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INDUCTION OF CALLUS ON VARIOUS EXPLANTS OF ARUGULA PLANT (*ERUCA SATIVA* MILL.) USING THE GROWTH REGULATORS (2,4-D AND KINETIN)

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Abstract

The research was conducted at the plant tissue culture laboratory of Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University during the years (2019-2020). The research was conducted to study the effect of the vegetative part and some regulators on the induction of callus from Arugula plant (*Eruca sativa* Mill) in vitro. The experiments included sterilization of the different explants, where three explants were used in this study (Hypocotyl, stems, and cotyledonary leaves) and the effect of adding different concentrations of growth regulators: Auxin (Dichlorophenoxy acetic acid (2,4-D)) at a concentration of (0.0- 0.0, 1.0, 2.0, 3.0 and 4.0 mg.L^{-1}) and Kinetin Kin at a concentration of (0.0, 05, 1.0, and 1.5 mg.L^{-1}) to the culture media (MS) for induction of callus from explants. The results showed the efficacy of Sodium hypochlorite solution (NaOCl) in sterilizing the different explants, where the 2% concentration for 15 min gave the lowest percentage of pollution amounted to (10%) for the explants under study. As for the effect of Auxin growth regulators, where the concentrations (2,4-D at 0.0, 1.0, 2.0, 3.0, and 4.0 mg.L^{-1}) were used with Kinetin at concentrations of (0.0, 05, 1.0, and 1.5 mg.L^{-1}) in the culture media (MS) for induction of callus from all explants used in the study. where the combination (2.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kinetin) gave the highest fresh weight for the callus from the Hypocotyl, stems, and cotyledonary leaves amounted to (40.8, 8.2, 85.9 mg), respectively. While the combination (3.0 2,4-D mg.L^{-1} + 0.0 mg.L^{-1} Kinetin) gave the highest dry weight of the induced callus from the Hypocotyl and stems amounted to (1.908 and 0.625 mg), respectively. The combination (2.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kinetin) gave the highest dry weight of induced callus from the cotyledonary leaves amounted to (8.76 mg).

Keywords: callus, arugula plant, *Eruca sativa* Mill, growth regulators.

Introduction

Arugula plant (*Eruca sativa Mill*) is a winter Annual plant spread in the Middle East and the Mediterranean basin, it is incompatible and pollination is conducted by insects (Barazani *et al*., 2012; Westberg *et al*., 2013; Ogran *et al*., 2016). Arugula plant is used in nutrition as fresh vegetables because it contains basic nutrients and spices as well as pharmaceuticals (Hadi *et al*., 2017). Therefore it is an important plant in complementary and alternative medicine (Kamel, 2014). Where it has been proven that the consumption of these plants contributes to improving the health of the consumer because of what it contains the chemical materials need in the body, therefore advised to include it in the nutritional programs of humans (Holst and Williamson, 2004). Consumption of such vegetables that contain glucosinolates and flavonols contributes significantly to reducing the percentage of infection with some cancerous diseases (Higdon *et al.*, 2007). Improving the health of blood vessels and the heart (Podsedek, 2007). The explants for dicotyledon plants often respond to the induction of callus when appropriate conditions are available, in contrast to Monocotyledon plants where the induction process of callus is more difficult (Mahmoud, 2017). Cell division and callus formation can be stimulated by plant hormones (Taiz and Zeiger, 2002). The plant growth regulators, whether created inside the plant or commercially manufactured, are effective in inducting callus, where they are added in small quantities, causing a change in plant growth and its development when adding it in certain stages of plant growth (Paridaen, 2009; Hamza and Ali, 2017). The most used growth regulators in plant tissue culture technology are Auxins and cytokines. Auxins are weak organic acids with an unsaturated indole ring, and it has important roles in bio-processes within plants with minimal concentrations (George *et al*., 2008), where it affects the elongation of the cells, tissues and plant organs elongation, in addition to its role in stimulating cell division, especially the Meristematic tissue during the development of callus. Cytokinins are nitrogenous bases with high molecular weights that are given in low concentrations, causing a number of physiological effects, where they stimulate the division, differentiation of plant cells, and the growth of axillary buds (Delloloio, 2007). The study aims to Induction of callus from different parts of the Arugula plant by using different combinations of growth regulators while noting the best vegetative part in terms of the fresh and dry weight of the induced callus.

