop of 1N Na OH and the volume was made up to 100 ml with sterilized distilled water stored in a refrigerator as stock for use.

### 2.4 CULTURE INCUBATION CONDITIONS

Cultures were maintained in incubation room at 25± 2ºC with a photoperiod of 16 hour dark and 8 hours light, at 1000 lux light intensity provided by cool white fluorescent lamp.

### 2.5 STERILIZATION

#### 2.5.1 STERILIZATION OF EQUIPMENT AND GLASS WARES

All operations for *in vitro* culture were carried out inside a horizontal laminar air flow cabinet with HEPA filters. The hood sterilized by an ultraviolet light for at least 30 min, then hood surface was wiped clean with 70 % ethanol, prior to use. All instruments, glassware's and other accessories were sterilized in autoclave at 121ºC with 15 psi for 15 min. Instruments like forceps and scissors were sterilized autoclaving and further by dipping in 70 % ethanol and flaming prior to use.

#### 2.5.2 STERILIZATION OF SEED

*Cassia senna* seed were washed under running tap water until it cleaned , treated with 70% alcohol for 1min with gentles shaking, rinsed with sterile distilled water, then sterilized for 15 min in 20% Clorox solution (containing 5.25% of sodium hypochlorite) fervid with few drops of liquid soap. Finally seeds were rinsed three times with sterile distilled water and then incubated under16 hours light / 8hours dark , light intensity of 1000 lux and temperature about 25 ±2 ºC to establish sterile culture.

### 2.6 IN VITRO CULTURE AND CALLUS INDUCTION

Sterilized seeds were cultured on culture jars contain 25 ml media (10 seed/ jar), under aseptic condition at the laminar airflow chamber. Culture jars were sealed and labeled carefully and finally kept in the incubation room under controlled condition of temperature (25±2 ºC) and light 16h light/8h dark, the growth was monitored weekly. For callus induction two explants, namely cotyledon and hypocotyls segment explant from ten day old in vitro micro plant were incubated in a culture jar (5x9 cm) containing 25 ml of MS media supplemented with different concentrations of auxin 2,4-D

and NAA (0.0,1.0,1.5,2.0 and 2.5 mg/l). Four explants per jar. Cultures were maintained in a growth room at 25°C±2°C temperature and 16h light/ 8h dark photoperiod. Callus induction observed regularly and day of initiation was recorded.

### 2.7 STATISTICAL ANALYSIS

Day of callus initiation was observed regularly .The final data was recorded after four weeks included callus percentage, callus color, callus texture and callus degree (callus degree was evaluated on scale of (0-4) where 0 mean no callus, while 1-4 for increasing callus formation till three time the size of the original explants). Data of callus degree were statistically analyzed using analysis of variance (ANOVA) and explained as mean ± standard error for Snedeco*r et al* [5]

## 3 RESULT

### 3.1 STERILIZATION OF SEED, *IN VITRO* CULTURE AND EXPLANT PREPARATION

Two type of medium were used for seed germination, sterilization percentage was 100% with the two type of media, seed begin germination after day with and two day and survival rate was 60 and 87 for MS and B5 respectively ( table 1). In vitro micro plant (figure 1) were used as source of explants for callus induction .

### 3.2 CALLUS INDUCTION

For callus induction cotyledon and hypocotyls explant from *cassia senna* micro plant were cultured on MS medium supplemented with different concentrations of 2, 4-D and NAA (table 2), On Murashige and Skoog basal media (without growth regulators) no callusing obtained. Cotyledon and hypocotyls explants showed callusing on MS media supplemented with NAA and 2, 4-D at all concentration. *The hypocotyl and cotyledonary leaf segment explants were taken from ten day old well established seedlings., callusing was observed after nine day from the both explant .The both explant induce compact callus with all treatment . For callus color ,cotyledon explant produced yellowish callus with the both auxin ,on the other hand hypocotyls explant produced green light green callus* .In hypocotyls explants callus started at the cut ends and later in whole of hypocotyls segment cover with callus, within four week the entire explants turned into mass compact, color between yellowish and light brawn with 2, 4-D and NAA. *Callus growth percentage of cotyledonary leaf segments is varied (60-100) as general , while the callus growth percentage of hypocotyl segment explant is varied (75-100), Highest percentage of callus 100% was observed in medium supplemented with 2.5 mg/l NAA and 2.4-D as general. The highest callus degree (2.55±0.11) was obtained in MS media supplemented with 2.5 mg/l NAA by cotyledon explant , followed by (2.45± 0.15) induced in MS media fortified with 2.5 mg/l NAA(the same treatment) from hypocotyl explant (figure 3 and 4 ), during same period of time(14 day)*

## 4 DISCUSSION

On Murashige and Skoog basal media (without growth regulators) no callusing obtained, this finding is agreement with Parveen and Shahzad [6], Parveen et al [7]. Cotyledon and hypocotyls explants showed callusing on MS media supplemented with NAA and 2, 4-D at all concentration I agree with Parveen et al [8], Hasan *et al* [9], Iman et al [10]. In hypocotyls explants callus started at the cut ends and later in whole of hypocotyls segment cover with callus, these result is agreement with Parveen, and Shahzad [6], Sharad and Raka [11] and within four week the entire explants turned into mass compact, color between yellowish and light brawn with 2, 4-D and NAA I agree with Agrawal and Sardar [12] about callus texture and color. Many author works reported similar result for percentage of callus but from varied species of cassia and varied explant , I agree with Hasan *et al* [9] , Veena *and Pratima* [13] , Agrawal and Sardar , [12] , Rouibi et al [14]. *The highest callus degree (2.55±0.11) was obtained in MS media supplemented with 2.5 mg/l NAA by cotyledon explant , followed by (2.45± 0.15) induced in MS media fortified with 2.5 mg/l NAA(the same treatment) from hypocotyl explant (figure 3 and 4 ), during same period of time(14 day)* , this finding is in agree with Parveen et al [7].

