Sprout results characterization of in vitro shoots encapsulation broccoli using sodium alginate

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Abstract. One of the in vitro culture techniques to obtain planting material in vitro is through artificial seed technology. The purpose of this research was to obtain a description and test the effects of sodium alginate on in vitro shoot encapsulation in broccoli. Research result for 7 days of storage the highest germination rate of 10 percent in treatment S1 (2 milligram per litre 6-*Furfuryl amino purine* (KIN)) which was encapsulated with 2 percent sodium alginate (E1). During the 28 days storage the germination rate increased significantly to 30 percent in the S2 treatment (1 milligram per litre KIN plus 0.5 milligram per litre naphthalene acetic acid (NAA)) and S3 (2 milligram per litre KIN plus 0.5 milligram per litre NAA) which was also encapsulated with E1. The percentage of synthetic seed germination rate correlated closely and positively with the number of seeds form sprouts (r is equal to 0.87). Based on the description data and selection results number of seeds germinating, two encapsulation treatments were selected, that is treatment S2 and S3 encapsulated with E1, which has a sprout stem shape round is not straight. The selected encapsulation treatment can be used for synthetic seed formation by coating.

1. Introduction

Broccoli plants (*Brassica oleracea*) are sub-tropical vegetables that are popular throughout the world, including Indonesia. This plant is popular because it plays an important role in fulfilling community nutrition with good nutrition and rich in vitamin C and dietary fiber. Fresh broccoli contains a lot of substances that are useful for the human body. In particular, broccoli contains many nutrients with anti-cancer properties, such as glucoraphanin, glukobrassisin and a small amount of selenium [1]. Glucoraphanin in broccoli can be hydrolysed into an anti-cancer sulforaphane (SFN) compound, a good anti-cancer compound, as shown in cancers of the stomach, liver, lung, breast, kidney, bladder and oesophagus [2-4]. Sulforaphane has been identified as one of the important bioactive compounds associated with health benefits after consuming these vegetables and has been shown to play a strong role in suppressing tumours [5]. Therefore, efforts to develop this plant from year to year continue to increase. This is a good indication because broccoli does have a very prospective development potential. However, there are various obstacles in producing seeds naturally, for example the self-incompatibility (SI) properties of this plant. In flowering plants, the nature of SI is a universal mechanism for avoiding self-fertilization and inbreeding, thus maintaining their genetic diversity [6]. In vitro culture is one of the solutions to obtain planting material instead of natural plant hybrid seeds. One of the in vitro culture techniques to obtain this planting material in vitro is through artificial seed technology. The idea of artificial seeds was put forward by Murashige [7], but the first report on its development was published

several years later. Artificial seeds (also called somatic seeds, synthetic seeds, clonal seeds, synseeds, somseeds) are defined as an alternative to botanic seeds analogue consisting of somatic embryos surrounded by artificial coats. This definition, also popular in these days, is based on the similarity of somatic embryos with zygotic embryos in morphology, physiology and biochemistry [8, 9]. Previously, Kamada [10] defined artificial seed as 'a capsule prepared by coating a cultured matter, a tissue piece or an organ which can grow into a plant body and nutrients with an artificial film'. This artificial seed concept comprised of 'an external film for strengthening the seed' which possibly implies the seed coat and 'an internal film for encapsulating nutrients required for growth of the cultured matter and plant hormones for controlling germination', a layer that probably simulates the endosperm tissue [11]. The currently used broader definition of synthetic seed is 'an artificially encapsulated somatic embryo, shoot or any other meristematic tissue which can develop into a plant under in vitro or in vivo conditions [12]. There are two types of artificial seeds are known: desiccated and hydrated. The first type of artificial seed produced from plant material is encapsulated in polyoxymethylene glycol followed its desiccation. In this context, the desiccated artificial seeds can only be produced in plants with desiccation-tolerant propagules. The hydrated artificial seeds are produced by encapsulating plant material in hydrogel coats. The second type of synseeds is produced in those species in which the propagules are recalcitrant and/or sensitive to desiccation [13]. This method has been employed as a suitable alternative for the use of somatic embryos [14] and became an important asset in micropropagation for example, broccoli which can be valued as a medicinal plant can also play a role in fulfilling nutrition. For this reason, testing is needed to determine the sprout characteristics of in vitro shoot encapsulation broccoli produced can be useful as a tool for synthetic seed formation by coating. This paper reports on the description and effect of sodium alginate on synthetic seed formation from in vitro shoots of broccoli.

