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Effect of plant growth regulators on Helianthus annuus L. callus induction

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ABSTRACT: The present study aimed to investigate the effect of different plant growth regulator namely 2,4 dichlorophenoxy acetic acid (2,4-D) and α-Naphthalene Acetic Acid (NAA) on callus induction of Helianthus annuus L.
Callus culture were initiated from three explants namely cotyledon leaf, hypocotyl and root segments explants of in vitro Helianthus annuus L seedling.
Both auxins 2,4-D and NAA stimulate callus induction, however 1.5 mg/L 2,4-D proved to be more effected for induction of callus. Among all treatment media containing 1.5 mg/l 2,4 D showed highest response of callus degree (3.44±0.12) with cotyledon leaf segment explant, maximum amount of callus explained by hypocotyl explant was (3.25±0.14), observed on MS media supplemented with 2.0 mg/l of both 2,4 D and NAA , for root segment explants maximum callus was (3.13±0.15), observed on MS media supplemented by 2.0 mg/l NAA . The callus from cotyledon leaf segment explants was creamy color with granular nature in media fortified with 2,4 D , while in media fortified with NAA callus was green color with watery nature and root induction observed . The callus from hypocotyl segment explants was yellow color with watery nature with 2,4 D , while in media fortified with NAA callus was green color with granular nature and root induction was observed. For root explant callus was brown color with both NAA and 2,4 D , with granular nature on MS media supplemented by 2,4 D and watery with root induction on MS media fortified with NAA.

KEYWORDS: 2,4 Dichlorophenoxy acetic acid (2,4-D), Root segment, explant, Hypocotyls, Helianthus annuus L, Callus induction.

1 INTRODUCTION

Composite family member sunflower (Helianthus annuus L.) has great importance in industrial crops with its high oil content , the technology for processing sunflower oil in to biodiesel oil has been developed lately , and as result the necessity of good quality sunflower oil is increase rapidly [1].

The genus Helianthus belong to the subdivision Tubiflorre and tribe Helianthae of the composites family these genus belong to Asteraceae family and composed of 49 species subspecies within 12 annual and 37 perennial species [2] and [3].

Thus in breeding programs, increase disease resistance and high oil content have been the main aim for sunflower [4]). In recent years, biotechnological techniques such as tissue culture have been used for improvement of sunflower .

The major producing countries for sunflower seeds are Argentine, European Union, Russian federation, Ukraine, USA, India and China, these seven countries of world produce about 76% of the total production [5] .

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Sunflower is one of the world major oil seed crops it important in industrial crops , the technology for processing sunflower oil in to biodiesel oil [1]

sunflower oil preparation of unsaturated fatty acid high oleic sunflower oil are excellent renewable raw material for industrial purpose it use as food oils, The high oxidation performance of fatty acid combined with low Content satiric acid make them suitable for industrial application like meta working fluids [5].

The Object of this study is to determine the effect of growth regulators on callus induction from varied explant of sun flower.

2 MATERIALS AND METHODS

This study was carried out in plant tissue culture laboratory, Department of Biology and Biotechnology, Faculty of Science and Technology, AL Neelain University, Sudan.

2.1 SOURCE OF SEEDS

Seeds of sunflower used in this study purchased from local market, Khartoun, Sudan.

2.2 PREPARATION OF MEDIA AND GROWTH REGULATOR

The medium used in this study was Murashige and Stooge [6], (MS) media in standard component : macro and micro element, vitamins, 7.0 g/l agar, and 3% sucrose. The pH adjusted to 5.8 ± 0.02.

The growth regulators used in this study are two types of auxin namely 2,4 dichlorophenoxy acetic acid (2,4-D) and Naphthalene Acetic Acid (NAA), 50 mg powder of appropriate auxin was dissolved in few drops 1N NaOH, then the volume made up to 50 ml by hot distilled water, mixed using magnetic stirrer, and store at 4ºC till use.

2.3 STERILIZATION OF SEEDS

The seeds were washed by running tap water, then sterilized by 70% alcohol for 30 second, washed three times by sterilized distil water to remove the trace of alcohol, and them sterilized by Clorox solution (15% concentration) supplemented with few drop of liquid soap for 2o min then the seeds washed five times with sterilized distil water to remove the trace of sterilization material, all this steps were carried out under the laminar air flow cabinet. Finally the seed were dried in sterilized tissue filter paper.

