Artificial seed viability of sugarcane (*Saccharum officinarum* L. cv. Mex 69-290) under conditions of Huimanguillo-Tabasco, Mexico

Viabilidad de la semilla artificial de caña de azúcar (*Saccharum officinarum* L. cv. Mex 69-290) bajo condiciones de Huimanguillo-Tabasco, México

Geiner Francisco Alvarez Sánchez [1], Sergio Salgado García [1*], David Jesús Palma López [1], Luz del Carmen Lagunes Espinoza [1], Hipólito Ortiz Laurel [2], Samuel Córdova Sánchez [3]

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Abstract

To develop an artificial seed of sugar cane using sodium alginate and starch, it was possible to bring shoots resistance and protection in addition to a germination of 100 and 84%, respectively. In order to improve the technology of the artificial seed of sugarcane, two experiments were carried out to evaluate different polymer concentrations (sodium alginate and starch) and determine the maximum storage time for the artificial seed, using a completely random design, and a factorial 5x5 completely random design. The study variables were as follows: physical condition, rheological, mechanical test and seedling emergence. The results obtained have allowed to us to conclude that the best physical condition, resistance and seedling emergence, were obtained with 2% (w/v) sodium alginate and 15% (w/v) starch, corroborating that the initially proposed encapsulation is reliable for the artificial seed elaboration. The seed viability at the fifth day of elaboration was the best choice with a seedling emergence of 100% at the 30 days of planting. Therefore, artificial seed can only be stored for five days to ensure a 100% seed germination.

Keywords
artificial seed • sugar cane • viability • encapsulated • germination

1 Student of the Master of Science in Agri-Food Tropical Production-Grupo MASCAÑA Colegio de Postgraduados Campus Tabasco. Km 3.5 Per. Carlos A. Molina S/N. 86500 H. Cárdenas, Tabasco, México. * salgados@colpos.mx
2 Colegio de Postgraduados Campus Córdoba-Grupo MASCAÑA. Carretera Federal Córdoba - Veracruz km 348. Congregación Manuel León. 94946 Amatlán de los Reyes. Veracruz. México.
3 Universidad Popular de la Chontalpa- Cuerpo Académico de Química Verde y Desarrollo Sostenible. Km 2. Carretera Federal Cárdenas-Huimanguillo. 86500 H. Cárdenas. Tabasco. México.

Resumen

Al desarrollar la semilla artificial de caña de azúcar, utilizando alginato de sodio y almidón, fue posible brindar resistencia y protección a las yemas, además de una germinación de 100 y 84% respectivamente. Con la finalidad de mejorar la tecnología de la semilla artificial de caña de azúcar, se realizaron dos experimentos para evaluar diferentes concentraciones de polímeros (alginato de sodio y almidón) y determinar el tiempo máximo de almacenamiento de la semilla artificial, para ello, se utilizó un diseño completamente al azar y un diseño factorial 5x5 completamente al azar. Las variables de estudio fueron: estado físico, prueba reológica, prueba mecánica y emergencia de plántulas. Los resultados obtenidos permiten concluir que, el mejor estado físico, resistencia y emergencia de plántulas, se obtuvieron con alginato de sodio al 2% (p/v) y almidón al 15% (p/v), corroborando que el encapsulado inicialmente propuesto es confiable para elaborar la semilla artificial. La viabilidad de la semilla a los cinco días de elaborada fue la mejor obteniendo una emergencia de plántulas de 100% a los 30 días de siembra. Por lo tanto, la semilla artificial solo puede almacenarse cinco días para asegurar una emergencia de plántulas del 100%.

Palabras clave
semilla artificial • caña de azúcar • viabilidad • encapsulados • germinación

Introduction

In Mexico, the sugarcane industry is an activity of high social impact, due to jobs generation and to be deeply rooted in the economy and culture of the country (1, 14). The sugarcane plantation in Mexico is a semi-mechanical activity by combining manual and mechanized operations (11, 14), however, the labor used in this system is increasingly expensive and difficult to achieve, which indicates the need for a fully mechanical operation, whose main advantage is the reduction of labor and operating costs (12, 13).

Even when using the planters' technology employing whole stems or sugarcane pieces, the efficiency of precision mechanized planting had not been achieved (12). During the process of obtaining sugarcane pieces with harvesters and in the transshipment to the seeders, the buds are mechanically damaged, this reduces the seed germination percentage, and after almost three decades of experience, it is still estimated that only 70% of commercially planted shoots manage to germinate (17).

Due to this problem, the technology of artificial seeds arises, which are vegetal structures of normally asexual origin, encapsulated with a gelling substance that protects it and that have the capacity to regenerate a plant completely identical to its parent. This technology includes two parts: a system of micro propagation through somatic embryos and another through buds (6, 7, 8, 9).

The technique of somatic embryogenesis has been the most widely used to obtain sugar cane seedlings *in vitro*, with the objective of seeding them semi-mechanically. The technique involves encapsulating a somatic embryo in a gelling matrix, which must protect it and allow it to be manipulated during storage, transport and planting, as well as to facilitate germination (5, 9).

