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EFFECT OF SODIUM ALGINATE AND CALCIUM CHLORIDE ON ARTIFICIAL SEEDS PRODUCTION OF RED CABBAGE PLANTS

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Abstract

This experiment was carried out at tissue culture laboratories in Iraqi Ministry of Agriculture from period 15/5/2019 till 1/9/2019. The seed of red cabbage variety Riassa, shoot tip resulting from somatic embryos produced by the direct method were used. Artificial seeds were produced by making a combination of artificial seed components from sodium alginate (SA) solution with concentrations of (1, 2 and 3)% and $Cacl_2$ solutions with three concentrations (10, 15 and 20) $g.L^{-1}$, measurements were taken (germination percentage, plantlets number and plantlets height). The results showed that using a concentration of 2% of sodium alginate added to a concentration of $15gm.L^{-1}$ of calcium chloride gave a significant superiority to the studied traits, as it gave the highest seed germination of 95% , highest plantlets number of 18 plantlets and highest plantlets height of 5.6 cm.

Key words: Sodium alginate, calcium chloride, artificial seeds, red cabbage

Introduction

Artificial seeds are defined as an industrial component that resembles natural seeds in terms of presence of main embryo and surrounding suitable environment for foodstuffs such as carbohydrates, proteins and fats. The embryo is also surrounded by a cover for the purpose of protection and upon germination it grows into a root and vegetable system (Asmah *et al.,* 2011), in 1986, the scientist Redenbaugh discovered the gelatinous materials used in the production of artificial seeds containing a single somatic embryo among them is Sodium alginate (SA) (Kumar, 2011). Kumar is called them artificial seeds or embryonic seeds, the most important obstacle to micro-proliferation by somatic embryos or plant parts are the delicacy of plant parts and their intolerance to any external stimulus, Therefore, it requires the provision of nutrients and a protective layer for easy carrying and storage without damage (Nongdam, 2016), (Al Shamari, 2014) found when using three concentrations of SA (2, 2.5 and 3)%, containing somatic embryos of cauliflower, with an addition of MS medium containing 30 $g.L^{-1}$ sucrose, was distilled in calcium chloride solution at concentrations of (5, 10 and 15) $gm.L^{-1}$ for 20 minutes and transferred to the germination medium. It was found that a concentration of 2% of SA with a concentration of 15 $g.L^{-1}$ gave the highest germination percentage of 76%. Through the study carried out by (Ghanbarali *et al.,* 2016) on the potato plant, he used micro-tubers, where he made artificial seeds for these tubers using concentrations of sodium alginate (2.5, 3 and 3.5%) with two concentrations of calcium chloride (1 and 1.5 %). found that sodium alginate at 3% with 1% of calcium chloride added to liquid MS media with full strength was better in eruption rate for the two study cultivars, (EL-abher, 2018), in a study that it conducted on three varieties of potato (Ditta, Santana and Diamond) for the purpose of multiplying potatoes with somatic embryos produced from callus, where an experiment was conducted to produce artificial seeds for plant sprouts produced from somatic embryos in which three concentrations of sodium alginate were used at (2, 3 and 4)% and five concentrations of calcium chloride are (1.5, 3, 5, 20 and 40)% for 3-5 minutes. It was found that sodium alginate of 4% with 1.5% of calcium chloride was better in Plant germination, noting that all the concentrations used helped germination. Therefore, the aim of the study is to use artificial seed technology to propagate of red cabbage plant.

Materials and Methods

This experiment was carried out at tissue culture laboratories in Iraqi Ministry of Agriculture from period 15/5/2019 till 1/9/2019. The seed of red cabbage variety Riassa were provided by (DAEHN FELDT) company. This variety needs 85-90 days for growing in the field as well as it has a big heads and external leaves.

Industrial seed production

Preparation and sterilization of sodium alginate (SA)

Prepare the SA solution with concentrations of (1, 2, and 3) % by dissolving (10, 20 and 30) $g.L^{-1}$ of this substance in 1000 ml flasks and complete its volume to one liter of liquid MS medium free of hormones and add 30 $g.L^{-1}$ of sucrose with continuous stirring using a magnetic mixer and adjusting the pH to 5.7 ± 0.1, (sterilizing the SA substance using the Autoclave steam sterilizer at 121°C and a pressure of 1.4 kg. Cm^{-3} for a period of 20 minutes may lead to the material losing its gelability (Al Shamari, 2014). Therefore, used the sterilization method described by (Rihan, 2014), which included the following stages:

1. Heat the mixture to a temperature of 80°C using a water bath for (15) minutes to kill the micro-organisms, but not spores.

