Eco. Env. & Cons. 26 (2) : 2020; pp. (546-549)
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ISSN 0971–765X

Effect of NAA, BA, and explant on *Linumusita tissimum* L. callus induction *In vitro*

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(Received 10 November, 2019; accepted 15 December, 2019)

ABSTRACT

This study was conducted initiation callus from explants (shoot tips, cotyledon, Hypocotyl, roots) of seedling *Linumusita tissimum* L. cultured on MS medium supplemented different concentrations of NAA with 1.00 mg/LBA after 4 weeks and callus content of protein after 8 weeks, Also the Results are shown as follow: callus produced from cultured shoot tips on MS medium supplemented 1.0 mg/L NAA and 1.0 mg/L BA highest wet weight of callus 0.91 g/explant and gave highest percentage 90% after 4 weeks, callus produced from cultured cotyledons leaves on MS medium supplemented 0.25 mg/L NAA and 1.0 mg/L BA highest wet weight of callus 1.08 g / explant and gave highest percentage 100% after 4 weeks, Hypocotyl cultured on MS medium supplemented 0.25 mg/L NAA and 1.0 mg/L BA after 4 weeks gave highest percentage and weight, 100% and 0.25 g/explant, roots cultured on MS medium supplemented 0.25 mg/L NAA and 1.0 mg/L BA after 4 weeks gave highest percentage and weight, 100% and 2.61g/explant.

Key words : *Linumusita tissimum, Callus induction, Callus induction, Iraq*

Introduction

Prepare technology tissue culture from biotechnology that have an important role in serving human, especially in the Plant propagation. A lot advantages of the most important access to large numbers from plants free from a etiology and same mother plant in a short time and any time of the year furthermore use of these technology in areas search and applied including education and improving plant and produce medical drugs, medicines and rapid clonal propagation which is of application that are great significance that are by following different methods differentiation and training formal such as the composition of adventitious buds and stimulating the growth axillary buds and induction somatic embryogenesis as well as a study growth aspects and plant development and secondary metabolites. Linaceae family back to order Geraniales and particularly plants in the form herbs or bushes and family consist of 9 species where 200 type widespread in temperate, for plant flax from important plants that grow to produce timely oil for human consumption as well as commercial or industrial use, the human used for the purpose nutrition.

For plant flax bush length from (1- 4) foot contain 5 pairs from chromosomes (2n = 30) for plant Flax grow a winter crop in hot zones.

There are concerns about how to meet the growing demand for food while protecting ecosystems and biodiversity, Phytohormones are considered the

most important endogenous substances for modulating physiological and molecular responses, a critical requirement for plant survival as sessile organisms, Phytohormones act either at their site of synthesis or elsewhere in plants following their transport (AL-Taey, 2017; AL-Taey and Majid, 2018; Kadhim *et al*., 2019). Cytokinines known to significantly improve the growth of crop plants grown , there are many researchers who studied Cytokinines ability to improve the plant growth by inducing cell division and regulate cell metabolism and activated many specific enzymes (Al-Taey and Saadoon, 2012). Tissue culture technique has played a role in its ability to propagate plants and to obtain a large number of them in a short time and at low costs compared to traditional propagation methods. Suitable protocols have been developed for multiplication and improvement of different species (Kadhim *et al.*, 2019)

The study aims to Induced the callus from different parts of seedlings to get the best part and the better concentration of hormones.

Determination protein content in callus from the different explants of seedling and the best treatment of callus inductions.

Materials and Methods

The seeds were surface sterilized by washing powder regular and seeds were washed with tap water for 10 -15 minutes, then transferred to heparin 15% (v/v) sodium hypochlorite solution for 15 minutes (commercial bleach Clorox contain 6% sodium hypochlorite) and finally rinsed 3 times with sterile distilled water. The sterilized seeds were placed on basal MS medium free hormones, after 4 weeks on growth seedlings. The following explants were taken from them (shoot tips, cotyledon, Hypocotyl, roots) planted on MS medium supplemented different concentrations of NAA (0.0, 0.25, 0.5, 1.0) with 1.00 mg/L BA after 4 weeks from induction of callus had been moved on MS medium free hormones for the purpose differentiation, and callus content of protein after 8 weeks from induction of callus used for growing in the glass bottles capacity (250 mL put it out 40 mL) from food medium at pH 5.7 which its covered with aluminum foil then sterilize the food medium by autoclave for 20 min at 121 co and under pressure 1.04 kg/cm^2, The cultures were kept in growth chamber at 25 ± 2 co under a 16 h photoperiod (supplied by fluorescent tubes) and 8 h dark periods with a light intensity of 300 lux, The experiments were arranged in factorial experimental based on completely randomized design (C.R.D.) with ten replicates per each treatment and one explant per replicate.

Results and Discussion

Table 1 shows callus produced from cultured shoot tips on MS medium supplemented 1.0 mg/L NAA and 1.0 mg/L BA highest wet weight of callus 0.91 g/explant and gave highest percentage 90% after 4 weeks, Figure (1- A). These results may be explained by the fact that auxins play a role in induction of callus and the addition of cytokinin to the medium with auxin lead to increased callus growth.

