Plant Archives Vol. 19, Supplement 2, 2019 pp. 1266-1269 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF SOME GROWTH REGULATORS (IAA AND BA) IN THE INDUCTION OF CALLUS FROM THE APICAL MERISTEM FOR SAGE (*SALVIA OFFICINALIS* L.) PLANT *IN VITRO*

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Abstract

The study was conducted in the laboratory of plant tissue culture belonging to the Department of Plant Production Techniques, Al-Furat Al-Awsat Technical University, Al-Mussaib Technical College, Babylon province from October 2018 to March 2019, in order to study the effect of some growth regulators: Auxins such as Indole acetic acid (IAA) with concentration of (0.0, 0.5, 1.0, 2.0, 4.0 mg.L^{-1}), cytokines such as Benzyl adenine (BA) with concentration of (0.0, 0.5, 1.0, 2.0, 4.0 mg.L^{-1}), and their interaction in the induction of callus from the Apical meristem for sage (*Salvia officinalis* L.) plant in vitro and their effect in the formation of callus. The plant parts were sterilized with different concentrations of Sodium hypochlorite (NaOCl) (0.0, 5, 7, 10 mg.L^{-1}) at different periods of (0, 5, 10, 15) and after selecting the best concentration and the best culturing period. The Apical meristem was cultured in the MS nutrient media. In the experiment designed according to the Completely randomized design (CRD), with five replicates. The averages were compared using the least significant difference (LSD) below the probability level of 0.05. The results showed that the best concentration of Sodium hypochlorite is 10% in 15 min to be used in the sterilization of the plant part. The growth regulator (IAA) with the concentration of (4 ml.L^{-1}) was excelled by giving it the highest average percentage for callus induction from the Apical meristem amounted to (76 mg) and the highest average fresh weight for callus at concentration of (1 mg.L^{-1}) with a fresh weight amounted to (445 mg) and the highest average dry weight for callus at concentration of (4mg.L^{-1}) with a dry weight amounted to (36.3 mg). As for the interaction, the growth regulators (IAA and BA) gave the highest percentage of callus induction with a combination of (1 mg.L^{-1}) of IAA and (1 mg.L^{-1}) of BA, which amounted to (100 mg). The growth regulator (BA) with a concentration of (2, 4 mg.L^{-1}) was excelled by giving it the highest percentage of callus induction, which amounted to (80, 80), respectively, and the highest percentage for dry weight amounted to (421 mg) for the treatment (4 mg.L^{-1}). The treatment (2 mg.L^{-1}) gave the highest average for dry weight amounted to (31.9 mg).

Keywords: Apical meristem, Micropropagation, Growth regulators, Sage plant.

Introduction

Sage plant (*Salvia officinalis* L.) belongs to the Lamiaceae family of plants containing many natural oils (El-Feky and Abulthana, 2016), it is an anti-inflammatory (Baur, 2012), It is an anti-cardiovascular, anti-cancer, and anti-Alzheimer (Miraj and S.Kiani, 2016). The sage contains flavonoids which contain substances that have the potential to prevent cancer where they affect tumor growth at different stages, especially in both stages of germination and development (Sanders, C.L.2003). Apigenin is considered an anti-carcinogen and it is found in the leaves of the sage plant (Dordevic, S., Cakic, M.and, S.2001). Salvia genus includes more than 900 species spread around the world and is one of the largest genera of the Lamiaceae family (Perry et al., 1999). Plant tissue culture means the technical or mechanism in which a cell, tissue or member of the mother plant is isolated under sterile conditions and culturing it in sterile artificial nutrition media, while incubating the cultured part in controlled conditions of temperature, light, and humidity to achieve the desired objective (Hartmann et al. , 2002). The in vitro propagation process is divided into four stages: the germination stage, the multiplication stage, the rooting stage and the acclimatization stage (Puorhit, 2003). A callus is a group of undifferentiated bronchial cells that arise on the cutting or Wounds areas of the plant parts.