2. Mixture was placed at room temperature for 5 hours to allow the spores to grow.

3. Mixture was heated at 90°C in water bath for (15) minutes to kill the growing spores.

4. Leave it overnight and then heat it at 90°C in water bath for 15 minutes to be sure.

Prepare calcium chloride solution ($CaCl_2$)

$Cacl_2$ solutions were prepared with concentrations (10, 15 and 20) $g.L^{-1}$ by dissolving 10, 15, 20 $g.L^{-1}$ of calcium chloride in 1000 cm^3 flasks and complete their volume to one liter by adding distilled water with continuous stirring using a mixer. The magnetic field was covered with cotton wool, sterilized with a steam autoclave at 121°C and a pressure of 1.4 $kg.cm^2$ for 20 minutes and kept in the cultivation room until it was used in subsequent experiments.

Preparation of explants

Shoot tips resulting from somatic embryos were used by direct method of red cabbage, where shoot tip were removed using fine tweezers and blades and primordial surrounding the shoot tips were removed so that the length of shoot tips was within the range of 2-2.5 mm using an electron microscope. The process was carried out inside cabin flow chamber.

Microencapsulation and industrial seed production

Encapsulation process was performed using the following technique (Al Shamari, 2014):

1. Prepared explant from the previous experiment was well mixed with SA inside glass vessels by magnetic mixer.

2. Explant was withdrawn using a pipette with a 2-4 mm hole to ensure that one explant was placed inside the capsule, then the mixture of the explant and SA was dropped into the calcium chloride and the capsules were left for 15 minutes.

3. The capsules were taken out and placed inside small diameter strainers and washed with sterile distilled water three times to get rid of the calcium chloride residues Fig. 1.

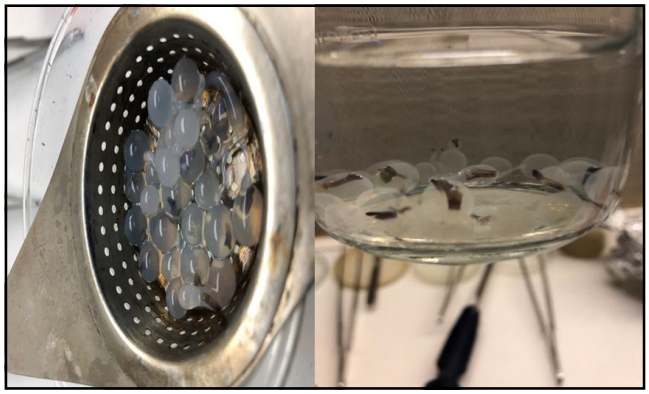

Fig. 1: Artificial seeds after the process of microencapsulation.

4. Artificial seeds were sown in 250 ml glass bottles containing MS media added to it, 1mg. L^{-1} of the two hormones (IBA and Kin) with the addition of 30 $g.L^{-1}$ of sucrose and 7 $g.L^{-1}$of agar. Where 2 artificial seeds were planted in each bottle. All of the above operations were carried out inside the Laminar flow cabin, Fig. 2.

The seedlings were incubated in the incubator under a temperature of 24°C ± 1, 16 hours light and 8 hours of darkness. After a month had passed, measurements were taken (germination percentage, number of fully grown plantlets and plantlets height).

Statistical Analysis

Completely Randomized Design (CRD) was applied with ten replicates for each experiment. Data were analyzed using Genstat software. Test of least significant differences (LSD) at 5% level of probability was used to compare the calculated averages of traits (El-sahookie and Wuhaib, 1990).

Fig. 2: Artificial seed germination.

Results and Discussion

Effect of SA and $CaCl_2$ on germination percentages of artificial seeds: The results of table 1 showed the effect of SA and $Cacl_2$ and their interaction on germination percentage of industrial seeds, as concentration of 2% of SA was significantly higher than other treatments, as it gave the highest germination percentage of 60%, while lower percentage was in concentration 3% of 10.33%. As for $CaCl_2$ treatments, concentration 15 gm. L^{-1} significantly higher than other concentrations, as it gave the highest germination percentage of 45.67 %, while 20 $gm.L^{-1}$ gave the lowest germination percentage of 22 %. The interaction between SA and $CaCl_2$ significantly affected in germination percentage especially when interaction between 2% SA and 15 $gm.L^{-1}$ $CaCl_2$, as it gave the highest germination percentage of 95%, while the interaction treatment 3% SA and 20 $gm.L^{-1}$ $CaCl_2$ did not give any germination percentage.

Table 1: Effect of SA and $CaCl_2$ on germination percentage of artificial seed (%).