Table 2 shows callus produced from cultured cotyledons leaves on MS medium supplemented 0.25 mg/L NAA and 1.0 mg/L BA highest wet weight of callus 1.08 g/explant and gave highest percentage 100% after 4 weeks Fig. 1-B). These results are explained in light of what is stated in the interpretation of the results of Table 1.

Table 3 shows callus produced from cultured Hypocotyl on MS medium supplemented 0.25 mg/L NAA and 1.0 mg/L BA after 4 weeks gave highest percentage and weight, 100% and 0.25 g/ex-

Table 1. The effect NAA and BA and combinations in induction of explant cultureof shoot tips for flax *Linumusitatissimum* L. on MS medium after 4 weeks.

NAA (Mg/L)	BA(Mg/L)	Induction of callus%	Wet weight of callus (g)	Strength and color of callus
0.0	0.0	20 b	0.39 d	Light green steel
0.25	1.0	80 a	0.89 b	Light green steel
0.5		70 a	0.45 c	Light green steel
1.0		90 a	0.91 a	Light green steel

*Values with similar characters for each factor or their interactions individually are not significantly different according to the Dunkin Multipliers test below the 5% probability level

plant, Figure (1- c). These results are explained in light of what is stated in the interpretation of the results of Table 1.

Table 4 shows callus produced from cultured roots on MS medium supplemented 0.25 mg/L NAA and 1.0 mg/L BA after 4 weeks gave highest percentage and weight, 100% and 2.61g/explant, Figure (1- D). These results are explained in light of what is stated in the interpretation of the results of Table 1.

Table 5 shows May explain the different response of different explants (shoot tips, leaves, hypocotyl, root) to induction of callus basedon concentration of growth regulators is the process of induction of cal-

Table 2. The effect NAA and BA and combinations in induction of callus from explant culture of cotyledons leaves for flax *Linumusita tissimum* L. on MS medium after 4 weeks.

NAA (Mg/L)	BA (Mg/L)	Induction of callus%	Wet weight of callus (g)	Strength and color of callus
0.0	0.0	30 B	0.11 D	green steel
0.25	1.0	100 A	1.08 A	green steel
0.5		80 A	1.03 B	green steel
1.0		80 A	0.74 C	green steel

* Values with similar characters for each factor or their interactions individually are not significantly different according to the Dunkin Multipliers test below the 5% probability level.

Table 3. The effect NAA and BA and combinations in induction of callus from explant culture of Hypocotyl for flax *Linumusita tissimum* L. on MS medium after 4 weeks.

NAA(Mg/L)	BA(Mg/L)	Induction of callus%	Wet weight of callus (g)	Strength and color of callus
0.0	0.0	50 b	0.22 C	Light green steel
0.25	1.0	100 a	0.25 A	Light green steel
0.5		80 a b	0.23 B	Light green steel
1.0		70 a b	0.12 D	Light green steel

* Values with similar characters for each factor or their interactions individually are not significantly different according to the Dunkin Multipliers test below the 5% probability level.

Table 4. The effect NAA and BA and combinations in induction of callus from explant culture of roots for flax *Linumusita tissimum* L. on MS medium after 4 weeks.

NAA (Mg/L)	BA (Mg/L)	Induction of callus%	Wet weight of callus (g)	Strength and color of callus
0.0	0.0	80 A	0.55 C	Solid white cream
0.25	1.0	100 A	2.61 A	Light green steel
0.5		90 A	1.33 B	Light green steel
1.0		80 A	0.29 D	Solid white cream

* Values with similar characters for each factor or their interactions individually are not significantly different according to the Dunkin Multipliers test below the 5% probability level.

Table 5. The content of the protein in the callus developed from different explants cultivation of the flax *Linumusita tissimum* L.

NAA (Mg/L)	BA (Mg/L)	Content of the protein Mg/g			
		Shoot tips	Cotyledons	hypocotyl	Root
0.25	0.0		1.13	0.64	0.73
1.0	1.0	0.10			

* Values with similar characters for each factor or their interactions individually are not significantly different according to the Dunkin Multipliers test below the 5% probability level.

lus depends on several factors, including the conditions of agriculture and the type and source of the explants used in addition to the central content of agriculture from growth regulators.

The results showed that best treatment of the protein content at the cultivation of different explants of flax plant seedlings *Linumusitatissimum* L. on MS medium, where protein content was in the callus was developed from the cultivar shoot tips (0.01) mg/g with the 1.0mg/L BA concentration , while the protein content was 1.13, 0.64, 0.73 mg/g in the developed callus.

From the cultivation of the explants of the cotyledons leaves, hypocotyl and root respectively when the treatment 0.25 mg/L NAA with 1.0 mg/L BA, the cause of the superiority of the leaves in their protein content may be due to the fact that they are newly grown and their content of metabolic compound is high, Including amino acids and thus increase protein.

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