2.4 IN VITRO CULTURE

The disinfected seeds were inoculated in to culture jars, containing 25 ml MS medium, this process was carried out under sterilized condition , the inoculated tubes were incubated for about ten days in 25°C±2°C temperature, under 16 h light and 8 h dark.

2.5 PREPARATION OF EXPLANTS

Three type of explants from in vitro micro plant namely cotyledon hypocotyls and root segment explant used for callus induction, laminar air flow chamber was sterilized overnight by UV light, then double sterilized with (70%) alcohol, micro plant carried out in filter paper and cutting to cotyledons , hypocotyls and roots segment, different explants were excised and cultured on MS medium supplemented with two types of auxin 2,4-D and NAA at different concentration (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l)

2.6 CULTURED INCUBATION CONDITIONS

All cultures were maintained at 25±2°C under 16/8h light/dark condition and 1000 lux cool light provided by florescent lamps.

2.7 CALLUS INDUCTION

Three explants used in this study were segmented from in vitro micro plant, the explants were inculcated in culture jars contain 25 ml MS medium supplemented by different concentration of hormones, 2.4-D and NAA were used at different concentration (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l), the cultured were maintained in growth room at 25°C in 16/8 h light/dark , the day of callus initiation recorded at time , and the final data were determined after four weeks of cultured including callus percentage, color, texture and callus degree.

2.8 STATISTICAL ANALYSIS

Data obtained are, day of callus initiation, callus color, callus texture and callus degree, the callus degree were subjected to statistical analysis using stander error ; means presents average ± stander error [7].

3 RESULTS AND DISCUSSION

3.1 STERILIZATION OF SEEDS AND MEDIA

The surface sterilized seeds exhibited100%, sterilization and 100% survival rate. The sterilization frequency is around 100% and the survival rate about 99%, after ten days of culture (Fig 1)

3.2 CALLUS INDUCTION

For callus induction, in vitro micro plant , ten day old is used as source of explant. Cotyledon leaf , hypocotyls , and roots segment explants were cultured on MS media supplemented with 2,4-D and NAA at different concentration (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) as shown in Table 1.

Root and hypocotyls explants became larger after three days of culturing, and the first sign of callus production was observed approximately in about three to six days as general, this finding is in agree with Dagustu et al [8].

The callus was induced in all MS media containing 2,4-D, callus percentage induced by hypocotyls was between 81-100%, callus percentage from cotyledons was between 81-100% and from roots was between 93-100% . The results indicated that MS media supplemented with 0.0, 0.5, 1.0, 1.5 and 2.0 mg/l 2,4-D is quite suitable for callus induction from roots, hypocotyls and cotyledons explants at different auxin concentration, the callus induction by hypocotyls was between 81-100%, the finding in agree with Ozyigit et al [9].

Highest callus degree (3.44±0.12) was produced by cotyledon at MS media fortified with 1.5 mg 2,4-D (Fig 2), followed by (3.25 ± 0.14) induced by hypocotyls in MS media supplemented by 2.0 mg/l 2,4-D , the finding is in agree with Inoka and Dahanayake [10].

In concise with this result, in different plants, Potato plant, high concentration of auxins (2,4-D) alone or in combination with cytokinins (BA or TDZ) at low concentration, was numerously used for callus induction by khadiga et al [11] ; Mutasim et al [12]; Trigonellafoenum-graecumL plant by Khadiga et al [13]; in different medicinal plant by khadiga et al [14]; [15]; and [16].

The texture and color of callus induced by 2,4-D varied depend on explants, cotyledon explant induce granular creamy color callus, hypocotyl obtained watery yellow color callus , while root segment explant exhibit granular brown color callus. On MS media fortified with NAA cotyledon leaf explant obtained was watery green color callus, hypocotyl explant granular green color callus with root induction was observed, while root segment explant exhibited brown watery callus with root induction. In MS media fortified with NAA, callus percentage induce by hypocotyls and roots about 100% , from cotyledon was about 31-100%, the finding is agree with Ozyigit et al [17].

