

EFFECT OF DIFFERENT SUBSTRATES, MICROORGANISMS AND BIOSTIMULANTS ON THE REPRODUCTION OF STRAWBERRY SEEDLINGS (*Fragaria L.*)

EFFECT OF DIFFERENT SUBSTRATES, MICROORGANISMS, AND BIOSTIMULANTS ON THE REPRODUCTION OF STRAWBERRY SEEDLINGS (*Fragaria L.*)

García López Luis Adolfo¹, Ortega López Eric¹, Solís Gordillo Luis Arturo¹, Márquez Juárez Jorge², Espinosa Velasco Sergio³

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¹ National Technological Institute of Mexico/Comitán Institute of Technology, luisgar213347@gmail.com, <https://orcid.org/0009-0008-3739-9760>

¹ National Technological Institute of Mexico/Comitán Technological Institute, ej25034@gmail.com, <https://orcid.org/0009-0009-2654-0623>

¹ National Technological Institute of Mexico/Comitán Institute of Technology, luis.sg@comitan.tecnm.mx, <https://orcid.org/0000-0003-2486-7162>

² National Technological Institute of Mexico/Comitán Technological Institute, jorge.mj@comitan.tecnm.mx, <https://orcid.org/0009-0005-5690-592X>

³ National Technological Institute of Mexico/Comitán Technological Institute, sergio.ev@comitan.tecnm.mx, <https://orcid.org/0000-0003-0986-4027>

Abstract -- At the Laboratory of Technological Innovation in Agriculture (Lab-ITA), Comitán Institute of Technology, the effect of three substrates (S1: 10% humus + 70% agricultural soil + 20% forest soil; S2: 20% humus + 60% agricultural soil + 20% forest soil; S3: 10% humus + 80% agricultural soil + 10% forest soil), two biostimulants (Raizorg® and GreenFish®), and two microorganisms (arbuscular mycorrhizae and Rhizobium spp.). The experimental design was a completely randomized 3x2x2 factorial arrangement, with 12 treatments and 14 replicates. The variables evaluated were: number of leaves, crown diameter, plant height, root length and volume, fresh and dry weight biomass, and percentage of mycorrhizal colonization in roots.

The data were analyzed using ANOVA and Tukey's test ($\alpha = 0.05$ and 0.10) in R software. Treatment 7 (S2 + Raizorg + mycorrhizae) stood out with the highest values for height (15.38 cm), number of leaves (12.77), fresh weight (28.95 g), and dry weight (6.88 g). Treatment 11 (S3 + Raizorg + mycorrhizae) recorded the greatest root length (20.92 cm) and mycorrhizal colonization (60%). Mycorrhizae significantly outperformed Rhizobium in promoting height and above-ground biomass ($p = 0.0284$). The interaction between factors was key: the Raizorg + mycorrhizae combination maximized development, while Raizorg + Rhizobium resulted in the lowest performance.

Keywords-- Biostimulants, colonization, development, mycorrhizae, seedlings, substrates.

Abstract -- At the Laboratory of Technological Innovations in Agriculture (Lab-ITA), Technological Institute of Comitán, the combined effect of three substrates (S1: 10% humus + 70% agricultural soil + 20% forest soil; S2: 20% humus + 60% agricultural soil + 20% forest soil; S3: 10% humus + 80% agricultural soil + 10% forest soil), two biostimulants (Raizorg® and GreenFish®), and two microorganisms (arbuscular mycorrhizae and Rhizobium spp.) A completely randomized design with a 3x2x2 factorial arrangement, 12 treatments, and 14 replicates was used. Evaluated variables included number of leaves, plant height, crown diameter, root length and volume, fresh and dry biomass, and mycorrhizal colonization percentage.

Data were analyzed using ANOVA and Tukey's test ($\alpha = 0.05$ and 0.10) using R software. Treatment T7 (S2 + Raizorg + mycorrhizae) achieved the highest values for plant height (15.38 cm), number of leaves (12.77), fresh weight (28.95 g), and dry weight (6.88 g). Treatment T11 (S3 + Raizorg + mycorrhizae) recorded the greatest root length (20.92 cm) and mycorrhizal colonization (60%). Mycorrhizae significantly outperformed Rhizobium in promoting plant height and above-ground biomass ($p = 0.0284$). Factor interactions were critical: Raizorg + mycorrhizae maximized growth, whereas Raizorg + Rhizobium yielded the poorest performance.