Material and Method

The study was conducted in the laboratory of plant tissue culture belonging to the Department of Plant Production Techniques, Al-Furat Al-Awsat Technical University, Al-Mussaib Technical College, Babylon province from October 2018 to March 2019. The well-known neutron medium was used (Murasnige and Skoog, 1962). The ability of Sodium hypochlorite (NaOCl) was tested at different time intervals in the sterilization of the Apical meristem for sage plant by removing the Apical meristem from the sage plant with a Blade Surgical Scalpels and then placed in a 250 ml conical flask, A few drops of Dishwashing solution were added, washed well and placed under running water for 2 hours to remove dust and surface contaminants. It was then washed with distilled water several times. This part was then transferred to the Laminar air flow cabinet, where Sodium hypochlorite (NaOCl) at concentrations of (5%, 7%, 10%) was added to the beaker containing the vegetable part and each concentration individually for (5, 10, 15 min) with continuous stirring with the addition of one drop of Tween 20 to prevent surface tension. After the specific sterilization period was completed, the Apical meristem was washed well with distilled water three times to remove the residual effect of the sterile material, thus ready to be cultured in the nutrition media. Five replicates were used for each period of sterilization period in this solution. The experiment was conducted to knowing the effect of some growth regulators: Auxins such as Indole acetic acid (IAA) with concentration of (0.0, 0.5, 1.0, 2.0, 4.0 mg.L^{-1}), cytokines such as Benzyl adenine (BA) with concentration of (0.0, 0.5, 1.0, 2.0, 4.0 mg.L^{-1}), and their interaction in the induction of callus from the Apical meristem for sage (*Salvia officinalis* L.) plant in vitro and their effect in the formation of callus (fresh weight and dry weight). After completing the culturing process, the cultures were transferred to the growth chamber and the cultures were incubated in the special incubation room under 25$\pm$ 2 ºC and lighting 1000 lux for 16 hours/day. Observations were taken about the percentage of contamination for the cultured plant parts for the sage plant. The results of the percentage of contamination after two weeks of culturing were recorded on the basis of the

percentage of contamination to determine the best time period for sterilization. The fresh weight and dry weight of the callus were taken after five weeks of culturing using a sensitive balance where The fresh and dry callus cuttings were extracted and placed on filter paper. The remaining nutrition media was removed by using the Blade Surgical Scalpels according to the fresh weight for the callus. The fresh callus pieces were dried cut into an oven at 70 °C which it weighed at a constant weight.

Results and Discussion

The effect of sodium hypochlorite concentrations (NaOCl%) and period of sterilization (min) and their interaction in the percentage for contamination of the cultured parts for the sage plant after two weeks of culturing.

Table (1) indicates a decrease in the average percentage of contamination with an increase in the period of sterilization with sodium hypochlorite at a concentration of 10%. The percentage of contamination amounted to 100% in non-sterilized plant parts with sodium hypochlorite (control treatment), while the percentage of contamination in cultured plant parts decreased when using sodium hypochlorite at concentrations of (5%, 7%) which amounted to 50% and 38% at the two periods (5, 10 min), respectively without significant differences Between the two periods. The percentage of contamination was significantly decreased by increasing by the period of sterilization to amount 24% during the sterilization period was (15 min), the growth of the cultures was good. Most of the interactions gave the lowest percentages of contamination and the concentration of 10% for the period of 15 min with the lowest percentages of contamination amounted to 10%. The growth of cultures was good. This result agrees with (Khosh-Khuim, 1982) when using commercial sodium hypochlorite with a concentration of 10% in the sterilization of the lateral buds for 15 min. This period has been adopted in the sterilization of the used plant parts in the study.

Effect of IAA and BA concentrations (mg.L^{-1}) and their interaction in the percentage of callus induction after 45 days of culturing the Apical meristem in the nutrition media (MS).