However, obtaining sugarcane seedlings through this technique is highly costly, due to the need for reagents, materials, technicians and a specialized laboratory, to obtain it *in vitro*, to this is added, greenhouse acclimatization and the subsequent transplant to the field (6).

On the other hand, encapsulated buds have the same advantages as embryogenesis, in addition to being able to take them directly to the soil for their germination and to mechanize the sowing process. The axillary buds can be isolated from a portion of the stem and mixed with a biodegradable polymer. This can be achieved under non-sterile conditions by adding fungicides to avoid contamination (7).

Given these concerns, the artificial sugarcane seed was developed, which consists of a piece of sugarcane stem of 35 mm in length with a single shoot, disinfected and encapsulated with a mixture of ground sugar cane straw and a biodegradable polymer.

In fact, sodium alginate and starch bring them shoots resistance and protection (2), in addition, had achieved a seed germination of 100% using alginate of sodium and 84% using starch (3). Bearing this in mind, the objective of the present investigation was to evaluate the viability of artificial sugarcane seeds by comparing different concentrations of polymers for their elaboration and to determine the maximum storage time. In order to have a better management of the planting material and obtain higher percentages of germination.

Materials and methods

The experiments were carried out in the plant physiology laboratory of the Colegio de Postgraduados Campus Tabasco, Mexico. Sugarcane samples were taken from eight-month-old sugarcane plantations of the cultivar Mex 69-290, in the village C-34 from the municipality of Huimanguillo-Tabasco, Mexico whose geographic coordinates are as follows: 17°58'16'' N, -93°37'30'' W. This variety has a good index of nitrogen efficiency (Salgado *et al.*, 2017).

With the use of a machete, the sugar cane stems were cut, later, with the use of a hacksaw, pieces of stems with shoots of 35 mm in length were cut (20 mm of reserve from the foliar scar towards above and 15 mm at the bottom) of the middle section of the stem. Shoots were disinfected for 10 minutes in a solution of Malathion 50EC (Agroquímica Tridente) to 0,2% and Carbendazim (Prozycar® 500) to 0,1%, subsequently, have allowed to dry for 10 minutes.

Encapsulation improvement of the sugarcane artificial seed

The experiment was carried out in October 2016. A completely randomized design was used with nine treatments (table 1, page 30) and 20 replicates.

Encapsulation of the sugarcane shoots using sodium alginate + calcium chloride (T1, T2, T3 and T4)

Sodium alginate was mixed with 1 L of water in a beaker, stirring to avoid lumps formation. In the same way, calcium chloride was mixed in 1 L of water in a beaker. To the sodium alginate mixture, 300 g of dried ground sugarcane milled straw was added to form a paste, with which the shoots were manually covered.

Table 1. Encapsulation treatments used.
Tabla 1. Tratamientos de encapsulación utilizados.

Treatments (T)	Description
T1	Sodium alginate to 2% + calcium chloride to 10%
T2	Sodium alginate to 4% + calcium chloride to 10%
T3	Sodium alginate to 6% + calcium chloride to 10%
T4	Sodium alginate to 4% + calcium chloride to 13%
T5	Sodium alginate to 4% + calcium hydroxide to 10%
T6	Starch to 10%
T7	Starch to 12.5%
T8	Starch to 15%
T9	Starch to 17.5%

The encapsulated shoots were immersed into a calcium chloride solution for five minutes, and subsequently, placed in a plastic tray to dry for 72 hours in the shade (26°C approximately). The encapsulation thickness was approximately 5 mm.

Encapsulation of the sugarcane shoots using sodium alginate + calcium hydroxide (T5)

Sodium alginate was mixed with 1 L of water in a beaker, stirring to avoid lumps formation. In the same way, calcium hydroxide in 1 L of water was mixed in a beaker. To the sodium alginate mixture was added 300 g of dried ground sugarcane milled straw to form a paste, with which the shoots were manually covered. The encapsulated shoots were immersed into a calcium hydroxide solution for five minutes and placed into a plastic tray to dry them for 72 hours in the shade (26°C approximately). The encapsulation thickness was approximately 5 mm.

Encapsulation of sugar cane shoots using starch (T6, T7, T8 and T9)

The starch was dissolved into 250 mL of water in a beaker, subsequently 750 mL of hot water was added, was stirred until homogenization. To the starch mixture was added 300g of dried ground sugarcane milled straw to form a paste, with which the shoots were manually covered and placed into a plastic tray to let them dry for 72 hours in the shade (26°C approximately). The encapsulation thickness was approximately 5 mm.

Study variables

Physical state of the artificial seed

A visual analysis of the encapsulation was performed after 72 h of storage. An encapsulation in good physical condition should not present detachments or germination.

Mechanical strength test

To observe the strength or fragility of the encapsulated with good physical condition, they were placed in a plastic tray, which stirred from one place to another on 10 occasions, simulating the movement that takes place during the planting process.

