

Article

Effects on Germination and Plantlet Development of Sesame (*Sesamum indicum* L.) and Bean (*Phaseolus vulgaris* L.) Seeds with Chitosan Coatings

Nancy A. Godínez-Garrido¹, Jorge A. Torres-Castillo², Juan G. Ramírez-Pimentel¹, Jorge Covarrubias-Prieto¹, Francisco Cervantes-Ortiz¹ and Cesar L. Aguirre-Mancilla^{1,*} 

¹ I.T. Roque, Tecnológico Nacional de México, km 8 Carretera Celaya-Juventino Rosas, Celaya 38110, Mexico; nnancygg@gmail.com (N.A.G.-G.); juan.rp1@roque.tecnm.mx (J.G.R.-P.); jorge.cp@roque.tecnm.mx (J.C.-P.); francisco.co@roque.tecnm.mx (F.C.-O.)

² Instituto de Ecología Aplicada, Universidad Autónoma de Tamaulipas, División del Golfo 356, Col. Libertad, Ciudad Victoria 87019, Mexico; joatorres@docentes.uat.edu.mx

* Correspondence: cesar.am@roque.tecnm.mx

Abstract: In seed technology, the use of biocompatible materials, such as chitosan, has been demonstrated to improve the germination process and establishment of seedlings. This research is focused on the effect of a chitosan coating on the germination and development of sesame and bean plantlets. The seeds were treated with different coating techniques and combinations of chitosan: chitosan solutions at 0.1, 0.5 and 1% were used in film coating, chitosan flakes with particle sizes of 1.19 mm and 0.71 mm were used as a crusted coating, and chitosan flakes with a size of 1.19 mm were used for coating with acrylic resin. Images of the coatings were obtained by means of scanning electron microscopy; the effect on germination, germination speed, vigor index, length and root area of plantlets were also determined. Chitosan treatments increased germination by 26% in bean and 16% in sesame compared with the control; the germination speed index showed an increase of 61% in bean and 58% in sesame. The treatments with chitosan increased the length of the root in bean by 77%, and in sesame four times more, compared with the control treatments. Different forms of chitosan coatings improve germination and seedling establishment; however, the response to the type of coating at a given stage of seedling development will depend on the crop species.

Keywords: germination; scanning electron microscopy (SEM); seed quality; seed vigor



Citation: Godínez-Garrido, N.A.; Torres-Castillo, J.A.; Ramírez-Pimentel, J.G.; Covarrubias-Prieto, J.; Cervantes-Ortiz, F.; Aguirre-Mancilla, C.L. Effects on Germination and Plantlet Development of Sesame (*Sesamum indicum* L.) and Bean (*Phaseolus vulgaris* L.) Seeds with Chitosan Coatings. *Agronomy* **2022**, *12*, 666. <https://doi.org/10.3390/agronomy12030666>

Academic Editor: Jose Maria Barrero

Received: 11 February 2022

Accepted: 7 March 2022

Published: 10 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Chitosan (Ch) is a widely used biopolymer in the agricultural industry; its application is determined according to its properties, such as the degree of deacetylation and molecular weight. Chitosan has been used in agronomy for different purposes, including roles as an antimicrobial [1], antiviral [2], elicitor [3], and adjuvant [4] agent; in the gradual release of active ingredients [5]; as a soil amendment [6], abiotic stress reliever [7], promoter of growth [8], and absorbent [9]; and in agricultural water treatment [10] and postharvest coatings [11]. Advances in seed technology associated with the use of Ch have highlighted applications for agricultural uses, which must consider the origin, nature, application form and physical characteristics of Ch, as these could influence the functional responses and compatibilities of the seed's physiological performance [12–16]. The application of Ch as a coating might influence the way in which the seed comes into contact with Ch, for example, Ch applied to wheat seeds using the priming technique increased their germination by 18%, germination rate by 53% and seedling vigor by 27% [17]. In broad bean seeds, it was reported that the priming treatment with nanoparticle chitosan showed harmful effects on germination and seedling growth [18]. In rice, an increase was reported in both germination rate and growth parameters [19]. In pearl millet seed primed with chitosan, an increased

germination rate and vigor were reported (13% and 18%, respectively) [20]. Additionally, in chitosan film coatings on bean seeds, a 10% increase in germination and a 7% germination speed increase were reported [21]. In artichoke seed, the germination percentage increased by 11% when coated with chitosan film [22]. In soybeans, an increase in germination of 13% was shown [23].

In recent years, seed coating technology has incorporated the application of ingredients that are biocompatible with the environment to ensure a positive impact on the germination and establishment of crops [24,25]. The use of Ch in seed technology is part of the strategies that take advantage of its properties to formulate coatings that are part of the integral management of crops; its use is based on the improvement in the physiological and functional responses at the initial stages of crop development. Therefore, the objective of this research was to identify the structure and functionality of seed coatings with chitosan and to determine the effect of coatings on the germination and development stage of sesame seedlings (*Sesamum indicum* L.) and beans (*Phaseolus vulgaris* L.).

2. Materials and Methods

The sesame seed cultivar 'Zirándaro' was obtained from the 2016 harvest in Michoacán, Mexico, and the common bean cultivar 'Pinto Saltillo' was obtained from the 2016 harvest in San Luis Potosí, Mexico; germination tests were carried out in 2019. Chitosan was obtained from commercial chitin (Sigma-Aldrich, St. Louis, MO, USA), with the methodology of Ruiz-de-la-Cruz et al. [15] with some modifications. A first deacetylation was carried out with 1.75 M NaOH; subsequently, it was washed and left to dry at 50 °C for 24 h, before a second deacetylation with 17.5 M NaOH. The obtained product (flake) was washed and allowed to dry at 50 °C for 24 h. Once the chitosan was obtained, the degree of deacetylation (DD) was determined using the methodology of Yuan et al. [26]. To obtain different flake sizes, the chitosan was ground in a blender; subsequently, the different particle sizes were obtained using sieves with a mesh size of 0.71 and 1.19 mm.