2. Materials and method

The materials tested were 4 in vitro shoots of broccoli, namely S1, S2, S3, and S4 which were encapsulated using 3 levels of sodium alginate concentration, namely E1, E2 and E3. The treatments of S1, S2, S3, and S4 were used as explants to form synthetic seeds, derived from the results of shoot induction using a pair of cotyledon 14-day broccoli F-1 Lucky (Primasid) hybrid seed sprouts on MS media with 2 $mg.l^{-1}$ KIN (S1); 1 $mg.l^{-1}$ KIN + 0.5 $mg.l^{-1}$ NAA (S2); 2 $mg.l^{-1}$ KIN + 0.5 $mg.l^{-1}$ NAA (S3) dan 3 $mg.l^{-1}$ KIN + 0.5 $mg.l^{-1}$ NAA (S4). Before induction of S1, S2, S3, and S4, broccoli seeds were sterilized for 15 minutes in 10 and 20% Clorox solution while being shaken, rinsed with sterile water 3 times to remove the remnants of the Clorox solution and sowing on MS base media [15]. The basic media of MS for seed germination and treatment media for induction of S1, S2, S3, and S4 before being sterilized by autoclaving at 121ºC and 1.03 kPa for 30 minutes, the pH of the media was adjusted to 5,7. A pair of cotyledons from normal age 14-day sprouts were harvested, cut and placed horizontally on the surface of the induction treatment media S1, S2, S3, and S4. Induction of S1, S2, S3, and S4 using 220 ml jam culture bottles containing 30 ml media, sealed with aluminium foil, maintained at 22ºC and 16 hours of light using cool white fluorescent lamps which provide an intensity of 60 μmol m-2.s-1.

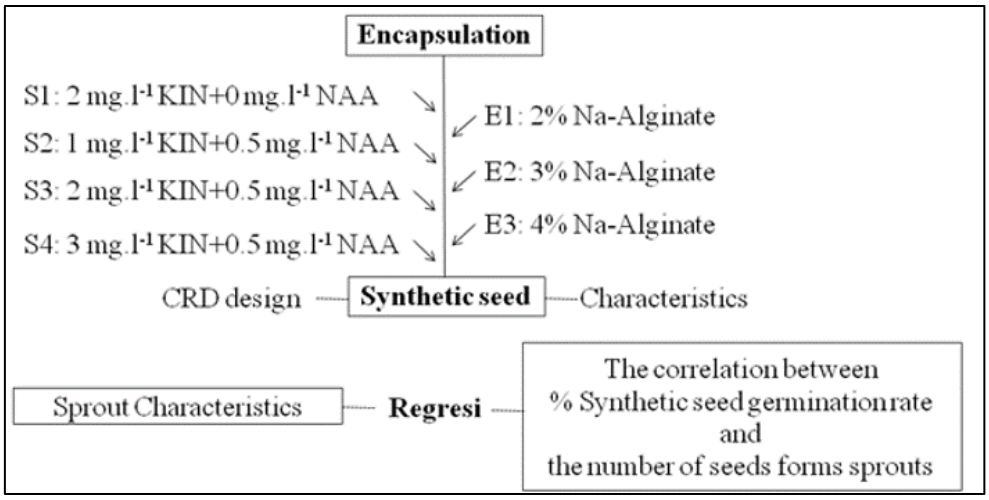

Figure 1. Flowchart of sprout results characterization of in vitro shoots encapsulation broccoli using sodium alginate.