Rheological test

With a pocket penetrometer (model E-280, AMS®, USA), a strength was exerted on the center of the encapsulates that passed the mechanical strength test and necessary effort was recorded (kg/cm^2) to break down or deform them.

Seedling emergence

Ten replicates of each treatment in good physical condition after the previous tests were selected and planted in a completely random plastic trays containing river sand. Irrigation was applied every second day to field capacity. 30 days after planting, a count of the seedling emerged was carried out per treatment.

Seedling vigor

30 days after panting, all the seedling emerged were extracted, in order to measure the roots length and stem height.

Statistical analysis

An analysis of variance was performed with a completely randomized design, and Tukey's multiple means comparison test, using the SAS version 9.3 package. To obtain an approximate normal distribution of the data, a transformation was performed by means of the arcsine function of the square root [$\sqrt{X}$ / (100)], for the variables evaluated in percentage (physical state, mechanical resistance and emergence of seedlings), for the rest of the variables (rheological test and seedling vigor) a logarithmic transformation was carried out.

Storage time of the artificial seed of sugarcane

The experiment was carried out in November 2016. A factorial 5x5 completely random design was used; 5 treatments (table 2) and 5 storage time with 25 replicates. The storage times were: 5, 8, 11, 14 and 17 days in the shade at room temperature (24°C approximately).

Encapsulation of sugarcane shoots using starch and sodium alginate + calcium chloride

For the encapsulation process of the sugar cane shoots, the same procedure described in the previous experiment was carried out for each corresponding polymer.

Study variables

Physical state of the artificial seed

For its evaluation, a visual analysis of the encapsulation was performed after 5, 8, 11, 14 and 17 storage days.

Seedling emergence

At each date of assessment of physical condition, were planted in a completely random design 5 replicates per treatment on plastic trays containing river sand. Irrigation was applied every second day at field capacity. 30 days after planting, in the phenological stage of germination, a count of the seedlings emerged was carried out per treatment.

Table 2. Encapsulation treatments used.

Tabla 2. Tratamientos de encapsulación utilizados.

Treatments (T)	Description
T1	Sodium alginate to 2% + calcium chloride to 7%
T2	Sodium alginate to 2% + calcium chloride to 10%
T3	Sodium alginate to 2% + calcium chloride to 13%
T4	Starch to 10%
T5	Starch to 15%

Seedling vigor

30 days after planting, all the seedlings that emerged were extracted, in order to measure the roots length and stem height.

Statistical analysis

An analysis of variance was performed with a factorial 5x5 completely randomized design, and Tukey's multiple means comparison test, using the SAS version 9.3 package. To obtain an approximate normal distribution of the data, a transformation was performed by means of the arcsine function of the square root [√X / (100)], for the variables evaluated in percentage (physical state and emergence of seedlings), for the rest of the variables (seedling vigor) a logarithmic transformation was carried out.

Results and discussion

Encapsulation improvement of the sugarcane artificial seed

Physical state of the artificial seed

The analysis of variance indicates highly significant differences between treatments. According to Tukey test, the best physical state of the artificial seed (100%) was presented in the T8 with 15% starch (table 3), this value exceeds 95% reported by Alvarez *et al.* (2016), when using 10% starch to encapsulate sugarcane buds; the mixture of 15% starch with ground cane straw, produces a polymer that allows better encapsulation of the buds, although its concentration is increased by 5%, this is cheaper than alginate and calcium chloride, which places it as the best polymer to encapsulate sugarcane buds.

Table 3. Physical tests of the encapsulated after 72 hours of storage.

Tabla 3. Pruebas físicas de los encapsulados después de 72 horas de almacenamiento.

Encapsulation treatments (T)	Physical state (%)	Mechanical strength (%)	Rheological test (kg/cm^2)
T1 Sodium alginate to 2 % + calcium chloride to 10%	95 b	100 a	4.5 a
T2 Sodium alginate to 4 % + calcium chloride to 10%	80.4 d	80 b	4.5 a
T3 Sodium alginate to 6 % + calcium chloride to 10%	70 e	40 d	4.5 a
T4 Sodium alginate to 4 % + calcium chloride to 13%	95 b	80 b	4.5 a
T5 Sodium alginate to 4 % + calcium hydroxide to 10%	85 d	80 b	3.6 b
T6 Starch to 10%	60 f	66.7 c	4.5 a
T7 Starch to 12.5%	93.3 bc	87.5 b	4.5 a
T8 Starch to 15%	100 a	100 a	4.5 a
T9 Starch to 17.5%	90.4 c	100 a	4.5 a
Average (%)	85.4	81.5	4.4
CV (%)	2.6	4.2	1.9
F probability of T	0.0001**	0.0001**	0.0001**
DMS	0.06	0.1	0.2

† Means with the same letter in the column are statistically equal. Tukey test (P≤ 0.05).