(a) Treatment preparation

For both types of seed, beans and sesame, eight coating treatments were used: 0.1% chitosan in distilled water (CS0.1), 0.5% chitosan in distilled water (CS0.5), 1% chitosan in distilled water (CS1), adherent acrylic resin ADH[®] manufactured by COSMOCEL[®] (San Nicolas de los Garza, NL, Mexico) (A), 1% chitosan in distilled water plus chitosan in flake 0.71 mm (CSD), ADH[®] plus chitosan in 0.71 mm flake (CDA), 0.71 mm flake chitosan (CD071), 1.19 mm flake chitosan (CD119) and a control with sterile distilled water (W). For each treatment, 100 seeds were used, with three repetitions.

(b) Seed coatings

In the case of film coating (CS0.1, CS0.5, CS1, and A), seeds were immersed in their respective treatment under agitation for 30–45 s; once coated, seeds were drained to remove the excess coating solution and then dried at room temperature (25 °C) for 48 h. The control treatment (W) seeds were immersed in distilled water under agitation for 30–45 s.

For the crust coating treatments (CSD and CDA), seeds were immersed in 1% chitosan in distilled water for CSD, or in acrylic adherent for CDA under stirring for 30–45 s; then, seeds were allowed to drain for 10 min to eliminate excess liquid. Later, seeds were mixed with the 0.71 mm chitosan flakes for 5 min until a uniform crust was obtained, and were left to dry at room temperature (25 °C) for 48 h.

Finally, for treatments CD071 and CD119, seeds were mixed in the respective chitosan flake sizes for a period of 5 min.

2.1. Scanning Electron Micrograph

To obtain the images with a scanning electron microscope (SEM), the coated seeds were subjected to dehydration in a critical point desiccator (Samdri-795, Tousimis, Rockville, MD, USA), then seeds were covered with a layer of gold with an Ionizer Fine Coat (Jeol

JFC-1100) to later obtain the images with the SEM (JEOL-JSM-6360LV) at the Institute of Marine Sciences and Limnology, Universidad Nacional Autonoma de Mexico (UNAM).

2.2. Physiological Evaluation

2.2.1. Seed Germination, Germination Speed and Vigor Index

To evaluate the germination of treated seeds, a completely randomized design was established. The technique, on paper, was used according to ISTA [27]. Germination was managed under controlled conditions, with a temperature of 25 ± 4 °C, in the growth chamber (Model 818, Thermo Scientific® Lab-Liner, Marietta, OH, USA). Germination was evaluated considering root protrusion, which was recorded every 24 h until 7 days after sowing. Germination speed was evaluated as proposed by Maguire [28]. Vigor index was obtained by multiplying the accumulated germination percentage by the total height of the seedling (14 days after sowing) divided by 100 [29].

2.2.2. Plantlet Evaluation

To evaluate the effect of treatments on the development of plantlet root and stem, germinated seeds were transplanted into pots with peat moss and vermiculite in a 2:1 *v/v* ratio (Green Forest México©, Premier®, Puebla, Mexico) to allow for development. Seven days after transplantation, the length of the main root was measured, starting from the neck to the root apex. Stem height was obtained by measuring from the root neck to the stem apex, and the total length of plantlets was obtained by measuring from the stem apex to the root apex. To determine the root area, digital images were taken at a resolution of 1280×720 pixels; the images were processed and analyzed to obtain the area using Java (ImageJ) software (ver. 1.50).

The obtained data were subjected to an analysis of variance and comparison of means using the Tukey test ($p \leq 0.05$) using the statistical package Statistical Analysis System version 9 (SAS® Institute, Inc., Cary, NC, USA).

3. Results

3.1. Micrographs of Chitosan-Coated Seeds

The chitosan used had a deacetylation degree of 89%. Regarding the images of sesame seeds obtained by SEM (Figure 1), treatments CS0.1, CS0.5, CS1, CSD and CDA showed differences in testa texture with respect to the control (W). A thin film was observed, that filled the rough texture that characterizes sesame seeds. On the other hand, CSD and CDA treatments showed chitosan flakes that were adhered in a dispersed way; this was attributed to the size of chitosan flakes. On the contrary, in CD119 and CD071 treatments, adhesion of chitosan to seed surface was scarce.

The SEM images of bean seeds (Figure 2) showed that the coatings formed a continuous film for treatments CS0.1, CS0.5, CS1 and A, and in these treatments the porosities in the margin of the hilum were fully filled by the coating. On the other hand, the CSD and CDA treatments showed that chitosan flakes were adhered in a dispersed way. Finally, for the CD119 and CD071 treatments, the images showed no adhesion of chitosan flakes to seed surface. The SEM images of coatings showed the ability of chitosan in solution to form firm, homogeneous and continuous films.