Table (2) shows the significant effect of the IAA on the increase in the percentage of the callus induction from the Apical meristem of the sage plant after 45 days of culturing. where the concentration of the IAA was significantly excelled on the control treatment, with no significant differences between them. The concentration of (4 mg.L^{-1}) gave the highest percentage for callus induction amounted to (76.0 mg) and the concentration of (2 mg.L^{-1}) amounted to (72.0 mg) and without significant differences between them compared to the control treatment which gave the lowest percentage amounted to (40 mg). The results of the same table showed that the use of the BA growth regulator had a significant effect on the increase in the percentage of callus induction. where the two concentrations of (4 mg.L^{-1} and 2 mg.L^{-1}) were excelled on the rest of the concentrations by giving it the highest percentage of callus induction amounted to (80 and 80 mg), respectively without significant differences between them While the control treatment gave the lowest percentage of callus induction amounted to (36.0 mg). The results of the table showed a significant effect of the interaction between the growth regulators IAA and BA in increasing the percentage of the callus induction. Most of the interactions gave the highest values amounted to (100, 80 and 60), whereas the control treatment and the interactions of (IAA 0.5 mg.L^{-1} and 0.0 BA mg.L^{-1}) and (1.0 mg.L^{-1} and 0.0 BA mg.L^{-1}) was not given any percentage of the callus induction. Cytokines are used in low concentrations to induce physiological effects in the cultured plant part and in balancing with Auxins where they help in the induction of callus (Wei and Xu, 1990). Many researchers have suggested that callus tissue can be inducted from different plant parts depending on the source of the plant part and the added growth regulators to the nutrition media and the balanced interaction increase the stimulation force of the callus (Evans, 1989). Several studies have been conducted to induct the callus from many medicinal plants using different interactions of plant growth regulators. Mahalakshmi et al., (2013) has succeeded in inducing the callus and the Perpetuating it from the Petiole for *Jatropha curcas*. Figure (1) illustrates the callus induction from the Apical meristem of the sage plant.

Fig. 1: illustrates the callus induction from the Apical meristem of the sage plant, (A) Callus growth on the plant part (B) Inducting the callus from the Apical meristem of the sage plant.

Effect of IAA and BA concentrations (mg.L^{-1}) and their interaction in the average fresh weight for callus after 45 days of culturing the Apical meristem in the nutrition media (MS).

Table (3) shows that the IAA concentrations had an important effect on increasing the fresh weight of the induced callus from the Apical meristem for the sage plant after 45 days of culturing. where the used concentrations of IAA were significantly excelled on the control treatment. The concentration of (1 mg.L^{-1}) gave the highest average of fresh weight amounted to (445 mg) while the control treatment gave the lowest weight amounted to (45 mg). Auxins has important in stimulating the flexibility of the cellular wall by

breaking the bonds of the cellular wall and returning it to new sites under the influence of bloating pressure, which contributes to increasing the cell size and its expansion (Taiz and Zieger, 2002). The use of different concentrations of BA had a significant effect on increasing the fresh weight for callus, where the concentration of (4 mg.L^{-1}) was significantly excelled on other concentrations by giving it the highest average weight for fresh weight amounted to (421 mg). The effect of cytokinins in the induction of callus is due to its active role in the work of attractions in their accumulated places to accelerate the transfer of water and nutrients, which lead to inducing the cultured cells in vitro to divide and grow, which lead to increases the weight of callus (George et al., 2008). Benzyl Adenine (BA) is considered the most important cytokines used in the tissue culture because it is most influential due to it contains more than double bonds (Krishamurthy et al., 1984), The interaction between the IAA and BA growth regulators had a significant effect on increasing the fresh weight for callus. where the interaction between (1 mg.L^{-1} IAA and 1 mg.L^{-1} BA) gave the highest average weight amounted to (848 mg) while the control treatment gave the lowest fresh weight amounted to (79 mg). These results agree with the previous studies' that the nutrient-free media of growth regulators does not encourage callus formation. It has been found that some plant parts used in culturing in vitro do not have the ability to form callus (Smith, 2000).