** Highly significant difference.

† Medias con la misma letra en la columna son iguales estadísticamente. Prueba de Tukey (P≤ 0,05).

** Diferencia altamente significativa.

The T1, T4 and T7 presented physical state greater than 93%. These results coincide with that reported by Arias *et al.* (2016), that the polymer 2% sodium alginate plus 10% calcium chloride, allow to obtain encapsulates of the artificial seed with physical states higher than 94%. The rest of the treatments are discarded since they present physical states of less than 90%.

Mechanical strength test

In the results of the analysis of variance can be observed that there are highly significant differences among encapsulation treatments. The Tukey test indicates that the treatments that presented 100% of their replicates in good condition after performing the test were as follows: T1, T8 and T9, respectively (table 3, page 32), corroborating what is reported by Álvarez *et al.* (2016) for T1, and surpassing 84% of physical state after carrying out the test with encapsulated of starch (at 10%), compared to 100% of the T8 and T9. In the case of T2 and T3, it is observed that, increasing the concentration of sodium alginate and maintaining the concentration of calcium chloride, does not favor the physical condition or the mechanical resistance of the encapsulates, indicating that the concentrations of the Polymers should be increased equally, as observed in T4, which obtained similar results to T1. On the other hand, substitution of calcium chloride by calcium hydroxide (T5), in an effort to reduce the processing costs, does not favor good physical condition or mechanical resistance to obtain only 85 and 80% of encapsulated in good physical state in each test respectively.

Rheological test

In the rheological test, the analysis of variance indicates highly significant differences among encapsulation treatments. The Tukey test indicates that the lowest resistance was obtained by T5 (3.6 kg/cm^2) because this treatment does not solidify properly. The rest of the treatments are statistically equal to each other, with an average resistance of 4.5 kg cm^{-2} (table 3, page 32). These results are similar to the 4.2 and 4.3 kg cm^{-2} reported by Álvarez *et al.* (2016) for 10% starch encapsulates and sodium alginate at 2% plus calcium chloride at 10% respectively. These results corroborate that the polymers T8 and T1, allow to elaborate an excellent artificial seed of sugarcane.

Seedling emergence (%)

The analysis of variance indicates that there are highly significant differences between the encapsulation treatments. The Tukey test indicates that the T1 and T8 treatments presented a 100% emergence of seedlings, followed by T2, T4 and T5, all of them with a 90% emergence of seedlings (table 4, page 34). These results are comparable and even superior in terms of the variability of the report by Muralles *et al.* (2009), who reported that, in a study under controlled conditions in North Florida, they obtained germination percentages of 73% using cuttings from the upper stem of sugarcane, while Galal (2016) reported a percentage of germination of 92.66% at 35 days using shoots planted in trays, surpassing the germination of traditional sowing in 8.43%. Of these results, T1 and T8 stand out, which obtained the best results in the physical state and the mechanical and rheological tests, which indicates that despite the hardness of the encapsulation, this does not prevent the development and seedling emergence of the sugarcane buds.

Table 4. Emergence and seedlings vigor resistance after 30 days of planting.
Tabla 4. Emergencia y vigor de plántulas a los 30 días después de la siembra.

Encapsulation treatments (T)	Seedling emergence (%)	Root length (cm)	Stem height (cm)
T1 Sodium alginate to 2% + calcium chloride to 10%	100 a	11.4 d	41.3 d
T2 Sodium alginate to 4% + calcium chloride to 10%	90.4 b	14.9 bc	33.3 d
T3 Sodium alginate to 6% + calcium chloride to 10%	80.4 c	14.3 c	40.7 cd
T4 Sodium alginate to 4% + calcium chloride to 13%	90.4 b	19.6 a	44.4 bcd
T5 Sodium alginate to 4% + calcium hydroxide to 10%	90.4 b	16.8 b	43.3 bcd
T6 Starch to 10%	71.2 d	13.8 c	44.6 bcd
T7 Starch to 12,5%	80.4 c	14.3 c	76.7 a
T8 Starch to 15%	100 a	10.2 d	64.6 abc
T9 Starch to 17,5%	80.4 c	14.6 bc	68.8 ab
Mean (%)	87	14.7	45.7
CV (%)	1.6	2.6	8.1
F Probability of T	0.0001**	0.0001**	0.0001**
DMS	0.04	0.1	0.5

† Means with the same letter in the column are equal statistically. Tukey test (P≤ 0.05)
** Highly significant difference.
† Medias con la misma letra en la columna son iguales estadísticamente. Prueba de Tukey (P≤ 0,05).
** Diferencia altamente significativa.

Seedling vigor

For the length of the root, the analysis of variance indicates highly significant differences between the encapsulation treatments. The Tukey test indicates that T4 obtained the highest root length (19.6 cm), while the lowest treatments were T1 and T8 (11.4 and 10.2 cm respectively), being statistically equal to each other. In this case it is observed that there is no relation between the length of roots and the emergence of the buds, and that perhaps the length of roots is influenced by the quantity of reserves of the sugarcane bud, which has not been possible control, despite collecting stems of similar diameter to extract the buds. The variety Mex 60-290 has an intermediate nitrogen consumption, requires 1.9 kg of N per t of stems (Salgado *et al.*, 2017).