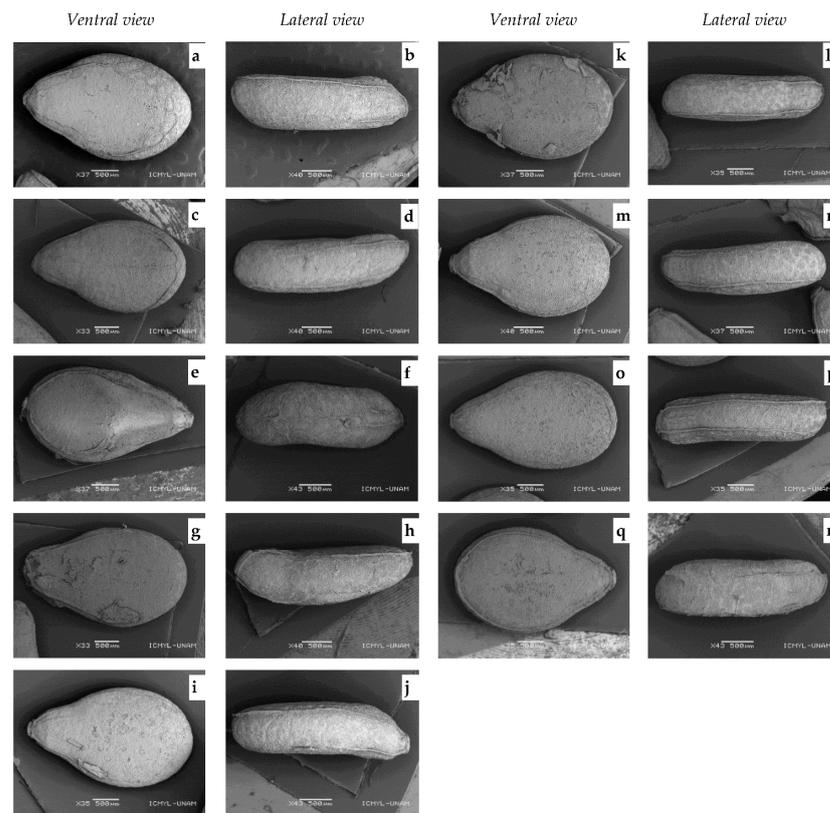


Figure 1. Micrographs of sesame seeds obtained by SEM, where (a,b) W; (c,d) CS0.1; (e,f) CS0.5; (g,h) CS1; (i,j) CSD; (k,l) CDA; (m,n) CD119; (o,p) CD071 and (q,r) A.

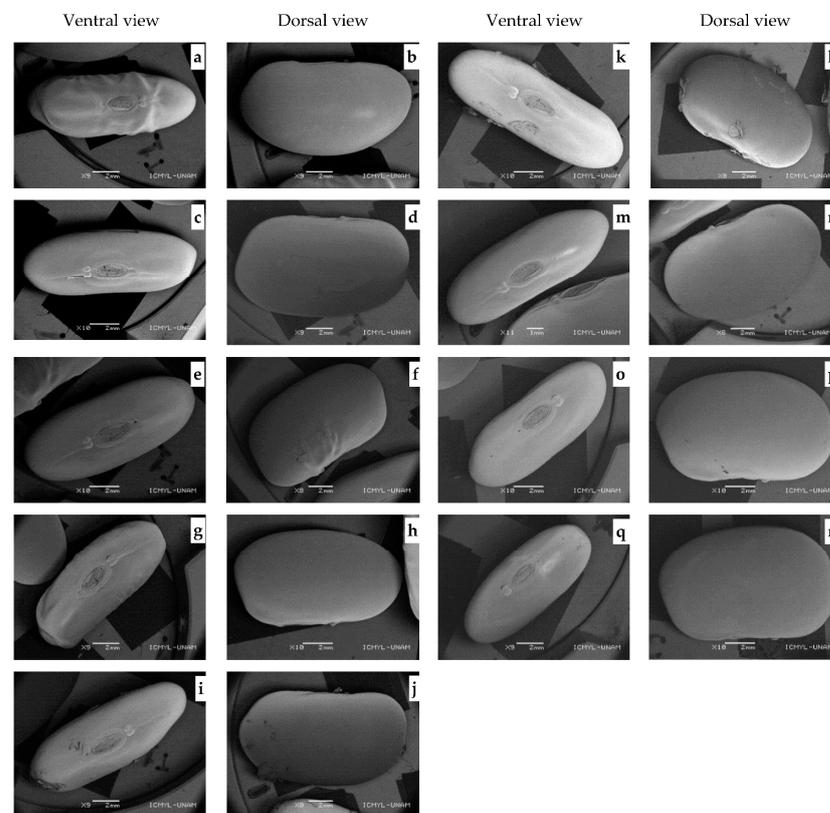


Figure 2. Micrographs of bean seeds obtained by SEM, where (a,b) W; (c,d) CS0.1; (e,f) CS0.5; (g,h) CS1; (i,j) CSD; (k,l) CDA; (m,n) CD119; (o,p) CD071 and (q,r) A.

3.2. Germination Capacity and Germination Speed

In the germination test for bean seeds, all the treatments were superior to the control; CS0.1, CS0.5 and CD119 treatments showed a 26% increase in germination; CS1 and CDA treatments were superior by 18 and 16%, respectively, and the CD071, CSD, CDA and A treatments were higher by 12% compared with the control (Figure 3A). On the other hand, for sesame seeds, the best treatments compared with the control were CS0.1, CS0.5 and CD119 with an increase of up to 16% in the germination percentage; CS1, CD071, CSD, CDA and A treatments showed a similar germination to the control (Figure 3B).

