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Research Article

A case study of a profitable mid-tech greenhouse for the sustainable production of tomato, using a biofertilizer and a biofungicide

Leobardo Serrano-Carreón [a],*, Sergio Aranda-Ocampo [b], Karina A. Balderas-Ruíz [a], Antonio M. Juárez [c], Edibel Leyva [d], Mauricio A. Trujillo-Roldán [e], Norma A. Valdez-Cruz [e], Enrique Galindo [a],*

[a] *Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Avenida Universidad 2001, Colonia Chamilpa, Cuernavaca, 62210 Morelos, México*

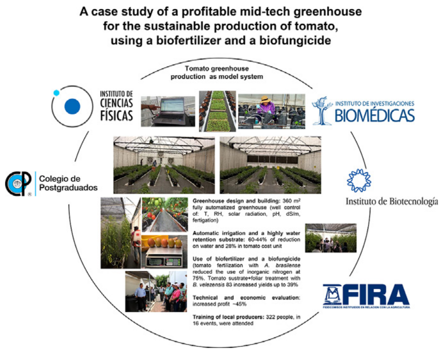
[b] *Posgrado en Fitosanidad-Fitopatología. Colegio de Postgraduados, Km 36.5 carretera México-Texcoco, C.P. 56230, Montecillo, Texcoco, Estado de México, México*

[c] *Instituto de Ciencias Físicas, Universidad Nacional Autónoma de México, P.O Box 48-3, 62251 Cuernavaca, Morelos, México*

[d] *Centro de Desarrollo Tecnológico Tezoyuca, Fideicomisos Instituidos en Relación con la Agricultura "FIRA", Km. 12.5 Carretera Jiutepec-Zacatepec, Crucero De Tezoyuca, Amatitlán, 62765 Emiliano Zapata, Morelos, México*

[e] *Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Av. Universidad 3000, Cd. Universitaria, Coyoacán, 04510, Ciudad de México, México*

GRAPHICAL ABSTRACT



A case study of a profitable mid-tech greenhouse for the sustainable production of tomato, using a biofertilizer and a biofungicide

Greenhouse design and building: 360 m^2 fully automatized greenhouse (well control of: T, RH, solar radiation, pH, dS/m, fertigation)

Automatic irrigation and a highly water retention substrate: 60-44% of reduction on water and 28% in tomato cost unit

Use of biofertilizer and a biofungicide (tomato fertilization with *A. brasilense* reduced the use of inorganic nitrogen at 75%. Tomato sustrate+foliar treatment with *B. velezensis* 83 increased yields up to 39%

Technical and economic evaluation: increased profit ~45%

Training of local producers: 322 people, in 16 events, were attended

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ABSTRACT

Background: Protected agriculture (PA) is an alternative allowing the control of environmental variables to produce healthy vegetables. Biofertilizers and biofungicides can reduce the chemical load of pesticides. There is abundant literature documenting individual aspects, such as control of environmental variables, irrigation, biological control, and cost assessments. However, there are no reports documenting integral approaches in which variables are considered altogether in a successful case study of mid-tech technology, suitable in middle-income countries like México. We tested if mid-tech greenhouses using biocontrol and biofertilization can increase profits, using tomato as a model system. This work provides considerations about middle-income countries' agriculture and the need for a multidisciplinary approach to offer cost-effective, sustainable alternatives to producers.
Results: This technology yielded up to 254 tons/ha·year of tomato, achieving reductions of 44–60% in water consumption, 25% in chemical nitrogen-fertilization, and 28% in the cost unit of production, increasing the profits by ~45% in relation to Mexican conventional greenhouses management.

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* Corresponding authors.
E-mail addresses: leobardo.serrano@ibt.unam.mx (L. Serrano-Carreón), enrique.galindo@ibt.unam.mx (E. Galindo).

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Pesticide reduction
Protected agriculture
Sustainable agriculture
Tomato

Conclusions: This case study has shown that it is possible to significantly increase profits in mid-tech greenhouse tomato production by increasing productivity and crop quality and decreasing the use of water and agrochemicals through greenhouse automatization, crop management, and beneficial bacteria applied to crops. **This manuscript includes a video**, supplementary to the main contributions of the project. **Please visit this URL:** https://youtu.be/uRBGgJqfkLE.