Encapsulation of S1, S2, S3, and S4 is carried out in alginate solutions (E1, E2 and E3) and 50 mM CaCl2.2H2O solutions. The encapsulation process is carried out by means of S1, S2, S3, and S4 harvested from culture bottles, placed in sterile Petridis and cut using a cutter to get explants (S1, S2, S3, and S4) measuring about 5 mm long. Insert the explant pieces into sodium alginate solution which contains the composition of the base media MS, sugar, sodium alginate and 1.00 $mg.l^{-1}$ IBA for roots, explants pipettes and drop them into a 50 mM CaCl2.2H2O solution, shake for 15 minutes for complexing between alginate and Ca, so capsules of S1, S2, S3, and S4 alginate are formed. Rinse capsules of S1, S2, S3, and S4 alginate with sterile water three times to remove remnants of 50 mM CaCl2.2H2O solution. Four levels of explant treatment (S1, S2, S3, and S4) and 3 levels of treatment of sodium alginate concentration were arranged in a Completely Randomized Design (CRD) with 2 replications. The data obtained were analysed statistically using the SAS 9.1 program.

3. Results and discussion

3.1. Synthetic seed germination rates the results of the treatment in vitro shoot encapsulation of broccoli

Synthetic seeds the results of in vitro shoot encapsulation of broccoli with sodium alginate that successfully germinate through alginate capsules are called in vitro encapsulation seeds (IES). Based on data on germination rates and the ability to germinate synthetic seeds, the response can be seen in all treatments for 7-28 days of in vitro storage as presented in Figures 1 and 2 and Tables 1 and 2.

In Figure 2, it appears that the percentage of synthetic seed germination rate after 7 days of storage shows a low response and there is even a treatment that has not shown a response at all compared to storage up to 28 days. During storage for up to 28 days, the seed germination rate increases sharply especially in the 2% Na-alginate encapsulation treatment compared to other encapsulation treatments. This can be seen from the germination rates of seeds S1, S2 and S3 for up to 28 days of storage compared to other germination rates. Figure 2 shows the ability to germinate synthetic seeds S1, S2, S3 and S4 in the treatment of 2% Na-alginate encapsulation for 7 and 28 days of storage in vitro.

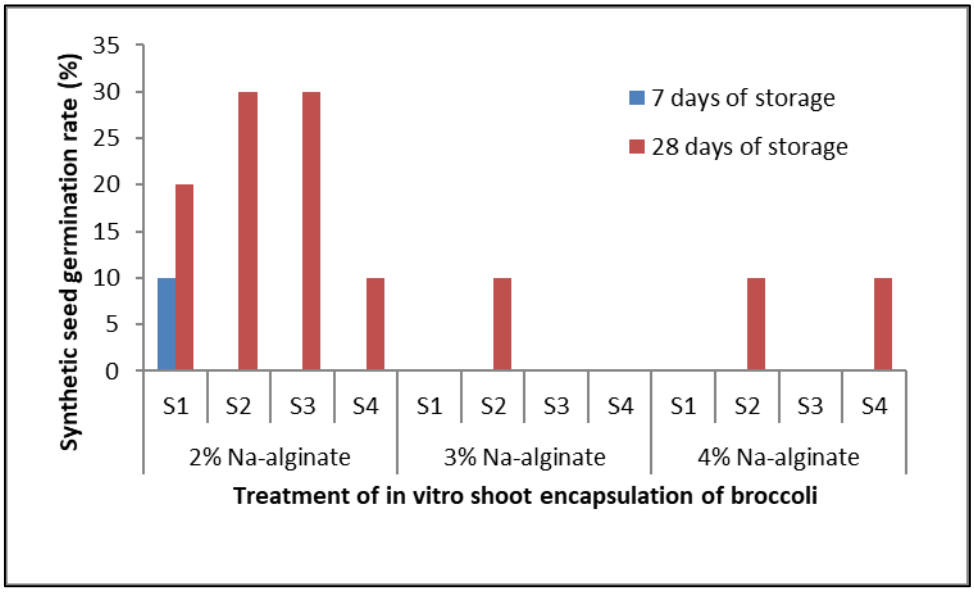

Figure 2. Percentage of synthetic seed germination results of in vitro shoot encapsulation broccoli after 7 and 28 days of storage in vitro.