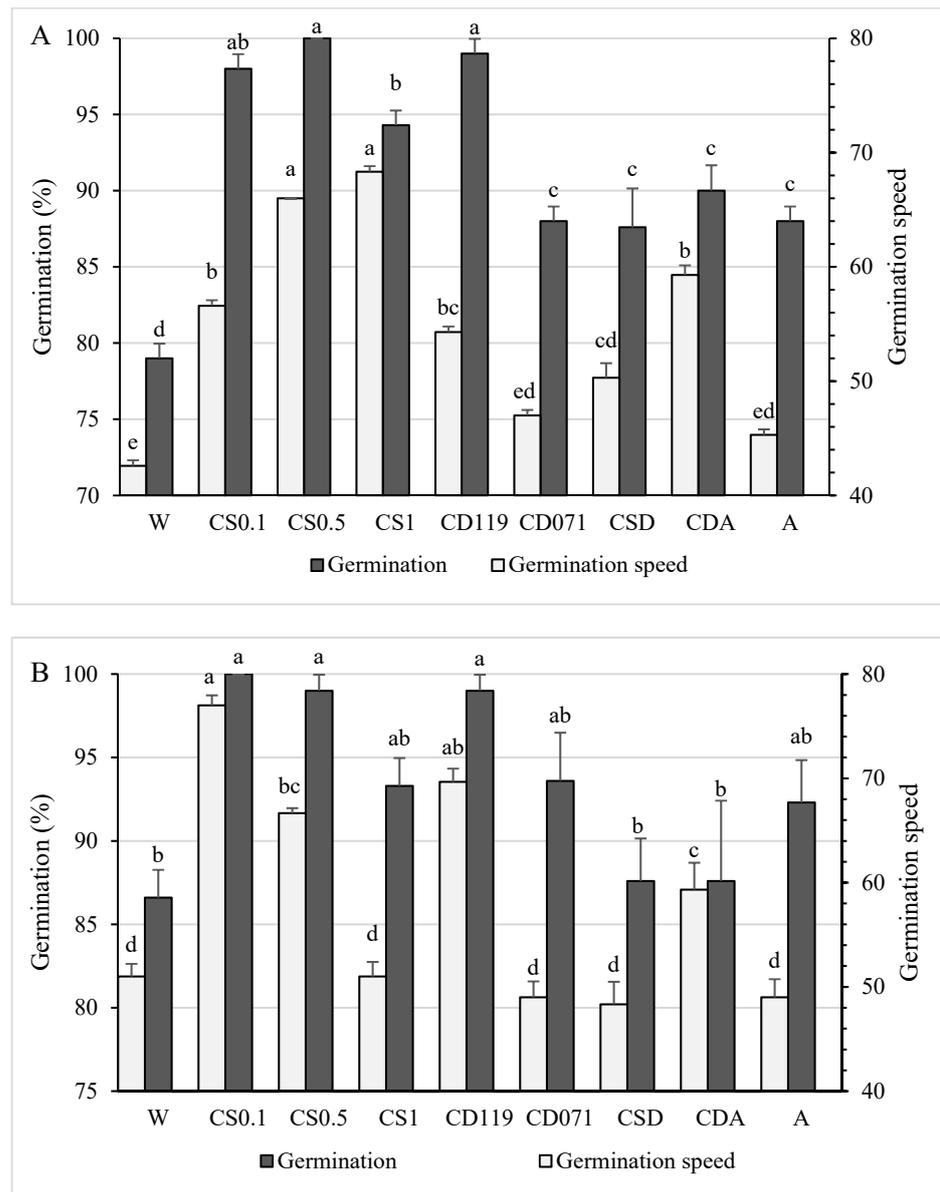


Figure 3. Effect of chitosan coating on germination and germination speed. (A) Bean seeds. (B) Sesame seeds. Means with the same letter indicates that they are statistically equal according to Tukey's test ($p < 0.05$).

In bean seeds, the CS1 and CS0.5 treatments were superior by 57% and 62%, respectively, in terms of germination speed compared with the control; CDA and CS0.1 treatments presented increases of 40 and 33%, respectively; while the CD119 and CSD treatments showed an increase of 28 and 19%, respectively, compared with the control treatment; and treatments CD071 and A did not show statistical differences when compared with the

control (Figure 3A). For sesame seeds, the best results for germination speed were for the CS0.1 treatment, with an increase of 58% compared with the control. The CD119, CS0.1 and CDA treatments increased by 43, 37 and 25%, respectively, compared with the control. In contrast, the CS1, CD071, CSD and A treatments did not show any differences compared with the control treatment (Figure 3B).

3.3. Root and Stem Growth and Vigor Index

Regarding the root length of bean plantlets, treatments CS0.1, CS0.5, CS1, CDA, CD119 and CD071 showed a growth-promoting effect, registering increases from 50% to 77% with respect to the control, while treatment A presented a lower root length, similar to the control treatment. Regarding root area in bean plantlets, CS0.5 treatment caused an increase of 2.6 times the area compared with the control; CDA, CSD, CD119 and CS071 treatments increased root area by between 68 and 90%; CS1 and A treatments did not show significant differences compared with the control (Figure 4A). In sesame, the CDA treatment increased root length of plantlets four times more than control treatment (W), CS0.5 and CSD treatments showed an increase of 2.4 times the root length of plantlets, while the CS0.1 treatment increased by 2.1 times. Regarding root area, all treatments, except treatment A, were superior to the control, but the CS0.5 and CDA treatments increased root area by 24 times more than the control (Figure 4B).

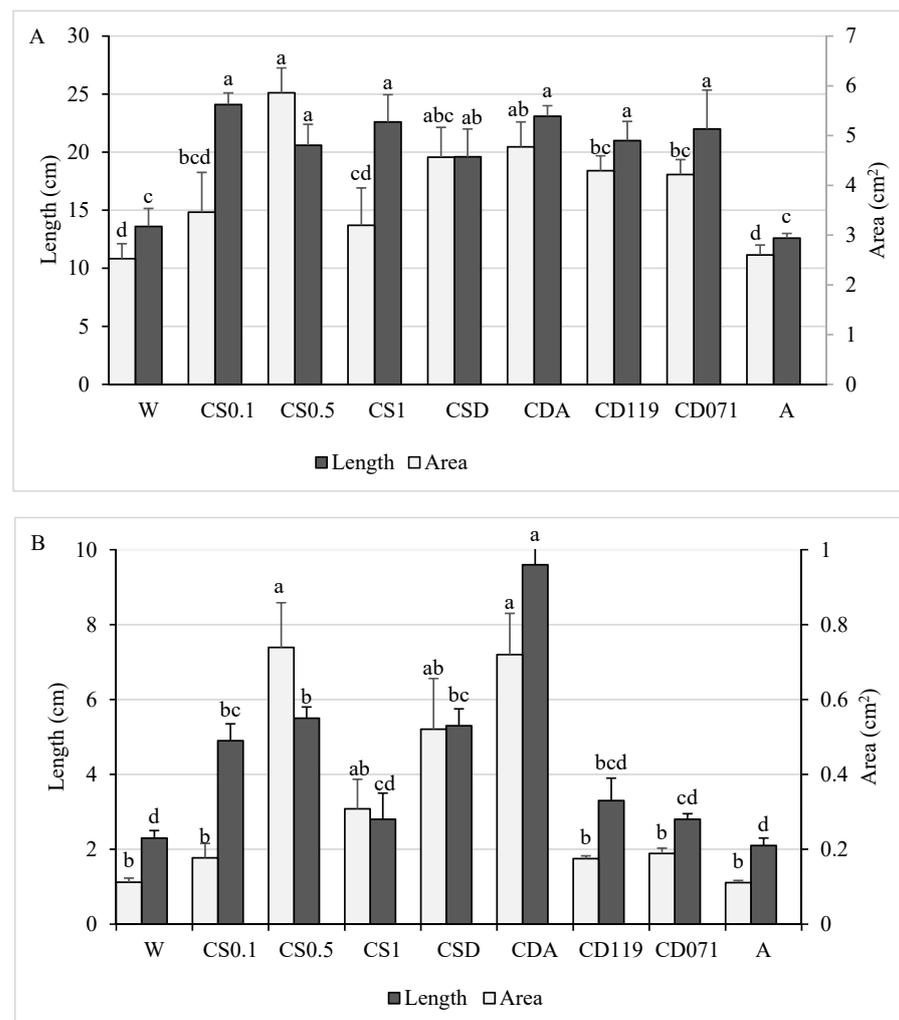


Figure 4. Effect of coatings with chitosan on bean and sesame seeds on their post-germination physiology. **(A)** Root length and root area of bean plantlets. **(B)** Root length and root area of sesame plantlets. Means with the same column letter indicate that they are statistically equal (Tukey, $p < 0.05$).