Higher callus degree induce in MS media supplemented with NAA was (3.25±0.11), induced in MS fortified with 2.0 mg/l , the texture is varied depend to explants also, it is granules and watery in addition to production of root in most treatments, it is granules with hypocotyls and watery with Cotyledon and root. Callus color is varied, it was white greenish to green in cotyledons, white to green with hypocotyls, and brown with roots. These finding is dis agree with Pandurang et al [18].

Varied results explained by Pandurang et al [18], NAA did not favored much callus induction at any of the concentration used, the variation may be due to the different of plant variety.

Callus obtained at all concentration of NAA by all explants formed high rooting. the cotyledon forming roots in 1.0-2.0 mg/l treatment, but hypocotyls and roots forming root plus callus at 0.5-2.0 mg/l NAA treatment (Fig 3 and 4).

Table 1. Effects of different concentrations of 2,4, dichlorophenoxy acetic acid (2,4-D) and Naphthalene Acetic Acid (NAA) on callus induction from cotyledon, hypocotyls and roots segment explant of the sunflower.

Growth regulators mg/l	Day of callusing	Percentage of callusing	Texture of callus	Callus color	Degree of callus
Cotyledon					
2,4-D 0.0	-	-	-	-	00.00
2,4-D 0.5	6	81.25%	Granules	Creamy	0.33± 0.11
2,4-D 1.0	5	100%	Granules	Creamy	1.38± 0.20
2,4-D 1.5	3	100%	Granules	Creamy	3.44±0.12
2,4-D 2.0	4	100%	Granules	Creamy	3.06±0.23
NAA 0.0	0	0.0%	-	-	00.00
NAA 0.5	6	31.25%	Watery	Green	0.63±0.18
NAA 1.0	6	62.5%	Watery	Green	0.15±0.14
NAA 1.5	5	93.75%	Watery+ root	Greenish	3.00±0.15
NAA 2.0	5	100%	Watery+ root	Greenish	1.31±0.18
Hypocotyls					
2,4-D 0.0	-	-	-	-	00.00
2,4-D 0.5	5	81.25%	Watery	Yellow	2.38±0.31
2,4-D 1.0	5	100%	Watery	Yellow	3.00±0.24
2,4-D 1.5	4	100%	Watery	Yellow	2.94±0.11
2,4-D 2.0	3	100%	Watery	Yellow	3.25±0.14
NAA 0.0	0	0.0%	-	-	00.00
NAA 0.5	5	100%	Granules	Green	2.06±0.17
NAA 1.0	5	100%	Granules+ root	Green	2.69±0.15
NAA 1.5	4	100%	Granules+ root	Green to white	3.00±0.06
NAA 2.0	4	100%	Granules+ root	Green to white	3.25±0.11
Root					
2,4-D 0.0	-	-	-	-	00.00
2,4-D 0.5	4	93.75%	Granules	Brawn	2.13±0.20
2,4-D 1.0	4	100%	Granules	Brawn	3.00±0.00
2,4-D 1.5	3	100%	Granules	Brawn	3.00±0.00
2,4-D 2.0	3	100%	Granules	Brawn	3.00±0.00
NAA 0.0	0	0.0%	-	-	00.00
NAA 0.5	5	100%	Watery	Brown	1.63±0.30
NAA 1.0	5	100%	Watery+ root	Brown	2.50±0.15
NAA 1.5	4	100%	Watery+ root	Brown	3.00± 0.11
NAA 2.0	4	100%	Watery + root	Brown	3.13±0.15