In Figure 2 and Table 1 it appears that, for 7 days storage the highest percentage germination rate is 10% obtained in treatment S1 which is encapsulated with 2%Na- alginate (E1). During 28 days of storage, the percentage of germination rate increased to 30% obtained in S2 and S3 treatments which were also encapsulated with E1. In Tables 1, S1, S2 and S3 each was obtained from the treatment of shoot induction of 2 mg.l^{-1} KIN + 0 mg.l^{-1} NAA (S1), 1 mg.l^{-1} KIN + 0.5 mg.l^{-1} NAA (S2), and 2 mg.l^{-1} KIN + 0.5 mg.l^{-1} NAA (S3).

Table 1. Percentage of synthetic seed germination the results of the encapsulation and in vitro shoot induction treatment of broccoli after 7 and 28 days of storage in vitro.

No	Encapsulation treatment (%)	Treatment of shoot induction (mg.l^{-1})		7 days storage (%)	28 days storage (%)
	Na-alginate	KIN	NAA		
1	E1 = 2	S1 = 2	0	10.000^{a}	20.000^{ab}
2	E1 = 2	S2 = 1	0.5	0.000^{b}	30.000^{a}
3	E1 = 2	S3 = 2	0.5	0.000^{b}	30.000^{a}
4	E1 = 2	S4 = 3	0.5	0.000^{b}	10.000^{ab}
5	E2 = 3	S1 = 2	0	0.000^{b}	0.000^{b}
6	E2 = 3	S2 = 1	0.5	0.000^{b}	10.000^{ab}
7	E2 = 3	S3 = 2	0.5	0.000^{b}	0.000^{b}
8	E2 = 3	S4 = 3	0.5	0.000^{b}	0.000^{b}
9	E3 = 4	S1 = 2	0	0.000^{b}	0.000^{b}
10	E3 = 4	S2 = 1	0.5	0.000^{b}	10.000^{ab}
11	E3 = 4	S3 = 2	0.5	0.000^{b}	0.000^{b}
12	E3 = 4	S4 = 3	0.5	0.000^{b}	10.000^{ab}

$^{a, b, ab}$ The average number followed by the same letters in the same column is not significantly different at the 5% level using the LSD test