Materials and Methods

This study was conducted in the laboratory of tissue culture belonging to the Department of Plant Production techniques, Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University. Arugula seeds were obtained from the local market in the city of Hilla. The seeds were planted in pots with a depth of 10 cm, filled with field soil and with more than one agricultural date, starting from 2/15/2019 to 1/2/2020 so that the explants can be available during the experiment stages with the required age. After seed germination, the explants were taken to the laboratory for sterilization, and the explants are:

A- Cotyledonary leaves with an area of (0.4 cm^2)
B- Hypocotyl with a length of (1 cm)
C- Stem with a length of (1 cm)

Surface sterilization for explants

The explants were placed under running water for 45 min to remove the soil and some surface pollutants. The explants were placed in a beaker and transferred to the Laminar flow cabinet (Vertical airflow). Different

concentrations of sodium hypochlorite solution (NaOCl) were then chosen to sterilize the explants, where the commercial blanching liquid (Sehat) used with a concentration of 6% active substance, where the concentration was reduced to a number of concentrations of (0.5, 1.0, 1.5, 2.0%) of sodium hypochlorite for different periods (10, 15, 20, 25 min) for each concentration, with the addition of a few drops of the dishwashing liquid, taking into account the continuous stirring of the beaker containing the explants and the sterilization solution. The explants were then washed three times with sterile distilled with stirring, the explants were then transferred to sterilized Petri dishes with alcohol and flame. The explants were removed by sharp blades sterilized with alcohol and flame. The explants were cultured, where the Cotyledonary leaves were with an area approximately (0.4 cm^2), Stem with a length of (1 cm), and Hypocotyl with a length of (1 cm), It was cultured in test tubes with dimensions of (12.5 cm long and 2.5 cm wide) and containing 10 ml of nutrition media (MS) (Murashige and Skoog, 1962). The data were taken after 10 days from cultivating, and the results of the percentage of contamination of cultures were calculated as follows:

The percentage of contamination = (number of tubes in which contamination appeared ÷ total number of tubes) x 100.

Nutrition media

The ready-made media consisting of MS salts was used, where it was obtained from the Indian company (HIMEDIA), where the media prepared by adding (4.9 $g.L^{-1}$) in a beaker containing 600 ml distilled water with adding 30 g of sucrose and mixing it until the sucrose melts, then growth regulators (Auxins and cytokinins) were added according to the studied treatments, the volume then completed to 1000 ml by adding distilled water and adjusting the pH to 5.7 ± 0.1. A 7.0 $g.L^{-1}$ of Agar-Agar was added according to the recommendation of the manufactured company (HIMEDIA), the medium was set under temperature a little below boiling temperature by placing it on a hot plate magnetic stirrer to homogenize the media and melting the Agar. The medium was then directly poured into the test tubes with a rate of 10 ml for each tube and all tubes containing the closed media completely were sterilized in Autoclave at a temperature of (121 °C) and pressure of (1.04 $kg.cm^{-2}$) for 15 min. The tubes containing the sterilized nutrition media were then taken out and left the media to cool and solidify at room temperature until use.

Induction of callus

The explants (Hypocotyl, stems, and cotyledonary leaves), that taken from the sterilized arugula plant, were used in the induction of callus. where the Cotyledonary leaves were with an area approximately (0.4 cm2), Stem with a length of (1 cm), and Hypocotyl with a length of (1 cm). The growth regulator Auxin (Dichlorophenoxy acetic acid (2,4-D)) at a concentration of (0.0- 0.0, 1.0, 2.0, 3.0 and 4.0 $mg.L^{-1}$) was used alone or in combination with the Kinetin Kin at a concentration of (0.0, 05, 1.0, and 1.5 $mg.L^{-1}$), Where explants are cultured within tubes and for each explant and hormonal concentration has 10 test tubes, with a factorial experiment according to a completely randomized design (CRD), where each test tube was considered a replicate and it was placed in each tube one explant. The culture process took place in an atmosphere of complete sterilization. The transplants were transferred to the growth chamber and incubated at a temperature of 25 ± 2 °C and illuminated for 16 hours and darkness for 8 hours and under the intensity of illumination of 1000 lux. After 35 days, the results of the transplants were taken from the fresh and dry weight for the induced callus. The percentage of callus induction was calculated according to the following formula:

$$\text{The percentage of callus induction (\%)} = \frac{\text{the number of explants that forming callus}}{\text{the total number of cultured explants}} \times 100$$

The results were compared according to the LSD test, with a 0.05 probability level, using the Genstat 12 program.