Regarding the stem length of bean plantlets, treatments formulated with chitosan stimulated stem growth by up to 30% compared with the control; only treatments W and A were statistically lower than coatings containing chitosan (Table 1). On the other hand, for sesame, the CDA treatment increased the length of the stem by up to double the size of the control, the CSD, CS0.1 and CD119 treatments achieved an increase from 81 to 77%, and, finally, the CS0.5 and CS1 treatments promoted an increase of 70 and 50%, respectively. Treatments CD071 and A did not show a statistical difference compared with the control (Table 1). For all treatments, the stem width showed no significant differences in both bean and sesame.

Table 1. Means comparison of stem length, plantlet length and vigor index in bean and sesame plantlets.

Crop	Treatment	SL		PL		VI	
Bean	W	23.3	b	38	b	32.3	c
	CS0.1	28.9	a	53.5	a	51.2	a
	CS0.5	26.3	a	56.1	a	55.5	a
	CS1	29.1	a	54.4	a	52.0	a
	CSD	30.1	a	50.7	a	42.6	b
	CDA	30.4	a	55.6	a	51.2	a
	CD119	29.8	a	53.8	a	53.8	a
	CD071	26.3	a	48.7	a	43.3	b
	A	16.2	b	30.2	b	25.7	d
Sesame	W	2.7	d	5.1	d	4.3	e
	CS01	4.8	ab	9.8	cb	9.4	bc
	CS05	4.6	abc	9.7	cb	9.6	bc
	CS1	4.1	abc	7	cbd	6.7	cd
	CSD	4.9	ab	10.2	b	8.6	bcd
	CDA	5.4	a	14.8	a	13.6	a
	CD119	4.8	ab	8.1	cbd	8.1	bcd
	CD071	3.7	cd	6.5	cd	5.8	cd
	A	2.5	d	4.7	d	4.0	e

SL: Stem length (cm); PL: Plantlet length (cm); VI: Vigor index of plantlets. Means with the same letter for each variable indicates they are statistically equal (Tukey, $p \leq 0.05$).

In relation to the vigor index, for beans, treatments CS0.5, CS0.1, CS1, CDA and CD119 surpassed the control with an increase from 58% to 71%; in the CSD and CD071 treatments, the vigor index increased by up to 34% compared with the control. In the case of sesame, all treatments with chitosan were statistically superior to the control, CDA was the best treatment, promoting an increase in the vigor index of three times more compared with the control, followed by the treatments CS0.5, CS0.1, CSD and CD119, which were from 1.9 to 2.25 times higher than the control. Finally, the CS1 and CD071 treatments achieved an increase of 55 and 34%, respectively, compared with the control. The data from this study allowed us to determine that there was a greater sensitivity to stimulation by chitosan for the cultivation of sesame.

4. Discussion

4.1. Scanning Electron Microscopy (SEM) of Seeds Coated with Chitosan

The importance of the uniformity of coatings is essential to ensure seed protection, the permanence of active ingredients and their mechanical properties. In this sense, studies such as SEM provide data on the characteristics of continuity and structure of coatings; as has been reported for the characterization of chitosan coatings in artichoke seeds where a smooth, homogeneous cover is reported, in addition to a shine given by the chitosan film [22]. On the other hand, Zeng et al. [23], report that chitosan coatings on seeds using the film technique generate a uniform protective layer, which covers the characteristic reliefs of the seed coat; these results agreed with the records in this study. Seed chitosan coating conferred homogeneity, firmness, and uniformity, as well as being colorless and compatible [22,30]; these types of coatings have shown their usefulness in postharvest

storage by extending the life of the seed [21]. In turn, the film allows for water absorption, retention and the gradual release of compounds of agricultural interest mixed with chitosan, due to the physicochemical characteristics of chitosan [31].

The surfaces of the seeds with film coatings (CS0.1, CS0.5, CS1) were observed to be smooth, colorless, without fissures, homogeneous and firm. The coverage capacity of the film is related to the surface texture of the seed coat, since the greater the roughness and porosity, the greater the adhesion of the chitosan [32]. In this way, the coatings applied using the crusting technique (without adherents) in this research proved to stimulate germination and germination speed.

4.2. Germination and Germination Speed

It has been reported that the application of 0.25% chitosan in solution as a seed coating, in addition to osmoconditioning, have managed to increase germination by more than 15% [17]. There are several factors that affect or diversify the success of chitosan coating technologies, including origin, degree of deacetylation, concentration, crop stress level, agricultural species, and even varietal responses at the genetic level [7,14,33–37], and that represent key points for these technologies. In this study, different responses were observed between agricultural species, since some treatments promoted germination or germination speed, while others did not show differences with respect to the control. In contrast, negative effects on germination speed have been reported in synthetic polymers, such as by de Barros et al. [38], where the super absorbent polymer treatment was exposed to sorghum seeds, resulting in the reduction of germination speed, while in another study [39], there was no effect on the speed of germination in onion seeds coated with chitosan using the priming technique. On the contrary, in this investigation, there was a stimulating response for germination speed, which agrees with that stated by Tovar et al. [40], who reported a positive effect on germination speed in treatments with moringa extract and chitosan mixed with iron oxide nanoparticles in corn seeds using the film technique. Similarly, Samarah et al. [41] found positive effects on germination speed in treatments with chitosan to bell pepper seeds using the film technique. It was thought that chitosan is processed at the cellular level in oligomers that have the chance to activate defense genes and other metabolic pathways. Considering this and the chance of increasing water availability due to the chitosan seed coating, there is a high probability of an increase in germination rate [42].