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INTRODUCTION

Agriculture faces serious problems of soil degradation; intensive farming systems and the unbalanced use of fertilizers are the main factors causing nutrient imbalances, decreasing soil fertility, lowering productivity, and reducing food quality [1]. The transition to sustainable agricultural systems requires the integration of biological inputs that improve resource use efficiency and reduce dependence on chemicals [2]. In this context, alternative substrates, beneficial microorganisms, and biostimulants are emerging as fundamental pillars for strengthening agroecology [3]. A primary consideration for sustainable production and food security concerns the negative impact of global warming or climate change. The adverse impact of this crisis on food security stems from the changes in temperature extremes and the distribution of precipitation and atmospheric humidity that we have witnessed for years, which cause stress in crops.

To mitigate these adverse effects, it has been recommended to utilize and promote the natural mechanisms that plants possess to acclimate to environmental changes and cope with stress [1].

Strawberry cultivation (*Fragaria L.*) has grown in importance in Mexico due to its high commercial and nutritional value; the country is the world's third-largest exporter of fresh strawberries and supplies 87.79% of U.S. imports [4]. For this reason, it is necessary to expand the production of pathogen-free strawberry seedlings. The rationale is that healthy seedlings are obtained from a seedbed that provides the physical, chemical, and nutrient conditions necessary for their development, thereby ensuring significant yields [5]. However, the quality of the initial seedlings is a limiting factor in productivity, as it depends on a well-managed "nursery" with optimal conditions. Substrates directly influence root establishment and nutrient availability [6]. Local materials such as forest soil and worm castings offer advantages in moisture retention and organic matter content, reduce costs, and promote the circularity of agricultural resources [7].

Currently, the use of growing media, microorganisms, and biostimulants in various forms and

production purposes is only a fraction of the set of techniques and tools available to us during this transition toward sustainable agriculture [4]. Biostimulants are defined as organic materials or microorganisms that improve nutritional efficiency, quality traits, and plant growth; additionally, they provide protection against various stress conditions. The positive effect that biostimulants have on crops is mainly due to the bioactive compounds they contain, which directly influence improved plant growth [8]. A key consideration for sustainable production and food security concerns the adverse impact of global warming or climate change, which many scientists describe as a climate emergency. To mitigate these adverse effects, it has been recommended to utilize and promote the natural mechanisms that plants possess to acclimate to environmental changes and cope with stress [2].

For their part, plant growth-promoting microorganisms, such as mycorrhizae, improve phosphorus uptake and confer tolerance to abiotic stress [9]. Rhizobacteria, although traditionally associated with legumes, have also shown positive effects on non-legume crops, including strawberries [10]. Furthermore, biostimulants contain bioactive compounds that modulate plant physiology, enhance rooting, photosynthesis, and biomass synthesis [2-8]. Microbial communities in soils drive between 80 and 90% of the biological processes occurring in the soil, due to their multiple ecological niches, including the mitigation of exogenous disturbances, promotion of plant growth, biocontrol activity, nutrient cycling, plant biomass production, soil structure and fertility, and the degradation of toxic compounds [11]. Recent studies have shown that inoculation with *Rhizobium* and *Phyllobacterium* increases the number of runners, fruit size, and vitamin C concentration in strawberries [10]. For their part, substrates directly influence root anchorage and nutrient availability [6]. Local materials such as forest soil and worm castings offer advantages in moisture retention and organic matter content, reduce costs, and promote the circularity of agricultural resources [7]. According to [7], forest litter, also known as forest mulch, reduces solar radiation, decreases surface evapotranspiration, and increases organic matter and biological activity near the surface, promoting greater root proliferation. For its part, worm castings provide N (1–2.6%), P (2–8%), K (1–2.25%), and a neutral pH

(6.8–7.2), making it a highly valued organic soil amendment [12].

Although agricultural soil has traditionally been used for direct cultivation, it can also serve as the main component in growing media mixtures for seedling production, provided it is supplemented with organic matter.

In this study, agricultural soil was collected at Ranchería Pamala in Comitán de Domínguez, Chiapas; it is of the loamy-clay-silt type, with good nutrient retention capacity but limited porosity and low organic matter content. Therefore, it was combined with vermicompost and forest soil to improve its physical structure, aeration, and biological activity, as recommended [7]. This approach allows for the use of local resources, reduces production costs, and promotes more sustainable production systems, especially in rural areas where commercial substrates (such as peat or coconut fiber) are difficult to access or economically unfeasible [5,6]. In Chiapas, agroclimatic conditions are suitable for strawberry cultivation [13], but challenges persist regarding the low quality of seedlings and the excessive use of agrochemicals in many parts of the country [4]. Although there is a history of the individual use of these inputs, few integrated studies have evaluated their interactions under local conditions.