SA (%)	$Cacl_2$ ($g.L^{-1}$)			
	Ca_{10}	Ca_{15}	Ca_{20}	mean
SA_1	23.00	32.00	44.00	33.00
SA_2	63.00	95.00	22.00	60.00
SA_3	21.00	10.00	0.00	10.33
mean	35.67	45.67	22.00	
L.S.D5%	SA	Ca	Inter	
	2.1	2.1	3.7	

Effect of SA and $CaCl_2$ on plantlets number: The results of table 2 showed the effect of SA and $Cacl_2$ and their interaction on plantlets number, as concentration of 2 % of SA was significantly higher than other treatments, as it gave the highest plantlets number of 10.33, while lower percentage was in concentration 3% of 0.67. As for $CaCl_2$ treatments, concentration 15 $gm.L^{-1}$ significantly higher than other concentrations, as it gave the highest plantlets number of 7.67, while 20 $gm.L^{-1}$ gave the lowest plantlets number of 3.67. The interaction between SA and $CaCl_2$ significantly affected in plantlets number especially when interaction between 2% SA and 15 $gm.L^{-1}$ $CaCl_2$, as it gave the highest plantlets number of 18.00, while the interaction between 3% SA and 15 , 20 $gm.L^{-1}$ $CaCl_2$ did not give any plantlets number.

Table 2: Effect of SA and $CaCl_2$ on plantlets number.

SA (%)	$Cacl_2$ ($g.L^{-1}$)			
	Ca_{10}	Ca_{15}	Ca_{20}	mean
SA_1	4.00	5.00	8.00	5.67
SA_2	10.00	18.00	3.00	10.33
SA_3	2.00	0.00	0.00	0.67
mean	5.33	7.67	3.67	
L.S.D5%	SA	Ca	Inter	
	1.4	1.4	1.8	

Effect of SA and $CaCl_2$ on plantlets height

Table 3 shows the effect of SA and $Cacl_2$ and their interaction on plantlets height, where the concentration of SA at 2% was significantly higher than other concentrations, which gave the highest plantlets height of 4.9 cm, while the concentration 3% gave the lowest height of 1.7 cm. Concerning $CaCl_2$, the concentration of 10 $gm.L^{-1}$ was significantly higher than other concentrations, as it gave the highest plantlets height of 4.97 cm, while the concentration of 20 $gm.L^{-1}$ gave the lowest height of 2.53 cm. 2% SA interaction with 15 $gm.L^{-1}$ of $Cacl_2$ were significant compared with other

Table 3: Effect of SA and $CaCl_2$ on plantlets height (cm).

SA (%)	$Cacl_2$ ($g.L^{-1}$)			
	Ca_{10}	Ca_{15}	Ca_{20}	mean
SA_1	5.00	3.50	3.30	3.93
SA_2	4.80	5.60	4.30	4.90
SA_3	5.10	0.00	0.00	1.70
mean	4.97	3.03	2.53	
L.S.D5%	SA	Ca	Inter	
	0.3	0.3	0.5	

treatments, as they gave the highest plantlets height of 5.6 cm, while the two interaction treatment of 3% SA with 15 and 20 $g.L^{-1}$ $CaCl_2$ did not give any results.

These results are in agreement with the results obtained by Rihan (2011), who conducted research on cauliflower using 2% SA and 15 $gm.L^{-1}$ $Cacl_2$ and the findings of Al Shamari (2014) on cauliflower at concentrations of 2% SA and 15 $gm.L^{-1}$ $Cacl_2$, while these results differed with the findings of Al-Dabbagh (2012), Siong (2012) and EL-abher (2018) in terms of the concentrations used from the encapsulation materials, while all researchers considered that the use of artificial seeds gave good results as a technique for plant propagation.

The superiority of the interaction treatment at 2% SA and 15 $gm.L^{-1}$ of $Cacl_2$ may be attributed to given a capsule with a good moisture content with a hardness similar to the emergence of plantlets, where these factors depend on the concentrations of the substances used in the capsule and the time period and that the basis of the gelatinization process and the capsule is the occurrence of ion exchange between the sodium element Na + in the sodium alginate (SA) and Calcium Ca+ in calcium chloride $Cacl_2$, as substitution of a monovalent ion with a divalent ion leads to an ionic junction between the carboxyl group and the polysaccharide molecules in alginate to form a polymeric compound, Knowing that alginate is a multiple sugar consisting of Glucuronic acid, Mannuronic acid and one carboxyl group for each sugar molecule (Rihan *et al.,* 2017), When using low concentrations of SA and $Cacl_2$, it may lead to the production fragile seeds are not solid and be difficult to handle or transport and circulate and thus affect on seed germination (Sarmah *et al.,* 2010), while high concentrations of these materials are produced solid seeds that the explants and somatic embryo are not able to emerge through them and thus lead to explant suffocation (George *et al.,* 2008).

Recommendations

Since the use of industrial seeds is considered one of the most promising techniques in vegetable plants propagating, and the lack of research on this technology in Iraq, we recommend increasing research on the production of industrial seeds on various vegetable crops, as it may be the main alternative to using real seeds in propagation of these crops.

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