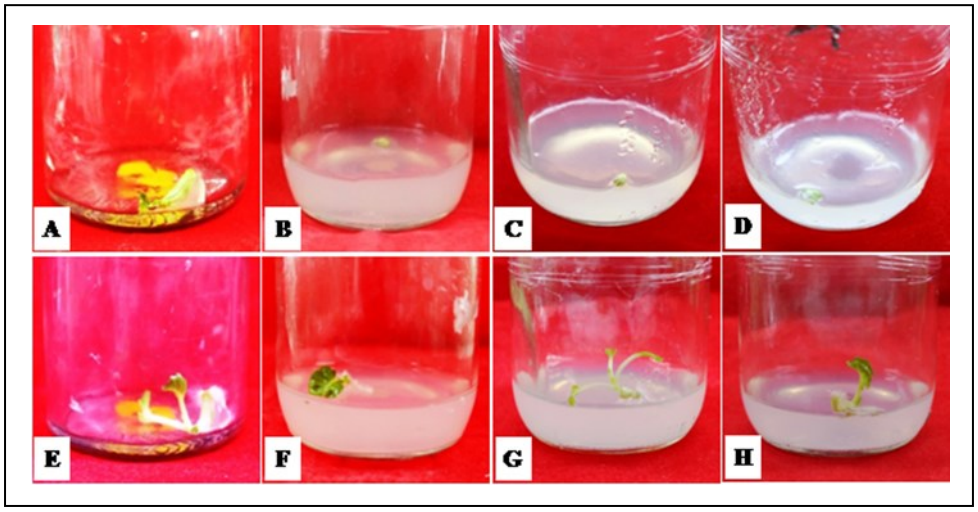

Figure 3. Ability to germinate synthetic seeds from in vitro shoot encapsulation broccoli using 2% Na-alginate (E1) after 7 days (A-D) and 28 days of storage in vitro (E-H). A and E sprouts from treatment E1 with 2 mg.l-1 KIN + 0 mg.l-1 NAA (S1); B and F sprouts from treatment E1 with 1 mg.l-1 KIN + 0.5 mg.l-1 NAA (S2); C and G sprouts from treatment E1 with 2 mg.l-1 KIN + 0.5 mg.l-1 NAA (S3); D and H sprouts from treatment E1 with 3 mg.l-1 KIN + 0.5 mg.l-1 NAA (S4).

Based on statistical analysis S2 and S3 treatments encapsulated with E1 were significantly different compared to other treatments but not significantly different from treatments S1 and S4 encapsulated with E1; S2 treatment encapsulated with E2; S2 and S4 treatments are encapsulated with E3. This means that the treatment of S2 and S3 which is also encapsulated with E1 can initiate and increase the percentage of synthetic seed germination. This occurs because of the loss of low capsule weight so that the ability to germinate synthetic seeds from S2 and S3 treatments encapsulated with E1 is better than other treatments although not significantly different compared to treatments S1 and S4 encapsulated with E1; S2 treatment encapsulated with E2; S2 and S4 treatments are encapsulated with E3.

3.2. Description of synthetic seeds resulting from in vitro shoot encapsulation broccoli

Based on the data description of the types of synthetic seed sprouts, it shows that there are two types of sprouts formed, namely axillary and adventitious. Axillary sprouts were obtained from the encapsulation treatments E1, E2 and E3. In treatment E1 obtained by S1; S2 and S4. In treatment E2 obtained by S1 and S2. In treatment E3 obtained by S2 and S4. Adventitious sprouts were obtained from E3 encapsulation treatment with S1 and S3. In the treatment of E1 encapsulation with S3 there are two types of sprouts formed, namely axillary and adventitious. Whereas based on the age parameters of sprouts formed, indicating that all treatments had a germination age formed between 6-56 days after storage (Table 2).

Visual observation in this research was carried out on the character of the stem colour and the shape of the synthetic seed sprout (Table 3). Based on the data from the observation of stem colour and sprout shape seed sprouts, it shows that all treatments have light green stem colour and sprout stem shape round is not straight, except in E2 treatment that is in the S3 and S4 treatments there is no visual observation because the seeds are unable germinate.

3.3. Number of sprouts

In observing the number of seeds forming sprouts (Table 4), the response for all treatments showed that the number of seeds forming the highest sprouts was 3 bottles obtained from the E1 encapsulation treatment i.e. in the treatment of S2 and S3. Based on statistical analysis, the treatment was not significantly different compared to other treatments. This means that, all in vitro shoot encapsulation broccoli treatments up to 28 days in vitro storage have not been able to increase the number of sprouts that have been formed significantly. So, the characters which are contained in sprouts will give responses and adjustments to the concentration of sodium alginate and the proportion of concentration of growth regulators in shoot induction after 28 days of in vitro storage.

Table 2. Description of synthetic seeds resulting from in vitro shoot encapsulation broccoli.