Therefore, the objective of this study was to evaluate the effect of different substrates, microorganisms, and biostimulants on the development of strawberry seedlings.

METHODS

Methodology

The experiment was conducted under shade net conditions at the Lab-ITA of the Comitán Institute of Technology (16°13'31"N, 92°05'21"W; 1,582 m a.s.l.), as shown in Figure 1.

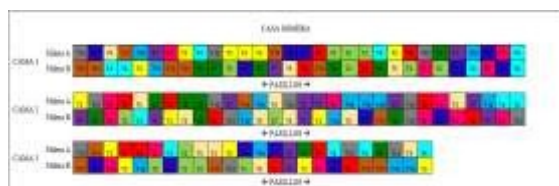


Figure 1. Sketch of the experimental design.
Source: Author's own work (2026)

A completely randomized design with a 3x2x2 factorial arrangement was used, where factor A was substrate (S1: 10% humus + 70% agricultural soil + 20% forest soil; S2: 20% humus + 60% agricultural soil + 20% forest soil; S3: 10% humus +

(80% agricultural soil + 10% forest soil), biostimulant factor B (Raizorg® and GreenFish®), and microorganism factor C (mycorrhizae, Rhizobium). A total of 12 treatments were generated, each with 14 replicates, for a total of 168 experimental units; each unit consisted of a 17x17 cm polyethylene bag containing one plant. The list of treatments evaluated is shown in Table 2.

Table 1. List of evaluated treatments.

Treatments	Abbreviation
(T1) Substrate 1 + Greenfish + Mycorrhizae	S1 + B1 + M1
(T2) Substrate 1 + Greenfish + Rhizobium	S1 + B1 + M2
(T3) Substrate 1 + Raizorg + Mycorrhizae	S1 + B2 + M1
(T4) Substrate 1 + Raizorg + Rhizobium	S1 + B2 + M2
(T5) Substrate 2 + Greenfish + Mycorrhizae	S2 + B1 + M1
(T6) Substrate 2 + Greenfish + Rhizobium	S2 + B1 + M2
(T7) Substrate 2 + Raizorg + Mycorrhizae	S2 + B2 + M1
(T8) Substrate 2 + Raizorg + Rhizobium	S2 + B2 + M2
(T9) Substrate 3 + Greenfish + Mycorrhizae	S3 + B1 + M1
(T10) Substrate 3 + Greenfish + Rhizobium	S3 + B1 + M2
(T11) Substrate 3 + Raizorg + Mycorrhizae	S3 + B2 + M1
(T12) Substrate 3 + Raizorg + Rhizobium	S3 + B2 + M2

Source: Author's own work (2026)

Description of the genetic material

The variety used was Albión, which belongs to a group with a lower response to photoperiod; that is, it requires adequate temperatures (above 12°C in the soil) to develop flower buds [13].

This variety is highly resistant to adverse weather conditions and diseases such as anthracnose, Verticillium, and Phytophthora, as well as pests such as spider mites [13]. Additionally, it exhibits slow but steady initial growth, with deep red fruits, firm flesh, and high sugar content (10–14° Brix)—desirable characteristics for international markets [14].

Crop Management or Experiment

Prior to establishing the experiment, the area was weeded by applying herbicides (glyphosate and paraquat at 10 mL·L⁻¹ and atrazine at 1 mL·L⁻¹); days later, it was covered with ground cover. Strawberry runners of the Albión variety were collected from mother plants at the Lab-ITA of the Comitán Institute of Technology and, prior to transplanting to the nursery, were soaked in a rooting solution (Nutri Root, Alga Root, Fulvidal, and Turbo Amino). They were subsequently transplanted to a Lab-ITA nursery bed and fertilized with a 20-30-10 fertilizer at 5000 ppm one week after planting. As a preventive measure, two foliar applications of Azubec (10 mL·L⁻¹) were made.