4.3. Root and Stem Development of Plantlets and Vigor Index

At present, advances in coating technology focus on the study of active ingredients that stimulate growth and high vigor in the seedling stage. Acharya et al. [43] used a film coating with silver nanoparticles on watermelon seeds (*Citrullus lanatus* L.), improving seedling growth by up to 35% in terms of stem length. Accinelli et al. [44] observed a stimulation of stem and root growth associated with the activation and survival of *B. subtilis*, in a study with bioplastics consisting of modified starch and chitin mixed with *Bacillus subtilis*, used as a coating for corn seeds. On the other hand, techniques such as pelleting with cellulose, diatomaceous earth and soy protein on broccoli seeds (*Brassica oleracea* L.) showed a 36% increase in stem and root growth. In addition, the vigor index exceeded the control by 30% [45]. Coatings with MWCNT have been shown to increase root growth and density in wheat [46]. The results of this study demonstrate chitosan's ability to stimulate the germination and germination speed of seeds, in addition to stimulating seedling development, with a higher potential than that reported for other polymers.

The results of this study also correlate with other findings, such as the stimulating effect on stem or root growth in the plantlet development stage, as reported in other studies [16,47,48]. This study shows the advantages and diversity of the responses to the application of chitosan as a seed coating, with different coating techniques being used to improve the physiological quality of seed in the germination process and plantlet development stage. There are also different degrees of sensitivity for each crop, acting as an active ingredient and additive.

5. Conclusions

In the present study, chitosan was highlighted as a functional agent for seed coating. In film treatments with low chitosan concentrations, germination and germination speed were improved for both species, while the pelletized coating technique for sesame showed better results in terms of plantlet development; thus, there is a different degree of sensitivity to the presence and concentration of chitosan for each crop species. Therefore, recommendations for the application of chitosan coatings, in addition to coating application technique, should be based on specific need; that is, the species to be worked on should be defined, in addition to the stage of crop development for which improvement is required.

Author Contributions: N.A.G.-G., C.L.A.-M. and J.A.T.-C. conceived and designed the experiments, and wrote the paper; J.G.R.-P., J.C.-P. and F.C.-O. contributed the reagents or materials and analyzed the data. All authors have read and agreed to the published version of the manuscript.

Funding: This research received funding from Tecnológico Nacional de México (TecNM), project number 10027.21-P.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Thanks to the National Council of Science and Technology (CONACyT, Mexico) for scholarship no. 575769 awarded to N.A.G.-G. for postgraduate studies in Agrifood Production Sciences at the TecNM-Instituto Tecnológico de Roque.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Oh, J.W.; Chun, S.C.; Chandrasekaran, M. Preparation and in vitro characterization of chitosan nanoparticles and their broad-spectrum antifungal action compared to antibacterial activities against phytopathogens of tomato. *Agronomy* **2019**, *9*, 21. [[CrossRef](#)]
2. Iriti, M.; Varoni, E.M. Chitosan-elicited plant innate immunity: Focus on antiviral activity. In *Research Progress in Oligosaccharins*; Yin, H., Du, Y., Eds.; Springer: New York, NY, USA, 2016; pp. 65–81. [[CrossRef](#)]
3. Merino, D.; Mansilla, A.Y.; Casalongué, C.A.; Alvarez, V.A. Preparation, characterization, and in vitro testing of nanoclay antimicrobial activities and elicitor capacity. *J. Agric. Food Chem.* **2018**, *66*, 3101–3109. [[CrossRef](#)] [[PubMed](#)]
4. Symonds, B.L.; Lindsay, C.I.; Thomson, N.R.; Khutoryanskiy, V.V. Chitosan as a rainfastness adjuvant for agrochemicals. *RSC Adv.* **2016**, *6*, 102206. [[CrossRef](#)]
5. De Oliveira, J.L.; Ramos Campos, E.V.; Pereira, A.E.; Nunes, L.E.; Da Silva, C.C.L.; Pasquoto, T.; Lima, R.; Smaniotto, G.; Polanczyk, R.A.; Fraceto, L.F. Geraniol encapsulated in chitosan/gum arabic nanoparticles: A promising system for pest management in sustainable agriculture. *J. Agric. Food Chem.* **2018**, *66*, 5325–5334. [[CrossRef](#)] [[PubMed](#)]
6. Xu, C.; Mou, B. Chitosan as Soil Amendment Affects Lettuce Growth, Photochemical Efficiency, and Gas Exchange. *HortTechnol. Hortte* **2018**, *28*, 476–480. [[CrossRef](#)]
7. Hidangmayum, A.; Dwivedi, P.; Katiyar, D.; Hemantaranjan, A. Application of chitosan on plant responses with special reference to abiotic stress. *Physiol. Mol. Biol. Plants* **2019**, *25*, 313–326. [[CrossRef](#)] [[PubMed](#)]
8. Chakraborty, M.; Hasanuzzaman, M.; Rahman, M.; Khan, M.A.R.; Bhowmik, P.; Mahmud, N.U.; Tanveer, M.; Islam, T. Mechanism of Plant Growth Promotion and Disease Suppression by Chitosan Biopolymer. *Agriculture* **2020**, *10*, 624. [[CrossRef](#)]
9. Vigneshwaran, S.; Sirajudheen, P.; Nikitha, M.; Ramkumar, K.; Meenakshi, S. Facile synthesis of sulfur-doped chitosan/biochar derived from tapioca peel for the removal of organic dyes: Isotherm, kinetics and mechanisms. *J. Mol. Liq.* **2021**, *326*, 115303. [[CrossRef](#)]
10. Picos-Corrales, L.A.; Sarmiento-Sánchez, J.I.; Ruelas-Leyva, J.P.; Crini, G.; Hermosillo-Ochoa, E.; Gutiérrez-Montes, J.A. Eco-friendly approach towards treating raw agricultural wastewater and river water through flocculation using Chitosan and bean straw flour as bioflocculants. *ACS Omega* **2020**, *5*, 3943–3951. [[CrossRef](#)]
11. Chen, C.; Nie, Z.; Wan, C.; Chen, J. Preservation of Xinyu Tangerines with an Edible Coating Using Ficus hirta Vahl. Fruits Extract-Incorporated Chitosan. *Biomolecules* **2019**, *9*, 46. [[CrossRef](#)]
12. Lan, W.; Wang, W.; Yu, Z.; Qin, Y.; Luan, J.; Li, X. Enhanced germination of barley (*Hordeum vulgare* L.) using chitoooligosaccharide as an elicitor in seed priming to improve malt quality. *Biotechnol. Lett.* **2016**, *38*, 1935–1940. [[CrossRef](#)] [[PubMed](#)]