Effect of IAA and BA concentrations (mg.L^{-1}) and their interaction in the average dry weight for callus after 45 days of culturing the Apical meristem in the nutrition media (MS).

Table (4) shows that there was a significant effect of the adding of IAA in the increase of the dry weight of the induced callus from the Apical meristem for the sage plant after 45 days of culturing. where the concentration of (4 mg.L^{-1}) was significantly excelled on the rest of the concentrations by giving it the highest dry weight amounted to (36.3 mg), while the control treatment gave the lowest dry weight amounted to (5.1 mg). As for the effect of cytokines BA, it was led to increasing the dry weight for the callus, where the concentration of (2 mg.L^{-1}) was significantly excelled on all other concentrations by giving it the highest dry weight amounted to (31.9 mg) while the control treatment gave the lowest average dry weight amounted to (8.3 mg). The supplying the nutrition media with appropriate interactions of Auxins and cytokines encourages cell division and growth and increases the production of amino acids and proteins, thus increasing the biomass for callus, which leads to increasing dry weight (Chavan *et al*., 2014).

Table 1: The effect of sodium hypochlorite concentrations (NaOCl%) and period of sterilization (min) and their interaction in the percentage for contamination of the cultured parts for the sage plant.

Concentration / Period	0	5%	7%	10%	Average of period
0	100	37.00	48.80	11.40	49.30
5	100	40.00	50.60	37.40	57.00
10	100	34.80	52.20	38.60	56..40
15	100	40.60	50 .00	10.40	50.25
Average of concentration	100	50.40	38. 60	24.45	
L.S.D 0.05	**Period =3.004, Concentration =3.004, Period × Concentration =6.009**				

Table 2: Effect of IAA and BA concentrations (mg.L^{-1}) and their interaction in the percentage of callus induction after 45 days of culturing the Apical meristem in the nutrition media (MS).

BA / IAA	0	0.5	1	2	4	Average of IAA
0	0.0	0.0	0.0	100.0	100.0	40.0
0.5	0.0	60.0	60.0	80.0	60.0	52.0
1	20.0	80.0	100.0	80.0	80.0	72.0
2	80.0	80.0	60.0	60.0	80.0	72.o
4	80.0	40.0	100.0	80.0	80.0	76.0
Average of BA	36.0	52.0	64.0	80.0	80.0	
L.S.D 0.05	**IAA = 22.45 BA = 22.45 IAA × BA= 50.19**					

Table 3: Effect of IAA and BA concentrations (mg.L^{-1}) and their interaction in the average fresh weight for callus after 45 days of culturing the Apical meristem in the nutrition media (MS).

BA / IAA	0	0.5	1	2	4	Average of IAA
0	0	0	0	65	162	45.
0.5	0	376	191	581	245	279
1	0	214	848	419	742	445
2	235	204	394	182	334	270
4	163	136	351	485	626	352
Average of BA	79	186	357	347	421	
L.S.D 0.05	IAA = 122.3 BA = 122.3 IAA × BA= 273.5					

Table 4: Effect of IAA and BA concentrations (mg.L^{-1}) and their interaction in the average dry weight for callus after 45 days of culturing the Apical meristem in the nutrition media (MS).

BA / IAA	0	0.5	1	2	4	Average of IAA
0	0.0	0.0	0.0	13.3	12.4	5.1
0.5	0.0	34.4	13.1	60.0	22.6	26.0
1	0.0	10.7	46.1	25.0	34.7	23.3
2	15.2	15.2	32.2	11.7	22.6	19.4
4	26.4	8.0	41.4	49.5	56.1	36.3
Average of BA	8.3	13.7	26.6	31.9	29.7	
L.S.D 0.05	IAA = 9.60 BA = 9.60 IAA × BA = 21.46					

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