Substrate Preparation

The substrates used were collected from specific areas: forest soil in Independencia, Chiapas; worm castings purchased from a producer in San Antonio el Milagro, Comitán; and agricultural soil (loamy-clay-silt) from Ranchería Pamala, also in the municipality of Comitán, which is of the same type. All substrates were sieved to remove impurities and mixed in defined proportions:

- S1: 10% humus + 70% agricultural soil + 20% forest soil
- S2: 20% humus + 60% agricultural soil + 20% forest soil
- S3: 10% humus + 80% agricultural soil + 10% forest soil

Each experimental unit consisted of a 17x17 cm polyethylene bag, weighed on a Truper digital scale (5 kg capacity) to ensure uniform volume, into which the previously acclimatized stolons were immediately transplanted. Irrigation was provided via a drip system (one dripper per bag).

Initial fertilization was performed 13 days after transplanting with an N-P-K ratio of 2-3-1 (MAP, NKS, and Aporta N-30), followed by a 3-1-1 ratio starting in week 4.

Pest control

Nine yellow sticky traps coated with honey were installed. , insecticides

(cypermethrin, imidacloprid, spirotetramat) and fungicides (Trikoplant®, Serenade®, carbendazim/Benomyl, Curabac, and ExterBac) were applied preventively and curatively. Notable among the monitored pests were thrips (*Frankliniella spp.*), spider mites (*Tetranychus urticae*), and whiteflies (*Bemisia tabaci*), while diseases included Botrytis cinerea, *Phytophthora cactorum*, and anthracnose (*Colletotrichum acutatum*).

Treatment Application Program

Applications of biostimulants and microorganisms were carried out according to specific schedules: Raizorg® via drench, 1–2 L·ha⁻¹ every 10 days; GreenFish® in alternating foliar/drench applications at 2 L·ha⁻¹ every 10 days; mycorrhizae at 3 kg·ha⁻¹ applied 16 days after transplanting, with top-dressing every 10 days; and *Rhizobium* at 1 L in 20 L of water (pH adjusted), applied every 20 days via drench.

Evaluated variables

At 61–65 days after transplanting, the following were measured: number of leaves, crown diameter (digital caliper), plant height using a (ruler), root length, and root volume—the latter was determined using the water displacement method described by [15]. To determine fresh and dry biomass, plants were weighed on an Adam LAB 254i analytical balance; and were placed in a Memmert Universal UN oven for 72 hours at 65 °C until a constant weight was reached, following the methodology of [16]. Finally, the percentage of mycorrhizal colonization was determined using the method of [17]; and to quantify the percentage of mycorrhizal colonization, the method of [18] was used with the following formula: Colonization (%) = $\frac{\text{Colonized segments}}{\text{Observed segments}} \times 100$.

Statistical Analysis

The data were analyzed using R software [19], ANOVA, and Tukey's test ($p \leq 0.05$ and 0.10) [20]; the assumptions of normality (Shapiro-Wilk) and homoscedasticity of variances (Levene) were verified.

DISCUSSION AND ANALYSIS OF RESULTS

The results of the experiment confirm that the vegetative development of the seedlings does not respond additively to the evaluated factors, but rather depends critically on their specific interactions.

Figure 2 ($\alpha = 0.05$) shows a significant interaction between biostimulant and microorganism (fb x fc) in dry weight. The crossed lines indicate that the effect of Raizorg depends on the type of microorganism applied; with mycorrhizae, Raizorg

increasing height to 15.38 cm and dry weight to 6.88 g (T7); however, when applied together with Rhizobium, it reduces biomass to 1.30 g in dry weight (T4).

This incompatibility between Raizorg and the Rhizobium cocktail suggests possible physiological competition or functional antagonism in non-leguminous crops. This is consistent with

specialization of Rhizobium in nodule formation in legumes, where it fixes atmospheric nitrogen. In strawberries, a non-nodulating species, its growth-promoting effect is limited compared to mycorrhizae, which form symbiotic structures (arbuscules) that expand the root absorption surface [21],