Results and Discussion

Results of sterilization on arugula explants for different periods

Table (1) shows the effect of different concentrations of sodium hypochlorite and sterilization period in reducing the percentage of contamination for explants, where the percentage of contamination amounted to (100%) in the non-sterilized explants, The percentage of contamination decreased when the sodium hypochlorite concentration increased. The percentage of contamination was 100 when using 0.5% of the sodium hypochlorite concentration for all periods. As for the concentration of 1% of sodium hypochlorite, The percentage of contamination decreased to 85% after 25 min of sterilization. While The percentage of contamination decreased to 20% and 15% at a concentration of 1.5% of sodium hypochlorite and for periods of (20, 25 min), respectively, but it led to the death of 10% of the explants at a period of 25 min. While the percentage of contamination amounted to (80, 10, 5, 5%) when using sodium hypochlorite at a concentration of 2% for the periods (10, 15, 20, 25 min), respectively, but there was death, blackening and lack of growth for the explants amounted to (0, 5, 35, 60), respectively. The results also showed that the period of sterilization has a severe impact on reducing contamination but within certain limits. Thus, the best results are when using the 2% concentration for 15 min. These results agree with (Shahzad, 2017) obtained on the various explants, (Al-Amri and Khalaf, 2017) on potato tubers.

Table 1: Effect of sterilization periods and on the percentage of contamination for the cultured Arugula explants.

The concentration of sodium hypochlorite (%)	Sterilization periods (min)	Percentage of contamination	Observations
0	25-10	100	All cultures are contaminated
0.5	10	100	All cultures are contaminated
	15	100	All cultures are contaminated
	20	100	All cultures are contaminated
	25	100	All cultures are contaminated
1.0	10	100	All cultures are contaminated
	15	100	All cultures are contaminated
	20	100	All cultures are contaminated
	25	85	
1.5	10	90	
	15	40	
	20	20	
	25	15	The death of 10% of cotyledonary leaves
2.0	10	80	
	15	10	The death of 5% of explants
	20	5	Blackening and death of 35% of explants
	25	5	Blackening and death of 60% of explants
$L.S.D_{0.05}$	23.18		

The percentage of callus induction for different explants of Arugula plant

Table (2) indicates the significant effect of using different concentrations of growth regulators (2,4-D and Kin) to induce callus from different explants of Arugula plant after five weeks of culture, where the results show that there is no induction of callus in the absence of growth regulators and for all explant and the percentage of induction was 0.0%. As for the Hypocotyl, the results of the same table showed a significant effect but not high for the interaction between the regulators of growth 2,4-D and Kin, and the interactions were (2.0 mg.L^{-1} 2,4-D + 1.0 mg.L^{-1} Kin, 3.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kin, and 3.0 mg.L^{-1} 2,4-D + 1.0 mg.L^{-1} Kin). which each of them gave a percentage of induction amounted to 85%, where It significantly excelled on all interactions at a concentration of (1.0 mg.L^{-1} 2,4-D and the control treatment). While the two interaction (1.0 mg.L^{-1} 2,4-D + 1.5 mg.L^{-1} Kin and 1.0 mg.L^{-1} 2,4-D + 1.0 mg.L^{-1} Kin) gave the lowest percentages of induction amounted to 30% for each of them. The previous studies indicate that the explants that are capable of forming callus increase when preparing culture media with growth regulators, Auxins, and cytokines, Where their interaction with suitable concentrations is important for the induction of callus, its growth, and development and to obtain a balance in the hormonal state within the explants (Chavan *et al*., 2014; Habib, 2018).