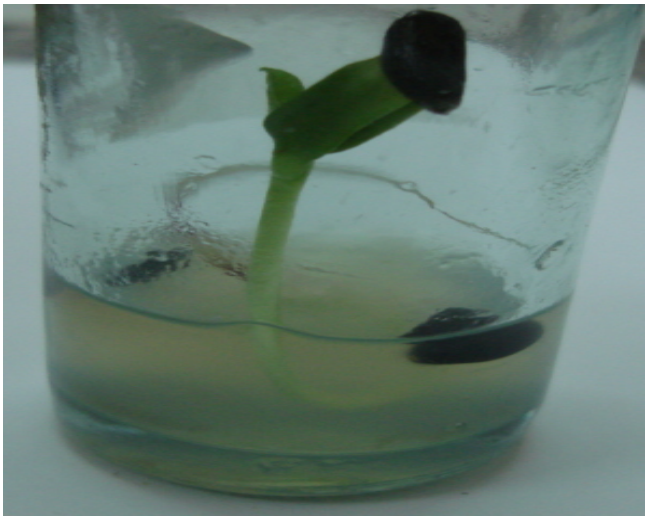

Figure 1: Establishment of sterile in vitro Sun flower micro plant

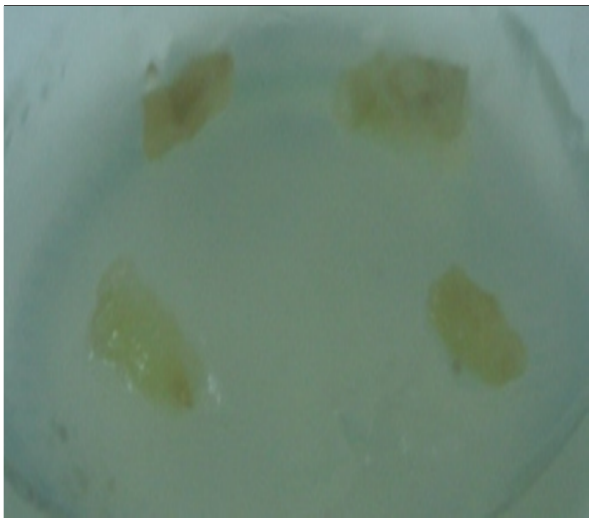

Figure 2: Callus induction from cotyledon explant in MS media supplemented by 1.5 mg/l 2.4-D

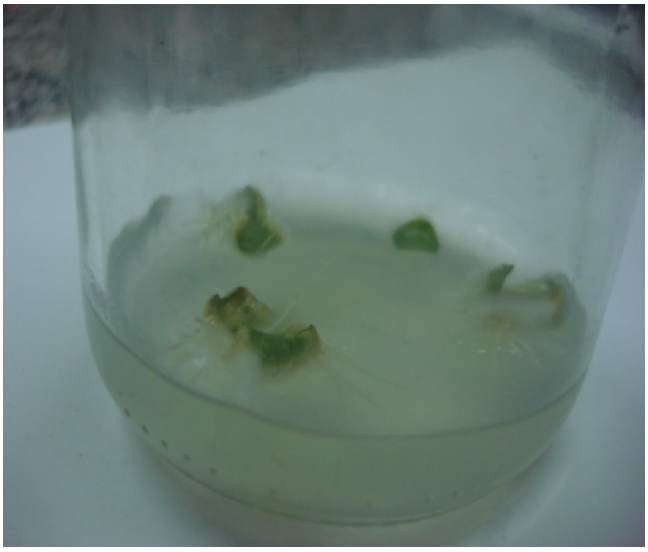

Figure 3: Callus induction from hypocotyl explant explant in MS media supplemented by 0.5 mg/l NAA

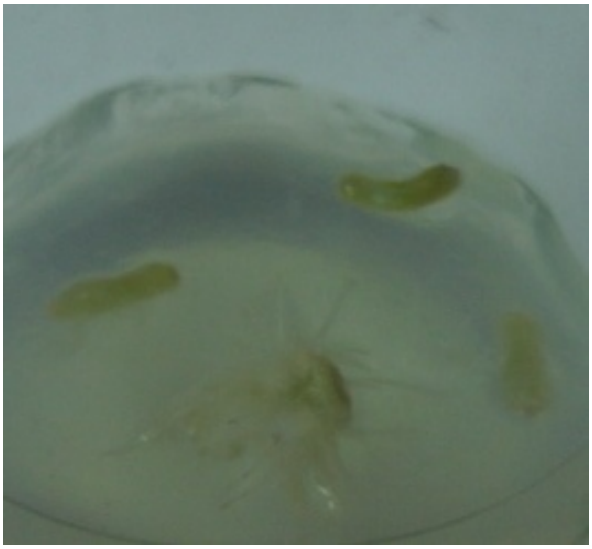

Figure 4 Callus induction from hypocotyl explant in MS media supplemented by 2.0 mg/l NAA.

4 CONCLUSION

In conclusion, callus was obtained from hypocotyl, cotyledonary leaf and root segments explant. Maximum callus degree was (3.44±0.12), induced by cotyledon explant on MS media fortified by 1.5 mg/L 2,4, D within three day . This protocol has capability for large scale production of phytochemical screening of medicinal plant .

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