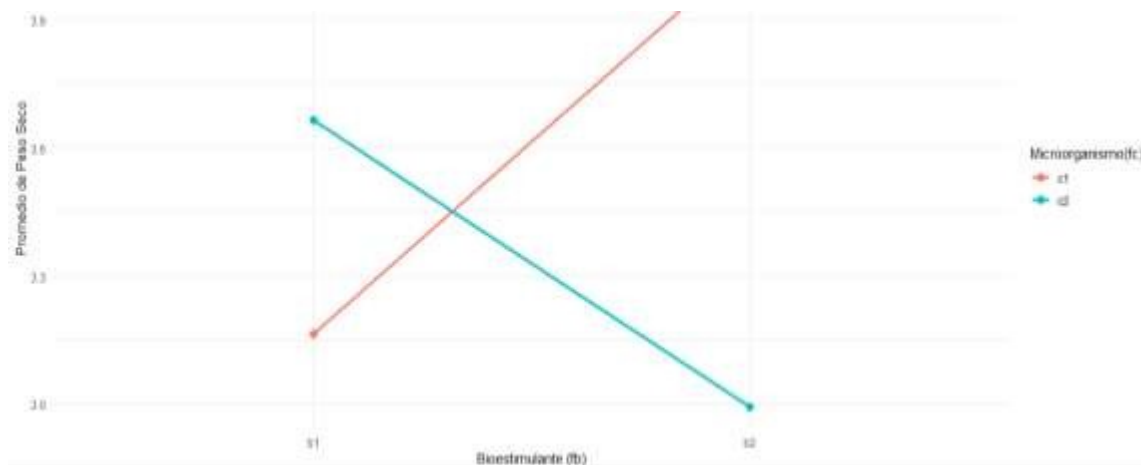


Figure 2. Interaction between biostimulant (fb) and microorganism (fc) on the average dry weight of strawberry seedlings.

Source: Author's own work (2026).

The superiority of mycorrhizae ($p = 0.0284$ for plant height) supports the findings reported by [21], who notes that the strawberry is a highly mycotrophic species whose physiological response to Rhizobium is limited, given that this genus is specialized for legumes [22].

Rhizobium maximizes root volume (8.20 cm^3 in T6), as reported by [23]. In contrast, Raizorg + Rhizobium yielded the lowest values.

Similarly, Figure 3 ($\alpha = 0.10$) reveals that the GreenFish +

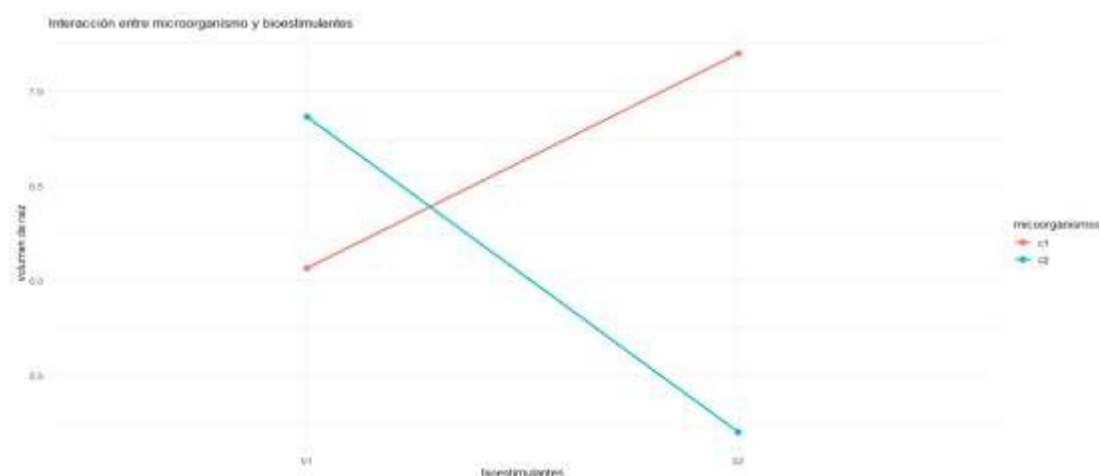


Figure 3. Interaction plot between Raizorg (B2) and Rhizobium (M2)

Source: Author's own work (2026)

Treatment 11 stood out in root length (20.92 cm) and mycorrhizal colonization (60%), suggesting a strategy of active soil exploration under conditions of lower organic matter availability. This finding is consistent with [24], who observed superior root responses in inoculated beans under saline stress.

Although statistical significance was not reached for the number of leaves ($p = 0.0764$), treatment T3 (S1

+ Raizorg + Mycorrhizae) recorded the highest average (12.77 leaves), while T4 (S1 + Raizorg + Rhizobium) was the lowest (10.22 leaves), suggesting that mycorrhizae may promote leaf development.