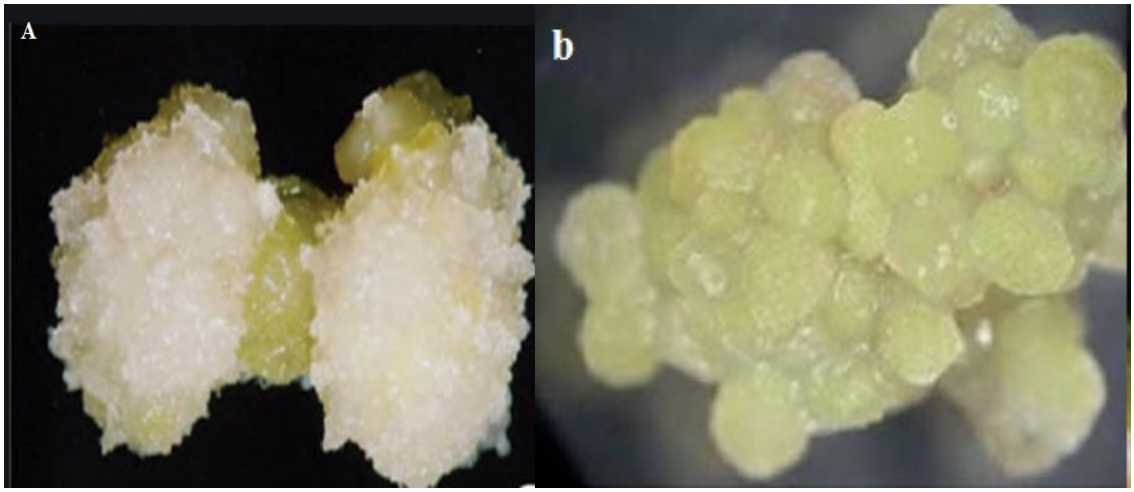

Fig. 1: Induction of callus from the Hypocotyl: (a) after 14 days of culturing, (b) after 35 days of culturing.

As for the stem, the results of the same table indicated a significant excelling for interaction between (2,4-D and Kin), where the interaction between (3.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kin) gave the highest percentage of induction callus amounted to (75%) excelling on the interaction (1.0 mg.L^{-1} 2,4-D + 1.5 mg.L^{-1} Kin). While the interaction (1.0 mg.L^{-1} 2,4-D + 1.5 mg.L^{-1} Kin) gave the lowest results, where the percentage of induction for callus amounted to (30%). These results agree with (Moradi *et al*., 2018) who did not show induction results when using concentrations of growth regulator (2,4-D) on *Crocus sativus* L. plant. Oxinate is considered an important growth regulator that participates in many physiological processes such as elongation, cell division, and apical dominance. As for plant tissue cultures, it is used to differentiate cells and plant organs. In general, when Auxin is used in small concentrations, it promotes the formation of roots. When it is used in high concentrations, it encourages the formation of callus. One of the most industrial Auxins used in tissue culture for induction of callus is 2,4-D, where it is considered common in plant tissue culture research (Bhojwani and Dantu, 2013).

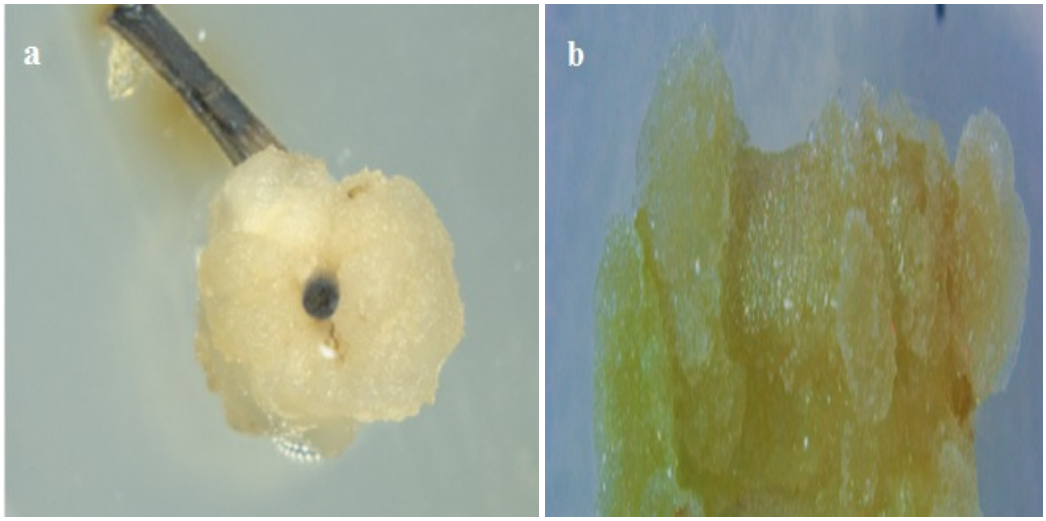

Fig. 2: Induction of callus from the stem: (a) after 14 days of culturing, (b) after 35 days of culturing.