The length of roots of T1 and T8 are less than those reported by Arias de la Cruz (2015), who reported 13.8 cm of root length for encapsulation with 10% starch and 14.0 cm root length with 2% sodium alginate plus chloride of calcium at 10%, at 45 days after planting.

With respect to, the stem height in the analysis of variance, can be observed that there are highly significant differences among encapsulation treatments. The Tukey test indicates that the highest stem height was obtained with the T7 (77.6 cm), followed by the T8 and T9 (64.6 and 68.8 cm respectively), being statistically equal. While the lowest stem height was obtained with T1 and T2 (41.3 and 33.3 cm respectively), being statistically equal to each other (table 4).

The differences between stem height could be explained, as mentioned above by the reserve of the buds, the polymer in this case only protects the buds from desiccation and possible physical damage during handling for planting.

The short observation period did not allow observing the relationship between root length and stem height, reported by Budi *et al.* (2016), who mention that the root system affects the growth of seeds and seedlings to reach the optimum height. The stem height of T1 and T8, could be considered adequate, if compared to the height reported by Arias de la Cruz (2015) 45 days after the development of the sugarcane plant, who found 59.5 cm of stem height for the starch encapsulation 10% and 58.8 cm of stem height with al 2% sodium alginate plus 10% calcium chloride.

Storage time of the sugarcane artificial seed

Physical state of the artificial seed

According to the results of the analysis of variance, highly significant differences were observed for the encapsulation treatment (T), storage days (SD) and their interaction T * SD (table 5).

The results of the Tukey test, for the encapsulation treatment, indicate that T2 and T3, presented 100% of the artificial seeds in good physical condition until 17 SD (table 5). These results corroborate that the encapsulation with 2% sodium alginate plus 10% calcium chloride reported by Alvarez *et al.* (2016), is sufficient to maintain a good physical condition up to 17 SD, and increase the concentration of calcium chloride, would be an unnecessary expense of the encapsulation material, on the contrary, by reducing the concentration of calcium chloride (T1), the percentage of encapsulates in good physical condition is reduced.

According to the results of the analysis of variance, highly significant differences were obtained for the storage days' factor (SD), where the highest percentage of artificial seed in good physical condition (98.4%) was obtained at five SD.

Table 5. Physical state (%) of the encapsulated at different Storage Days (SD).

Tabla 5. Estado físico (%) de los encapsulados a diferentes días de almacenamiento.

Encapsulation treatments (T)	Storage Days (SD)					T mean
	5	8	11	14	17	
T1 Sodium alginate to 2% + calcium chloride to 7%	96 b	95 b	93.3 b	90 b	80 b	90.8 c
T2 Sodium alginate to 2% + calcium chloride to 10%	100 a	100 a	100 a	100 a	100 a	100 a
T3 Sodium alginate to 2% + calcium chloride to 13%	100 a	100 a	100 a	100 a	100 a	100 a
T4 Starch to 10%	96 b	90 c	86.5 c	80 c	60 c	82.5 d
T5 Starch to 15%	100 a	95 b	93.3 b	90 b	80 b	91.6 b
Mean of Days of storage	98.4 a	96 b	94.6 b	92 c	84 d	
CV (%)	2.9					
F probability						
Encapsulation treatment (T)	0.001**					
Days of storage (SD)	0.001**					
Interaction (T*SD)	0.001**					
DMS (T)	0.03					
DMS (SD)	0.03					

† Means with the same letter in the column are equal statistically. Tukey test (P≤ 0.05)

** Highly significant difference.

† Medias con la misma letra en la columna son iguales estadísticamente. Prueba de Tukey (P≤ 0,05).

** Diferencia altamente significativa.

In fact, it was observed that the three best encapsulation treatments (T2, T3 and T5) achieved a better interaction at five days, with 100% of the artificial seeds in good physical condition, followed by the interaction at eight days where said treatments, obtained 100, 100 and 95% of the artificial seeds in good physical condition respectively (table 5, page 35).

The above indicates that the shelf life of the artificial seed can be from 5 to 8 days, depending on the treatment used. Sufficient time to carry out the mechanized planting, without damaging the artificial seed. On the contrary, T1 and T4, presented the lowest percentages of encapsulated in good physical condition, this can be attributed to the fact that, having a lower concentration of polymer, the humidity content could be higher, which would delay the drying process and would cause the sprouting of the yolk, in comparison with the rest of the treatments with higher concentration of polymers.

Seedling emergence (%)

According to the results of the analysis of variance, highly significant differences were observed for the encapsulation treatment (T), Storage days (SD) and their interaction T * SD (table 6).

The results of the Tukey test, for the encapsulation factor, indicate that the worst treatment was T3, which had no seedling emergence, attributed to the hardness of the encapsulation.