Table 2 presents the Pearson correlation matrix between morphological and biometric variables. These high correlations confirm a strong functional integration between the root and aboveground systems: greater root volume facilitates water and nutrient uptake, as demonstrated in [25], which in turn results in greater aboveground biomass, as demonstrated in [26]. However, mycorrhizal colonization did not correlate with root length ($r = 0.004$, $p = 0.973$), but it did show positive associations with above-ground variables (fresh weight = 0.45 and dry weight = 0.48), indicating that mycorrhizal symbiosis improves the functional efficiency of the root system rather than its physical extent, which is consistent with the literature on arbuscular mycorrhizal fungi (AMF), which highlights their role in optimizing resource use [26].

Table 2. Correlation among study variables

COEFICIENTES DE CORRELACIÓN								
Variable	Altura	dc	nh	pf	ps	vr	lr	Colonización
Altura	1.000	164	187	16	27	-47	-326	219
dc	164	1.000	651	454	462	318	431	-13
nh	187	651	1.000	492	410	265	296	-59
pf	16	454	492	1.000	958	943	590	138
ps	27	462	410	958	1.000	969	573	214
vr	-47	318	265	943	969	1.000	534	278
lr	-326	431	296	590	573	534	1.000	4
Colonización	219	-13	-59	138	214	278	4	1.000

Nota: dc=diámetro de corona; altura=altura de la planta; altura=altura; peso seco; vr=volúmenes de raíz; lr=longitud de raíz.

Source: Author's own work (2026).

CONCLUSIONS

The vegetative development of Albión strawberry seedlings does not respond additively to the individual application of inputs, but rather depends critically on the specific interactions between substrate, biostimulant, and microorganism. It is concluded that the combination of Raizorg® with arbuscular mycorrhizae maximizes growth

vegetative growth, with treatment T7 (S2 + Raizorg + Mycorrhizae) showing the highest values for height, number of leaves, and biomass (fresh and dry), while treatment T11 (S3 + Raizorg + Mycorrhizae) optimized root architecture with the greatest root length and colonization percentage (60%).

Substrate composition influenced biomass distribution: a higher humus content (S2) favored the aboveground portion, while a higher proportion of agricultural soil (S3) encouraged root exploration. Likewise, it was determined that mycorrhizae significantly outperformed Rhizobium spp. in promoting plant height and above-ground biomass, which is consistent with the non-leguminous nature of the strawberry. A physiological incompatibility or functional antagonism was identified between the biostimulant Raizorg® and Rhizobium, resulting in lower performance when combined, suggesting that this association should be avoided in strawberry crops. Finally, mycorrhizal colonization showed a positive correlation with above-ground variables, confirming that symbiosis improves the functional efficiency of the root system rather than its physical extent.

As future lines of research, it is recommended to evaluate the effect of the identified optimal treatments (T7 and T11) during the fruit production stage to determine whether the benefits observed in the seedling phase translate into higher yield and fruit quality. Furthermore, it is necessary to validate these results under field conditions that allow for the consideration of interactions with variable environmental factors.

Evaluate the effect of the identified optimal treatments (T7 and T11) during the fruit production stage to determine whether the benefits observed in the seedling phase translate into higher yield and fruit quality.

Conduct studies under field conditions that allow for the validation of the results obtained under controlled shade net conditions, taking into account interactions with variable environmental factors.

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TABLE OF CONTRIBUTION ROLES

Role	Author(s)
Conceptualization	Luis Arturo Solís Gordillo
Data Curation	Luis Adolfo García López "igual" Eric Ortega López "igual" Jorge Márquez Juárez "who supports."
Methodology	Luis Adolfo García López "same" Eric Ortega López "same" Luis Arturo Solís Gordillo "same" Jorge Márquez Juárez "who supports"

	Sergio Espinosa Velasco "who supports"
Project Management	Luis Adolfo García López "same" Eric Ortega López "same"
Resources	Luis Adolfo García López "same" Eric Ortega López "same" Luis Arturo Solís Gordillo "same"
Software	Jorge Márquez Juárez
Supervision	Luis Arturo Solís Gordillo "same" Jorge Márquez Juárez "same" Sergio Espinosa Velasco "same"
Validation	Luis Arturo Solís Gordillo "Principal" Jorge Márquez Juárez "equal" Sergio Espinosa Velasco "equal"
Display	Luis Adolfo García López "same" Eric Ortega López "same" Luis Arturo Solís Gordillo "who supports"
Draft (Original Draft)	Luis Adolfo García López "same" Eric Ortega López "same"
Editorial Staff (Review and Editing)	Luis Adolfo García López "same" Eric Ortega López "same"

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