The results of the same table were also shown for the cotyledonary leaves, where the two interactions (2,4-D 2.0 mg.L^{-1} + 0.5 mg.L^{-1} Kin and 2,4-D 3.0 mg.L^{-1} + 0.5 mg.L^{-1} Kin) have excelled on the rest of the interactions by giving them the highest percentage of callus induction amounted to (90%) for each of them. The two interactions (2,4-D 1.0

mg.L^{-1} + 1.0 mg.L^{-1} Kin and 2,4-D 1.0 mg.L^{-1} + 1.5 mg.L^{-1} Kin) gave the lowest percentage of callus induction amounted to (65%) for each of them. There is a lot of research and published reports for researchers in the field of botany confirmed that the induction of callus affects many genetic factors for the plant and the physiological state for the plant and the explant (He *et al*., 2019). The results agree with (Mastuti *et al*., 2017) on the cotyledonary leaves for the *Physalis angulata* L. plant, where he concluded through its study that Auxins, in particular 2,4-D, is one of the growth regulators most encouraging the induction of callus, and when working on a combination with Kin, it gave the best percentage for induction of callus from the cotyledonary leaves.

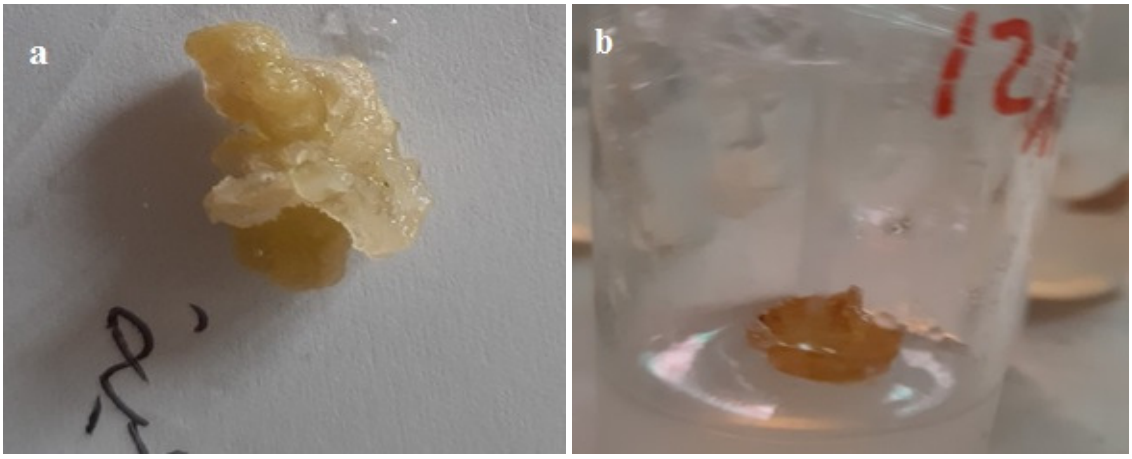

Fig. 3: Induction of callus from the cotyledonary leaves: (a) after 14 days of culturing, (b) after 35 days of culturing.

Table 2: Effect of 2,4-D and Kin concentrations and their interaction on the percentage of callus induction (%) for the different arugula explants after five weeks of culture.

2,4-D	Kin	Hypocotyl	Stem	Cotyledonary leaves
0.0	0.0	0.0	0.0	0.0
1.0	0.0	45	45	70
	0.5	45	40	75
	1.0	30	40	65
	1.5	30	30	65
2.0	0.0	70	60	80
	0.5	80	70	90
	1.0	85	70	85
	1.5	70	60	75
3.0	0.0	70	65	85
	0.5	85	75	90
	1.0	85	70	80
	1.5	70	50	70
4.0	0.0	45	40	70
	0.5	55	65	80
	1.0	55	60	75
	1.5	45	45	70
Average		60.321	55.312	76.562
L.S.D$_{0.05}$		39.41	40.30	34.14

Effect of 2,4-D and Kin Concentrations on the fresh weight of induced callus from different Arugula explants