Table 6. Seedling emergence of artificial seeds planted after 5, 8, 11, 14 and 17 Storage days (SD).

Tabla 6. Emergencia de plántulas a partir de semillas artificiales sembradas después de 5, 8, 11, 14 y 17 días de almacenamiento.

Encapsulation treatments (T)	**Seedling emergence (%)**					**T mean**
	5 SD	**8 SD**	**11 SD**	**14 SD**	**17 SD**	
T1 Sodium alginate to 2 % + calcium chloride to 7%	80 b	60 c	60 b	0 a	0 a	40 b
T2 Sodium alginate to 2 % + calcium chloride to 10%	100 a	80 b	0 d	0 a	0 a	36 c
T3 Sodium alginate to 2 % + calcium chloride to 13%	0 c	0 d	0 d	0 a	0 a	0 d
T4 Starch to 10%	100 a	80 b	80 a	0 a	0 a	52 a
T5 Starch to 15%	100 a	100 a	40 c	0 a	0 a	48 a
Mean of Days of storage	76 a	64 b	38 c	0 d	0 d	
CV (%)	3.1					
F probability						
Encapsulation treatment (T)	0.001**					
Days of storage (SD)	0.001**					
Interaction (T*SD)	0.001**					
DMS (T)	0.01					
DMS (SD)	0.01					

† Means with the same letter in the column are equal statistically. Tukey test (P≤ 0.05).

** Highly significant difference.

† Medias con la misma letra en la columna son iguales estadísticamente. Prueba de Tukey (P≤ 0,05).

** Diferencia altamente significativa.

The decrease in the emergence of the seedlings after eight days can be attributed to the dehydration and loss of the nutrient reserve due to the period of inactivity of the shoots, in this respect Carneiro *et al.* (1995), mention that the organic reserve of the buds, it influences the time of germination and development of the seedlings.

For the Storage days' factor, it was observed that, with the artificial seeds with five SD before planting, the highest percentage of seedling emergence are obtained (76 %) (table 6, page 36). Which is very favorable, since Shrivastava *et al.* (2008), indicate that, in order to achieve a good germination, sugarcane should not be allowed to remain unplanted more than two days after cutting, this demonstrates the favorable effect of the encapsulation by providing an adequate means for the shoots to maintain its viability.

T * SD interaction, it is observed that the highest percentage of seedling emergence (100%) was obtained with T2, T4, and T5 at the five SD, and even at 8 SD for T5 (table 6, page 36).

In general, a significant decreasing in seedling emergence was observed after 11 SD, similar results reported by Shrivastava *et al.* (2008), where, after a period of inactivity of 10 days, the shoots of the upper and lower part of the stem did not germinate and only a few of the middle section manage to germinate.

Therefore, the best viability of the artificial seed is observed at five SD where is possible to observe a 100% seedling emergence, being higher than that reported by Galal (2016), who showed that the shoots planted in plastic trays can reach a viability percentage of 95%. These results corroborate that the polymers T2 and T5, allow to elaborate an excellent artificial seed of sugarcane.

Seedling vigor

According to the results of variance analysis for root length, highly significant differences were observed for the treatment of encapsulation (T), storage days (SD) and interaction T * SD (table 7, page 38).

The results of the Tukey test for the encapsulation factor indicate that the largest root length was obtained with T4 (10.6 cm), with T3 being the worst (table 7, page 38), as it did not show root growth due attributed to the encapsulation hardness by increasing the concentration of calcium chloride.

The artificial seed of T2 and T5, allow a good growth of roots, as already discussed previously in the previous stage. What corroborates the viability of this technology. For the Days of Storage factor, it was observed that, with the artificial seeds with five SD before planting, the highest root length was obtained (9 cm), as the SD increases, the root length is reduced, this is attributed to the loss of nutrient reserve of the buds by dehydration due to days of inactivity after the cut of the stem. T * SD interaction, is observed that in general with the artificial seeds seeded after five SD, a greater root length is obtained, and when increasing the SD before planting, the length of the root system decreases (table 7, page 38).

With respect to the height of the stem, the results of the analysis of variance showed highly significant differences for treatment of encapsulation (T), storage days (SD) and interaction T * SD (table 8, page 38).

The results of the Tukey test for the encapsulation treatment factor, indicates that the highest height of the stem was obtained in T4 with an average of 41.9 cm, in contrast, T3 presented the worst performance when not developing the stem (table 8, page 38), attributed to the hardness of the encapsulation due to the increase in chloride concentration of calcium (13%) that prevented the softening of the cover during irrigation and the hydration of the buds.

Table 7. Root length of artificial seeds planted after 5, 8 and 11 Storage days (SD).

Tabla 7. Longitud de raíz de semillas artificiales sembradas después de 5, 8 y 11 días de almacenamiento.