13. Saharan, V.; Pal, A. *Chitosan Based Nanomaterials in Plant Growth and Protection*; Springer: New Delhi, India, 2016; pp. 33–41. [[CrossRef](#)]
14. Peña-Datoli, M.; Hidalgo-Moreno, C.M.; González-Hernández, V.; Alcántar-González, E.; Etchevers-Barra, J. Maize (*Zea mays* L.) seed coating with chitosan and sodium alginate and its effect on root. *Agrociencia* **2016**, *50*, 1091–1106.
15. Ruiz-de-La-Cruz, G.; Aguirre-Mancilla, C.L.; Godínez-Garrido, N.A.; Osornio-Flores, N.M.; Torres-Castillo, J.A. Chitosan mixed with beneficial fungal conidia or fungicide for bean (*Phaseolus vulgaris* L.) seed coating. *Interciencia* **2017**, *42*, 307–312.
16. Iglesias, M.J.; Colman, S.L.; Terrile, M.C.; Paris, R.; Martín-Saldaña, S.; Chevalier, A.A.; Álvarez, V.A.; Casalongué, C.A. Enhanced Properties of Chitosan Microparticles over Bulk Chitosan on the Modulation of the Auxin Signaling Pathway with Beneficial Impacts on Root Architecture in Plants. *J. Agric. Food Chem.* **2019**, *67*, 6911–6920. [[CrossRef](#)] [[PubMed](#)]
17. Hameed, A.; Sheikh, M.A.; Hameed, A.; Farooq, T.; Basra, S.M.A.; Jamil, A. Chitosan seed priming improves seed germination and seedling growth in wheat (*Triticum aestivum* L.) under osmotic stress induced by polyethylene glycol. *Philipp. Agric. Scientist.* **2014**, *97*, 294–299.
18. Abdel-Aziz, H. Effect of Priming with Chitosan Nanoparticles on Germination, Seedling Growth and Antioxidant Enzymes of Broad Beans. *Catrina* **2019**, *18*, 81–86. [[CrossRef](#)]
19. Stanley-Raja, V.; Senthil-Nathan, S.; Chanthini, K.M.P.; Sivanesh, H.; Ramasubramanian, R.; Karthi, S.; Shyam-Sundar, N.; Vasantha-Srinivasan, P.; Kalaivani, K. Biological activity of chitosan inducing resistance efficiency of rice (*Oryza sativa* L.) after treatment with fungal based chitosan. *Sci. Rep.* **2021**, *11*, 1–15. [[CrossRef](#)]
20. Manjunatha, G.; Roopa, K.; Prashanth, G.N.; Shekar Shetty, H. Chitosan enhances disease resistance in pearl millet against downy mildew caused by *Sclerospora graminicola* and defence-related enzyme activation. *Pest. Manag. Sci.* **2008**, *64*, 1250–1257. [[CrossRef](#)]
21. Godínez-Garrido, N.A.; Ramírez-Pimentel, J.G.; Covarrubias-Prieto, J.; Cervantes-Ortiz, F.; Pérez-López, A.; Aguirre-Mancilla, C.L. Chitosan coating on bean and maize seeds: Release of agrochemical fungicide and post-storage condition. *J. Seed Sci.* **2021**, *43*, e202143036. [[CrossRef](#)]
22. Ziani, K.; Ursúa, B.; Maté, J.I. Application of bioactive coatings based on chitosan for artichoke seed protection. *Crop Prot.* **2010**, *29*, 853–859. [[CrossRef](#)]
23. Zeng, D.; Luo, X.; Tu, R. Application of bioactive coatings based on chitosan for soybean seed protection. *Int. J. Carbohydr. Chem.* **2012**, *2012*. [[CrossRef](#)]
24. De Gregorio, P.R.; Michavila, G.; Ricciardi Muller, L.; de Souza Borges, C.; Pomares, M.F.; Saccol de Sá, E.L.; Pereira, C.; Vincent, P.A. Beneficial rhizobacteria immobilized in nanofibers for potential application as soybean seed bioinoculants. *PLoS ONE* **2017**, *12*, e0176930. [[CrossRef](#)] [[PubMed](#)]
25. Krishnamoorthy, V.; Rajiv, S. Tailoring electrospun polymer blend carriers for nutrient delivery in seed coating for sustainable agriculture. *J. Clean. Prod.* **2018**, *177*, 69–78. [[CrossRef](#)]
26. Yuan, Y.; Chesnutt, B.M.; Haggard, W.O.; Bumgardner, J.D. Deacetylation of chitosan: Material characterization and in vitro evaluation via albumin adsorption and pre-osteoblastic cell cultures. *Materials* **2011**, *4*, 1399–1416. [[CrossRef](#)]
27. ISTA. *International Rules for Seed Testing*; International Seed Testing Association: Zurich, Switzerland, 2004; 243p.
28. Maguire, J.D. Speed of Germination—Aid in selection and evaluation for seedling emergence and vigor. *Crop Sci.* **1962**, *2*, 176–177. [[CrossRef](#)]
29. Qiu, Y.; Amirkhani, M.; Mayton, H.; Chen, Z.; Taylor, A.G. Biostimulant seed coating treatments to improve cover crop germination and seedling growth. *Agronomy* **2020**, *10*, 154. [[CrossRef](#)]
30. Tian, F.; Chen, W.; Cai'E, W.; Kou, X.; Fan, G.; Li, T.; Wu, Z. Preservation of Ginkgo biloba seeds by coating with chitosan/nano-TiO₂ and chitosan/nano-SiO₂ films. *Int. J. Biol. Macromol.* **2019**, *126*, 917–925. [[CrossRef](#)]
31. Lian, H.; Peng, Y.; Shi, J.; Wang, Q. Effect of emulsifier hydrophilic-lipophilic balance (HLB) on the release of thyme essential oil from chitosan films. *Food Hydrocoll.* **2019**, *97*, 105213. [[CrossRef](#)]
32. Foster, L.J.R.; Ho, S.; Hook, J.; Basuki, M.; Marçal, H. Chitosan as a biomaterial: Influence of degree of deacetylation on its physiochemical, material and biological properties. *PLoS ONE* **2015**, *10*, e0135153. [[CrossRef](#)]
33. Hameed, A.; Sheikh, M.A.; Hameed, A.; Farooq, T.; Basra, S.M.A.; Jamil, A. Chitosan priming enhances the seed germination, antioxidants, hydrolytic enzymes, soluble proteins and sugars in wheat seeds. *Agrochimica* **2013**, *57*, 97–110. Available online: <https://www.cabdirect.org/cabdirect/abstract/20133352106> (accessed on 2 August 2021).
34. Lizárraga-Paulín, E.G.; Miranda-Castro, S.P.; Moreno-Martínez, E.; Lara-Sagahón, A.V.; Torres-Pacheco, I. Maize seed coatings and seedling sprayings with chitosan and hydrogen peroxide: Their influence on some phenological and biochemical behaviors. *J. Zhejiang Univ. Sci.* **2013**, *14*, 87–96. [[CrossRef](#)] [[PubMed](#)]
35. Torres-Castillo, J.A.; Sinagawa-García, S.R.; Lara-Villalón, M.; Martínez-Ávila, G.C.G.; Mora-Olivo, A.; Reyes-Soria, F.A. Evaluation of biochemical components from *Pterophylla beltrani* (Bolivar & Bolivar) (*Orthoptera: Tettigoniidae*): A forest pest from Northeastern Mexico. *Southwest. Entomol.* **2015**, *40*, 741–751. [[CrossRef](#)]
36. Udhaya-Nandhini, D.; Somasundaram, E. Lipo-Chito Oligosaccharides Enhances Germination Tolerance of Maize to Salinity Stress. *Int. J. Curr. Microbiol. App. Sci.* **2017**, *6*, 437–443. [[CrossRef](#)]
37. Li, R.; He, J.; Xie, H.; Wang, W.; Bose, S.K.; Sun, Y.; Hu, J.; Yin, H. Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *Int. J. Biol. Macromol.* **2019**, *126*, 91–100. [[CrossRef](#)]