Table (3) indicates the interaction between regulators (D-2,4 and Kientin) that the combination (0.0 mg.L^{-1} 2,4-D + 0.0 mg.L^{-1} Kin) did not produce any positive results and did not form callus for any explants understudy after five weeks of culture. There was a significant difference for the fresh weight of the induced callus, where the two interactions (2.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kin and 2.0 mg.L^{-1} 2,4-D + 1.0 mg.L^{-1} Kin) were significantly excelled by giving it the highest fresh weight of callus amounted to (40.8 mg and 33.90 mg), respectively. While the lowest fresh weight of the callus was at the interaction of (1.0 mg.L^{-1} 2,4-D + 1.5 mg.L^{-1} Kin) which gave a weight amounted to (5.6 mg). These results were in agreement with (Narayanaswamy, 1977) that the high concentration of Auxins and the low concentration of cytokines have stimulated cell division and amplitude in a group of plants, which leads to an increase in callus induction and an increase in the fresh weight of callus. Also, the best results were obtained by (Hamza and Ali, 2017) to induce callus and increase its fresh weight from seed embryos of two genotypes from the Alfalfa Plant, where the interaction (2.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kin) gave the highest fresh weight of the induced callus. The results of the same table indicated that there were significant differences in the interaction between the regulators of growth 2,4-D and Kin in increasing the fresh weight of the induced callus from the stem. where the interactions (2.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kin and 3.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kin) gave the highest fresh weight of the callus amounted to (8.20 and 7.90 mg), respectively, which were significantly excelled on the

most interactions in the table. While the interaction (1.0 mg.L^{-1} 2,4-D + 1.5 mg.L^{-1} Kin) gave the lowest average weight of the induced callus. The different concentrations of Auxin were the factor directly affecting the average weight of the induced callus, and the interaction between auxin with cytokinin affected the fresh weight (Habib, 2018). These results agree with (Hassan *et al*., 2013) when using a 2.0 mg.L^{-1} concentration to get the best average weight of the induced callus from the stem of the potato plant. These results also agree with (Al-Taha *et al*., 2018), where it obtained the highest weight of callus from the *Epiphyllum truncatum* plant using the combination of (3.0 mg.L^{-1} Kin + 0.2 mg.L^{-1} BA). While the results of the same table indicated that there was a significant effect for the interaction between the growth regulators (2,4-D and kin) in increasing the fresh weight of the induced callus from the cotyledonary leaves after five weeks of culture. where the interaction (2.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kin) gave the highest fresh weight of induced callus amounted to (85.9 mg), which significantly excelled on all other interactions, except for the two interactions (2.0 mg.L^{-1} 2,4-D + 1.0 mg.L^{-1} Kin and 3.0 mg.L^{-1} 2,4-D + 0.0 mg.L^{-1} Kin), which had a fresh weight of callus amounted to (77.3 and 73.9 mg). While interaction (1.0 mg.L^{-1} 2,4-D + 1.5 mg.L-1 Kin) gave the lowest fresh weight of induced callus amounted to (9.9 mg). These results agree with (Alaiwi *et al*., 2012), where it obtained the highest fresh weight of induced callus at a concentration of (1.0 - 6.0 mg.L^{-1} 2,4-D without or with 0.5 mg.L^{-1} BAP) from the leaves of *Centaurea* species. These results agree with (Eari *et al*., 2017) when using multiple concentrations of (2,4-D and Kin) for induction of callus from leaves of the *Silybum marianum* L. Gaertn. Chen *et al*., (2011) also indicated that 2,4-D Auxin is necessary for the induction of callus and to influence the average weight of induced callus from the cotyledonary leaves of the *Eruca sativa* plant, but remains an ideal combination of induction and increasing fresh weight.

Table 3: Effect of 2,4-D and Kin concentrations and their interaction on the fresh weight of induced callus (mg) for the different arugula explants after five weeks of culture.

2,4-D	Kin	Hypocotyl	Stem	Cotyledonary leaves
0.0	**0.0**	**0.0**	**0.0**	**0.0**
1.0	**0.0**	6.90	5.90	12.2
	0.5	8.90	7.10	18.8
	1.0	7.50	6.00	10.1
	1.5	5.60	4.70	9.9
2.0	0.0	14.60	6.10	61.9
	0.5	40.80	8.20	85.9
	1.0	33.90	6.80	77.3
	1.5	13.80	5.20	32.8
3.0	0.0	15.90	7.10	73.9
	0.5	18.30	7.90	60.3
	1.0	13.10	7.00	38.5
	1.5	10.80	5.00	30.2
4.0	0.0	13.00	5.00	37.1
	0.5	18.70	6.10	42.2
	1.0	11.30	6.60	31.7
	1.5	10.20	4.80	20.9
Average		**15.21**	**6.22**	**40.244**
L.S.D$_{0.05}$		**8.906**	**2.081**	**21.57**

Effect of 2,4-D and Kin Concentrations on the dry weight of induced callus from different arugula explants