Encapsulation treatment (T)	Root length (cm)			T mean
	5 SD	8 SD	11 SD	
T1 Sodium alginate to 2% + calcium chloride to 7%	11.3 ab	9 a	2.4 c	7.5 c
T2 Sodium alginate to 2% + calcium chloride to 10%	10 b	7.8 a	2 c	6.6 d
T3 Sodium alginate to 2% + calcium chloride to 13%	0 c	0 b	0 d	0 e
T4 Starch to 10%	12.3 a	8.4 a	11.2 a	10.6 a
T5 Starch to 15%	11.3 ab	8 a	5.4 b	8.2 b
Mean of Days of storage	9 a	6.6 b	4.2 c	
CV (%)	2.7			
F probability				
Encapsulation treatment (T)	0.001**			
Days of storage (SD)	0.001**			
Interaction (T*SD)	0.001**			
DMS (T)	0.1			
DMS (SD)	0.07			

† Means with the same letter in the column are equal statistically. Tukey test (P≤ 0.05)

** Highly significant difference.

† Medias con la misma letra en la columna son iguales estadísticamente. Prueba de Tukey (P≤ 0,05).

** Diferencia altamente significativa.

Table 8. Stem height of seedlings planted after 5, 8 and 11 Storage days (SD).

Tabla 8. Altura de tallos de plántulas sembradas después de 5, 8 y 11 días de almacenamiento.

Encapsulation treatments (T)	Stem height (cm)			t mean
	5 SD	8 SD	11 SD	
T1 Sodium alginate to 2% + calcium chloride to 7%	35 c	41 ab	32.6 a	36.2 b
T2 Sodium alginate to 2% + calcium chloride to 10%	30.5 d	37.6 bc	17.6 c	28.5 c
T3 Sodium alginate to 2% + calcium chloride to 13%	0 e	0 d	0 d	0 d
T4 Starch to 10%	63 a	34.8 c	28.1 b	41.9 a
T5 Starch to 15%	50 b	43.2 a	18.2 c	37.1 b
Mean of Days of storage	35.7 a	31.3 b	19.3 c	
CV (%)	2.7			
F probability				
Encapsulation treatment (T)	0.001**			
Days of storage (SD)	0.001**			
Interaction (T*SD)	0.001**			
DMS (T)	0.07			
DMS (SD)	0.05			

† Means with the same letter in the column are equal statistically. Tukey test (P≤ 0.05).
** Highly significant difference.
† Medias con la misma letra en la columna son iguales estadísticamente. Prueba de Tukey (P≤ 0,05).
** Diferencia altamente significativa.

Figure 1. Details of the encapsulated after 72 hours of storage, rheological test and seedling. **a).** Encapsulated of Sodium alginate to 2 % + calcium chloride to 10 %. **b).** Encapsulated of starch to 15%. **c).** Encapsulated subjected to rheological test. **d).** Seedling with root system and stem with good plant development.

Figura 1. Detalle de los encapsulados después de 72 horas de almacenamiento, prueba reológica y plántula, **a).** Encapsulado de alginato de sodio al 2 % + cloruro de calcio al 10 %. **b).** Encapsulado de almidón al 15 %. **c).** Encapsulado sujeto a la prueba reológica. **d).** Plántula con raíces y tallo en buen desarrollo.

For the Storage days' factor (SD), it was observed that, with the artificial seeds with 5 SD before planting, the highest height of the stem (35.7 cm) was obtained. Subsequently, interaction T * SD, it was observed that the highest stem height, was obtained with the T4 in the five SD (table 8, page 38). As previously mentioned, the encapsulation can protect or in any case retard the emergence of buds, but does not influence its development, since this to be conditioned by the amount of reserves in the bud. This coincides with that reported by Nieves *et al.* (2003), who observed that differences in height and diameter of the stem of plants derived from *in vitro* cultures and plants derived from cuttings, planted in field conditions diminish with time and even disappear at 12 months of age.

In figure 1 (page 39), the detail of the encapsulated in good physical condition, the performance of the rheological test and a seedling with a portion of the encapsulation can be shown, demonstrating that the encapsulation does not impede the seedling germination and development.

Conclusions

The experiment focused on the improvement of the encapsulation of the artificial seed, using 2% sodium alginate + 10% calcium chloride and 15% starch, the best encapsulation hardness is obtained for the protection of the shoots without affecting the germination and development process of the seedlings. As for the experiment regarding the storage time of the artificial seed, 2% sodium alginate + 10% calcium chloride, maintains 100% of its artificial seed in good physical condition until 17 storage days. However, the optimum emergence of the seedlings (100%) was obtained with the seeds planted after five storage days.

For the 15% starch, the best physical condition (100%) was obtained after five storage days, while the optimum emergence of the seedling (100%) was obtained with the seeds planted to five and eight storage days. Therefore, the viability of the seed made with 2% sodium alginate + 10% calcium chloride and 15% starch, was better after five storage days, obtaining 100% germination at 30 days of planting. Given these concerns, it is concluded that artificial seeds can only remain at storage for five days to ensure a good physical condition, germination and emergence of 100% seedlings.