No	Encapsulation treatment (%)	Treatment of shoot induction ($mg.l^{-1}$)		Types of sprouts	Age of sprouts is formed (DAS)
	Na-alginate	KIN	NAA		
1	E1 = 2	S1 = 2	0	Axillary	6
2	E1 = 2	S2 = 1	0.5	Axillary	14
3	E1 = 2	S3 = 2	0.5	Axillary and adventitious	7
4	E1 = 2	S4 = 3	0.5	Axillary	7
5	E2 = 3	S1 = 2	0	Axillary	35
6	E2 = 3	S2 = 1	0.5	Axillary	14
7	E2 = 3	S3 = 2	0.5	-	-
8	E2 = 3	S4 = 3	0.5	-	-
9	E3 = 4	S1 = 2	0	Adventitious	56
10	E3 = 4	S2 = 1	0.5	Axillary	21
11	E3 = 4	S3 = 2	0.5	Adventitious	28
12	E3 = 4	S4 = 3	0.5	Axillary	21

DAS - days after storage

Table 3. Visual observation of stem colour and shape of synthetic seedling stem results from in vitro shoot encapsulation broccoli.

No	Encapsulation treatment (%)	Treatment of shoot induction ($mg.l^{-1}$)		Sprout stem colour	Sprout stem shape
	Na-alginate	KIN	NAA		
1	E1 = 2	S1 = 2	0	Light green	Round is not straight
2	E1 = 2	S2 = 1	0.5	Light green	Round is not straight
3	E1 = 2	S3 = 2	0.5	Light green	Round is not straight
4	E1 = 2	S4 = 3	0.5	Light green	Round is not straight
5	E2 = 3	S1 = 2	0	Light green	Round is not straight
6	E2 = 3	S2 = 1	0.5	Light green	Round is not straight
7	E2 = 3	S3 = 2	0.5	-	-
8	E2 = 3	S4 = 3	0.5	-	-
9	E3 = 4	S1 = 2	0	Light green	Round is not straight
10	E3 = 4	S2 = 1	0.5	Light green	Round is not straight
11	E3 = 4	S3 = 2	0.5	Light green	Round is not straight
12	E3 = 4	S4 = 3	0.5	Light green	Round is not straight

3.4. Relationship between percentage of seed germination rate and number of seeds forms sprouts
Based on the percentage data of synthetic seed germination (Figure 2) and the number of seeds forms sprouts up to 28 days in vitro storage (Table 4), it can be seen the correlation between the variable data on the percentage of synthetic seed germination with variable data observing the number of seeds forming sprouts up to 28 days in vitro storage as presented in Figure 4.

Table 4. Observation of the number of seeds forming sprouts treatment of in vitro shoot encapsulation of broccoli using sodium alginate up to 28 days in vitro storage.

No	Encapsulation treatment (%)	Treatment of shoot induction ($mg.l^{-1}$)		Number of sprouts (bottle)
	Na-alginate	KIN	NAA	
1	E1 = 2	S1 = 2	0	2[a]
2	E1 = 2	S2 = 1	0.5	3[a]
3	E1 = 2	S3 = 2	0.5	3[a]
4	E1 = 2	S4 = 3	0.5	1[a]
5	E2 = 3	S1 = 2	0	0[a]
6	E2 = 3	S2 = 1	0.5	1[a]
7	E2 = 3	S3 = 2	0.5	0[a]
8	E2 = 3	S4 = 3	0.5	0[a]
9	E3 = 4	S1 = 2	0	0[a]
10	E3 = 4	S2 = 1	0.5	1[a]
11	E3 = 4	S3 = 2	0.5	0[a]
12	E3 = 4	S4 = 3	0.5	1[a]

[a] The average number followed by the same letters in the same column is not significantly different at the 5% level using the LSD test