Table (4) indicates the effect of interaction between 2,4-D and Kin on the dry weight of the induced callus from the Hypocotyl after five weeks of culture, where the interaction (0.0 mg.L^{-1} 2,4-D + 0.0 mg.L^{-1} Kin) did not form any callus for all the explants used in the study. While the interaction (3.0 mg.L^{-1} 2,4-D + 0.0 mg.L-1 Kin) was significantly excelled on all interactions in the same table except for the interaction (4.0 mg.L^{-1} 2,4-D + 0.0 mg.L^{-1} Kin) by giving it the highest dry weight amounted to (1.908 mg), While the interaction (1.0 mg.L^{-1} 2,4-D + 1.5 mg.L^{-1} kin) gave the lowest dry weight. These results agree with (Al-Mukhtar, 2008) where he used 2,4-D to induce callus from the Hypocotyl for the *Papaver somniferum* L., and (Jebor *et al*., 2016) who concluded that 2,4-D concentrations were of great importance In dry weight of the induced callus from the *Pimpinella anisum* L. plant. The results of the same table also showed the effect of the interaction between 2,4-D and Kin on the dry weight of the induced callus from the Hypocotyl, where it indicated a significant difference in the dry weight of the induced callus at the interaction (3.0 mg.L^{-1} 2,4-D + 0.0 mg.L^{-1} Kin) where the average weight of callus for this interaction amounted to (0.625 mg). While interaction (1.0 mg.L^{-1} 2,4-D + 1.5 mg.L^{-1} Kin) gave the lowest dry weight of callus amounted to (0.089 mg). These results agree with (Eari *et al*., 2017) when using multiple concentrations of 2,4-D and Kin for induction of callus from the slices of roots and leaves of the *Silybum marianum* L. Gaertn. The interaction in the same table between the 2,4-D and Kin concentrations had a significant effect on increasing the dry weight of the induced callus from the Cotyledonary leaves. Where the interaction (2.0 mg.L-1 2,4-D + 0.5 mg.L-1 Kin) gave the highest dry weight of induced callus amounted to (8.76 mg), where It significantly excelled on most interactions except the interactions (3.0 mg.L^{-1} 2,4-D + 0.0 mg.L^{-1} Kin and 3.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kin). They did not significantly excel on them, which gave a dry weight of callus amounted to (7.39 and 6.75 mg), respectively. While interaction (1.0 mg.L^{-1} 2,4-D + 1.0 mg.L^{-1} Kin) recorded the lowest dry weight of callus amounted to (0.25 mg). It is considered one

of the functions of cytokinin to divide and multiply cells, and that in the presence of auxin with cytokines and balanced concentrations, the explant tends to form unspecialized cells, this, as a result, increases the fresh and dry weight of the callus (Ranabhatt and Kapor, 2018). The interaction between the appropriate concentrations of auxin and cytokinin that allows the explants to form callus (Motte *et al*., 2014). The results also agree with (El Nagar and Mekawi, 2014), where he was able to obtain the largest size of growing callus from the Cotyledonary leaves for the arugula plant when using a number of different growth regulators with multiple concentrations where the best combination gave the largest size of callus and the best percentage of induction was at a combination (2.0 mg.L^{-1} 2,4-D + 0.5 mg.L^{-1} Kin).

Table 4: Effect of 2,4-D and Kin concentrations and their interaction on the dry weight of induced callus (mg) for the different arugula explants after five weeks of culture.

2,4-D	Kin	Hypocotyl	Stem	Cotyledonary leaves
0.0	0.0	0.0	0.0	0.0
1.0	0.0	0.124	0.13	0.4
	0.5	0.169	0.149	0.92
	1.0	0.157	0.144	0.25
	1.5	0.095	0.089	0.28
2.0	0.0	0.876	0.403	5.94
	0.5	1.326	0.431	8.76
	1.0	1.39	0.483	6.34
	1.5	0.538	0.203	3.12
3.0	0.0	1.908	0.625	7.39
	0.5	0.714	0.284	6.75
	1.0	0.734	0.462	4.43
	1.5	0.454	0.26	2.9
4.0	0.0	1.69	0.455	4.34
	0.5	0.598	0.317	4.26
	1.0	0.509	0.429	2.88
	1.5	0.5	0.331	1.94
Average		0.74	0.34	3.81
L.S.D$_{0.05}$		**0.3746**	**0.1176**	**2.147**

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