References

1. Aguilar-Rivera, N.; Galindo, G. M.; Fortanelli, J. M.; Contreras, C. S. 2009. ¿Por qué diversificar la agroindustria azucarera en México? Revista de Globalización, Competitividad y Gobernabilidad. España. 3(1): 62-75 p.
2. Alvarez-Sánchez G. F., Arias-de la Cruz, H. L.; Salgado-García, S.; Córdova-Sánchez, S.; Ortiz-Laurel, H.; Castelán-Estrada, M.; García-de la Cruz, R.; Castañeda-Ceja, R. 2018. Development of artificial sugarcane seed CP-54 from three cultivars (cv MEX 69-290; cv MEX 68-P-23; cv CP 72-2086) using polymers in Tabasco. México. Acta Agronómica. 67(1): 94-100 p.
3. Arias de la Cruz, H. L.; Salgado García, S.; Córdova Sánchez, S.; García de la Cruz, R.; Ortiz Laurel, H. 2016. Germinación de semilla artificial CP-54 de caña de azúcar para la siembra mecanizada de precisión. Innovación Tecnológica para la Seguridad Alimentaria. Universidad Juárez Autónoma de Tabasco: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Villahermosa. Tabasco. México. 292-298 p.

4. Budi, S.; Redjeki, E. S.; Prihatiningrum, A. E. 2016. Effect variety and stratified plantlet nursery to the growth sugarcane (*Saccharum officinarum* L.) propagated in single bud. Research Journal of Seed Science. Indonesia. 9(2): 42-47.
5. Carneiro, A. E. V.; Trivelin, P. C. O.; Victoria, R. L. 1995. Utilização da reserva orgânica e de nitrogênio do tolete de plantio (colmo-semente) no desenvolvimento da cana-planta. Sci. Agric., Piracicaba. Brasil. 52(2): 199-209 p.
6. Cid, M.; González-Olmedo, J. L.; Lezcano, Y.; Nieves, N. 2006. Influencia del pectimorf sobre la calidad de la semilla artificial de caña de azúcar (*Saccharum* spp.). Cultivos tropicales. Cuba. 27(1): 31-34 p.
7. Galal, A. M. O. 2016. A new technique for planting sugarcane in Egypt. IIOAB Journal. 7(4): 15-21 p.
8. Morales, M. E de J.; Cano, J del S. 2012. Semillas sintéticas. El campo del futuro. Revista Ciencia y Desarrollo. México. 16-21 p.
9. Muralles, L.; Bolques, A.; Grant, H.; Queeley, G. 2009. Sugarcane eye propagation in northern Florida. Proc. Fla. State Hort. Soc. 122: 411-413 p.
10. Nieves, N.; Zambrano, Y.; Tapia, R.; Cid, M.; Pina, D.; Castillo, R. 2003. Field performance of artificial seed-derived sugarcane plants. Plant Cell, Tissue and Organ Culture. Cuba. 75: 279-282 p.
11. Ortiz-Laurel, H.; Rosas-Calleja, D.; Rössel-Kipping, D.; Salgado-García, S.; Debernardi de la Vequia, H. 2016. Efectividad y rentabilidad de técnicas de siembra de caña de azúcar (*Saccharum* spp.). Agroproductividad. México. 9(3): 40-47 p.
12. Rípoli. C. M. L.; Rípoli, C. T. C. 2010. Evaluation of five sugarcane planters. Eng. Agríc. Jaboticabal. Brazil. 30(6): 1110-1022 p.
13. Robotham, B. G. 2004. Sugarcane planters: characteristics of different types, soil disturbance and crop establishment. Sugar Cane Technology. 26: 9 p.
14. Salgado García S.; Lagunes, E. L. C.; Núñez, E. R.; Ortiz, G. C. F.; Bucio, A. L.; Aranda, I. E. M. 2013. Caña de azúcar: Producción sustentable. BBA, Colegio de Postgraduados-Mundi Prensa. México. D. F. 520 p.
15. Salgado García, S.; Izquierdo Hernández, J.; Lagunes Espinoza, L. del C.; Palma López, D. J.; Córdova Sanchez , S.; Ortiz Laurel, H.; Castelán Estrada, M. 2017. Consumo de nitrógeno por cultivares de caña de azúcar en Tabasco, México. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 49(1): 45-59.
16. Shrivastava, A. K., Solomon, S.; Srivastava, A. K.; Shukla, S. P.; Sawnani, A.; Darash, R.; Prajapati, C.; Singh, P.; Singh, P.; Rai, R. K.; Jain, R.; Srivastava, P.; Chatterjee, C.; Dube, B. K. 2008. Influence of progressive staling on germination of sugarcane. Sugar Tech. India. 10(2): 137-142 p.
17. Viveros, C. A.; Calderón, H. 1995. Siembra. En Cenicaña. El cultivo de la caña en la zona azucarera de Colombia. Cali. Cenicaña. 131-139 p.