38. de Barros, A.F.; Pimentel, L.D.; Araujo, E.F.; de Macedo, L.R.; Martinez, H.E.P.; Batista, V.A.P.; da Paixão, M.Q. Super absorbent polymer application in seeds and planting furrow: It will be a new opportunity for rainfed agriculture. *Semin. Ciênc. Agrár.* **2017**, *38*, 1703–1714. [[CrossRef](#)]
39. Singh, G.; Bhuker, A.; Mor, V.S.; Panghal, V.P.S. Seed Quality Enhancement through Priming in Onion (*Allium cepa*). *Seed Res. J.* **2018**, *45*, 1–3.
40. Tovar, G.I.; Briceño, S.; Suarez, J.; Flores, S.; González, G. Biogenic synthesis of iron oxide nanoparticles using *Moringa oleifera* and chitosan and its evaluation on corn germination. *Environ. Nanotechnol. Monit. Manag.* **2020**, *14*, 100350. [[CrossRef](#)]
41. Samarah, N.H.; AL-Quraan, N.A.; Massad, R.S.; Welbaum, G.E. Treatment of bell pepper (*Capsicum annuum* L.) seeds with chitosan increases chitinase and glucanase activities and enhances emergence in a standard cold test. *Sci. Hortic.* **2020**, *269*, 109393. [[CrossRef](#)]
42. Orzali, L.; Corsi, B.; Forni, C.; Riccioni, L. Chitosan in agriculture: A new challenge for managing plant disease. In *Biological Activities and Application of Marine Polysaccharides*; Shalaby, E.A., Ed.; IntechOpen: London, UK, 2017; pp. 87–96. [[CrossRef](#)]
43. Acharya, P.; Jayaprakasha, G.K.; Crosby, K.M.; Jifon, J.L.; Patil, B.S. Nanoparticle-mediated seed priming improves germination, growth, yield, and quality of watermelons (*Citrullus lanatus*) at multi-locations in Texas. *Sci. Rep.* **2020**, *10*, 1–16. [[CrossRef](#)]
44. Accinelli, C.; Abbas, H.K.; Shier, W.T. A bioplastic-based seed coating improves seedling growth and reduces production of coated seed dust. *J. Crop Improv.* **2018**, *32*, 318–330. [[CrossRef](#)]
45. Amirkhani, M.; Netravali, A.N.; Huang, W.; Taylor, A.G. Investigation of Soy Protein-based Biostimulant Seed Coating for Broccoli Seedling and Plant Growth Enhancement. *HortScience* **2016**, *51*, 1121–1126. [[CrossRef](#)]
46. Joshi, A.; Kaur, S.; Dharamvir, K.; Nayyar, H.; Verma, G. Multi-walled carbon nanotubes applied through seed-priming influence early germination, root hair, growth and yield of bread wheat (*Triticum aestivum* L.). *J. Sci. Food Agric.* **2018**, *98*, 3148–3160. [[CrossRef](#)] [[PubMed](#)]
47. Chamnanmanoontham, N.; Pongprayoon, W.; Pichayangkura, R.; Roytrakul, S.; Chadchawan, S. Chitosan enhances rice seedling growth via gene expression network between nucleus and chloroplast. *Plant Growth Regul.* **2015**, *75*, 101–114. [[CrossRef](#)]
48. Sathiyabama, M.; Manikandan, A. Application of Copper-Chitosan Nanoparticles Stimulate Growth and Induce Resistance in Finger Millet (*Eleusine coracana* Gaertn.) Plants against Blast Disease. *J. Agric. Sci.* **2018**, *66*, 1784–1790. [[CrossRef](#)]