***Table 1. Sterilization of Seed and Seed Germination on MS and B5 Media***

| Type of medium | Day of germinations | Sterilization percentage | Survival rate |
|---|---|---|---|
| MS media | After three days | % 100 | 60% |
| B5 media | After two days | %100 | 87% |

***Table 2. Effect of different concentration of 2,4-D and NAA on callus induction from cotyledon and hypocotyls segment explant of cassia senna, data were recorded after four weeks of culture.***

| Growth regulators mg/l | Explant | Day of callusing | % of callusing | Texture of callus | Callus color | Degree of callus |
|---|---|---|---|---|---|---|
| 0.0 mg 2,4 D | Cotyledon | - | 0.0 | - | | |
| 0.5 mg 2,4 D | | 15 | 80 | Compact | Yellowish | 1.25± 0.18 |
| 1.0 mg 2,4 D | | 17 | 80 | Compact | Yellowish | 1.15±0.17 |
| 1.5 mg 2,4 D | | 17 | 85 | Compact | Yellowish | 1.35± 0.18 |
| 2.0 mg 2,4 D | | 13 | 100 | Compact | Yellowish | 1.65 ±0.13 |
| 2.5 mg 2,4 D | | 14 | 100 | Compact | Yellowish | 2.1 ± 0.16 |
| 0.0 mg 2,4 D | Hypocotyls | - | - | - | - | 0.0 ±0.0 |
| 0.5 mg 2,4 D | | 9 | 75 | Compact | Light green | 1.1 ± 0.18 |
| 1.0 mg 2,4 D | | 11 | 80 | Compact | Light green | 1.15±0.17 |
| 1.5 mg 2,4 D | | 11 | 75 | Compact | Light green | 0.9 ±0.14 |
| 2.0 mg 2,4 D | | 15 | 75 | Compact | Light green | 1.6 ±0.23 |
| 2.5 mg 2,4 D | | 14 | 100 | Compact | Light green | 2.1 ±0.16 |
| 0.0 mg NAA | Cotyledon | - | - | - | - | 0.0 ±0.0 |
| 0.5 mg NAA | | 11 | 60 | Compact | Yellowish | 0.6 ± 0.11 |
| 1.0 mg NAA | | 14 | 80 | Compact | Yellowish | 1.15± 0.16 |
| 1.5 mg NAA | | 14 | 90 | Compact | Yellowish | 1.55±0.17 |
| 2.0 mg NAA | | 16 | 90 | Compact | Yellowish | 1.9 ±0.22 |
| 2.5 mg NAA | | 14 | 100 | Compact | Yellowish | 2.55±0.11 |
| 0.0 mg NAA | Hypocotyls | - | - | - | - | 0.0 ±0.0 |
| 0.5 mg NAA | | 11 | 85 | Compact | Light green | 1.2 ±0.15 |
| 1.0 mg NAA | | 12 | 85 | Compact | Light green | 1.05± 0.13 |
| 1.5 mg NAA | | 14 | 90 | Compact | Light green | 1.2 ±0.13 |
| 2.0 mg NAA | | 9 | 100 | Compact | green | 2.25± 0.14 |
| 2.5 mg NAA | | 14 | 100 | Compact | green | 2.45± 0.15 |

***Fig. 1. Establishment of sterile in vitro micro plant***

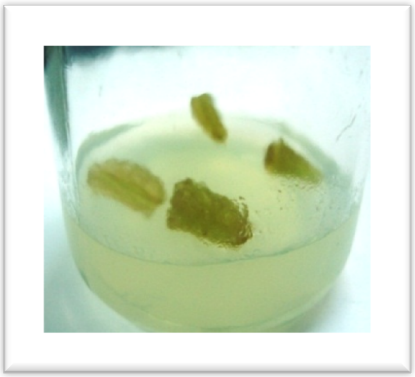

*Fig.2. Callus formation from hypocotyls explant after four week of culture on MS medium supplemented with 2.5 mg/l NAA .*

*Fig.3. Callus formation cotyledon explant after four week of culture on MS medium supplemented with 2.5 mg/l NAA.*
*Callus induction*

## 5 CONCLUSION

In conclusion, in vitro micro plant of *Cassia sp* were used as source for explant for callus induction ( *hypocotyl and cotyledonary leaf segment explants*). Maximum callus degree were induced by using 2.5 mg/l of both 2,4,D and NAA in short period of time (14 day) . This protocol has potential for large scale production of callus and subsequent secondary product of medicinal plant.

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