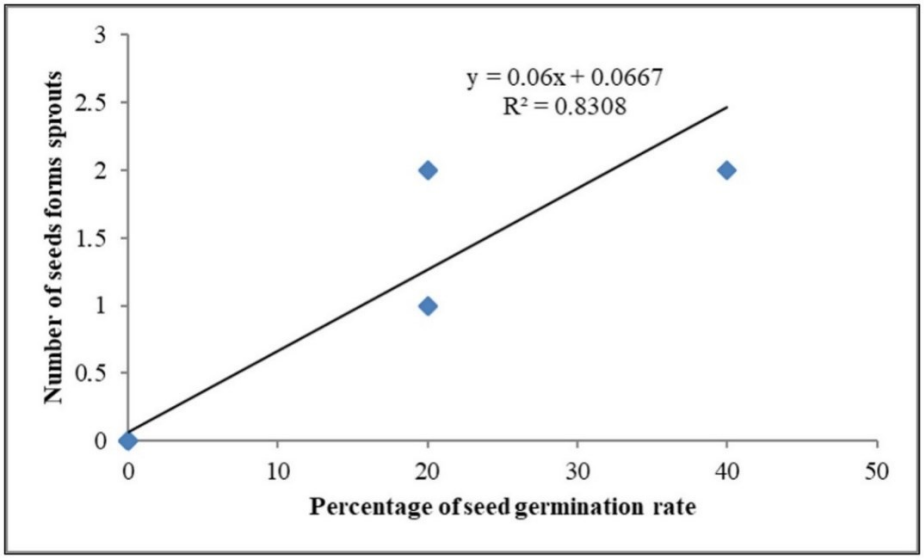

Figure 4. Relationship between percentage of seed germination rate and number of seeds forms sprouts up to 28 days in vitro storage. The value of R2 = 0.830 is obtained based on the results of regression correlation analysis using the SAS 9.1 program.

Based on the results of the regression analysis the relationship between the variable data on the percentage of synthetic seed germination rate with variable data on the number of seeds form sprouts up

to 28 days in vitro storage based on statistical tests showed that the percentage of synthetic seed germination rate correlated closely and positively with the number of seeds form sprouts (r = 0.87). This means that the higher the percentage of synthetic seed germination rate, the greater the number of seeds that form sprouts or vice versa. In Figure 4 above, it appears that the value R2 = 0.830; it can be interpreted that the data from variables percentage of synthetic seed germination rate that is equal to 83% while the rest can be caused by the influence of other variables not included in this research.

3.5. Selected encapsulation treatment

Based on the description data and selection results number of seeds germinating, two encapsulation treatments were selected, that is treatment S2 (1 mg.l^{-1} KIN + 0.5 mg.l^{-1} NAA) and S3 (2 mg.l^{-1} KIN + 0.5 mg.l^{-1} NAA) encapsulated with E1 (2% Na-alginate), which has a sprout stem shape round is not straight. The selected encapsulation treatment can be used for synthetic seed formation by coating.

4. Conclusions

Research result showed that, for 7 days storage the highest percentage of germination rate was 10% obtained at treatment S1 (2 mg.l^{-1} KIN + 0 mg.l-1 NAA) encapsulated with 2% Na-alginate (E1). During 28 days of storage, the percentage of germination rate increased significantly to 30% in S2 treatment (1 mg.l^{-1} KIN + 0.5 mg.l^{-1} NAA) and S3 (2 mg.l^{-1} KIN + 0.5 mg.l^{-1} NAA) which was also encapsulated with E1 compared to other treatments but not significantly different from treatments S1 and S4 (3 mg.l^{-1} KIN + 0.5 mg.l^{-1} NAA) encapsulated with E1; S2 treatment encapsulated with E2 (3% Na-alginate); S2 and S4 treatments are encapsulated with E3 (4% Na-alginate). The percentage of synthetic seed germination rate correlated closely and positively with the number of seeds form sprouts (r = 0.87). Based on the description data and selection results number of seeds germinating, two encapsulation treatments were selected, that is treatment S2 and S3 encapsulated with E1, which has a sprout stem shape round is not straight. The selected encapsulation treatment can be used for synthetic seed formation by coating (for next work).

5. References

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