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1. Introduction

1.1. The need for better and sustainable practices in agriculture

Agriculture is the world's largest industry as it employs more than one billion people worldwide and generates over 1.3 trillion dollars' worth of food annually [1]. The so-called "green revolution" was characterized by intensive agriculture practices in developed countries where the abuse of the use of chemical fertilizers and pesticides, monoculture production, intensive irrigation, and deforestation were regular practices [2]. This approach led to water and soil pollution, pollinators' distress, pest resistance, and human health problems. Irrigation now claims close to 70% of all freshwater appropriated for human use, and a 19% increase in agricultural water consumption is forecasted by 2050. Moreover, to meet food demand by 2050, worldwide production needs to increase by 70% [3]. Monoculture production can cause the accumulation of weeds and promote plant diseases and soil infertility due to a lack of crop rotation practices resulting in loss of soil nutrients and even deforestation [4]. Approximately 30–80% of the nitrogen applied to farmland lixiviate and contaminate water systems which, once into the oceans, cause, among other effects, the seaweed deluge hitting Caribbean shores [2]. Facing an increasing population expected to reach 9.6 billion people by 2050, industrial agriculture systems cannot ensure the availability of healthy and innocuous products to minimize the environmental, health, and social impacts. Therefore, the development of knowledge and techniques to attain sustainable agriculture practices is, in consequence, one of the biggest challenges of the 21st century [5]. One out of 17 Sustainable Development Goals (SDGs) of the 2030 Agenda for Sustainable Development is: "By 2030, to ensure sustainable food production systems and to implement resilient agricultural practices that increase productivity and production. It is also expected that sustainable agriculture practices may help reduce the damage in ecosystems and help maintain food production despite climate change, extreme weather, drought, flooding, and other disasters and that progressively it will improve land and soil quality" [6].

The Instituto Nacional de Estadística y Geografía (INEGI) of México reported in the National Agricultural Survey (Encuesta Nacional Agropecuaria, ENA) of 2017 that 110 millions of hectares (ha) were dedicated to agriculture in México in 2017, 79% of which correspond to irrigated land [7]. Farming land was distributed in 101,828 production units, from which 83% were productive open-air systems. Most of the productive units employed irrigation by gravity (70.8%), chemical fertilization (68.2%), chemical herbicides (66.9%), and pesticides (54.8%). In contrast, only 17,338 production units (17% of the total) corresponded to protected agriculture (PA) systems, most of them greenhouses (54%). Only 30.8% of the PA productive units used fertigation [7]. There is substantial room for improvement of Mexican farm productivity and the introduction of sustainable production systems.

1.2. The key aspects for achieving high quality, innocuous and sustainable production while maximizing the return on investment (the case of tomato)

Tomato is currently the most profitable agricultural product that México exports to the USA, accounting for almost 30% of its national production. According to the "Servicio de Información Agroalimentaria y Pesquera" (SIAP) of México the area planted with tomato in México for the agricultural year (AY) 2020 (October 2019-March 2021) was estimated at 45,284 ha, slightly lower compared to AY 2019 (47,372 ha) [8]. To produce this vegetable, different resources are required: water, fertilizers, and pesticides, both of synthetic and organic (or biological) origin; seeds, substrates, energy, plastics, and in the high-end producers, automated sensors and controls that help to achieve an efficient production [9]. The use of each of these resources must be analyzed in relation to its social, economic, and environmental impacts [9,10].

The assessment of these impacts is necessary to promote the adoption of good practices by the producer in PA and agribusiness and minimize the impact on the environment. Moreover, the final product must comply with the quality and innocuity requirements of the final consumer [10]. The current importing policies by regulatory agencies established for food in countries such as México impose reliable evidence that improvements have been achieved in the following aspects [11]:

- Water productivity (more kilograms of tomatoes per cubic meter of water)
- Reduction in the use of synthetic fertilizers and reduction in the chemical load of agro-toxic inputs
- Energy efficiency (more Kg of product per KWh)
- Reduction in carbon footprint
- Use of biodegradable and efficient plastics
- Waste reduction in irrigation supplements, ferrous waste, bags, substrates, disinfectants, packing boxes, among others

1.3. Protected agriculture: state of the art and Mexican situation

PA refers to buildings, sensors, actuators, and software that allow controlling the environmental variables and watering of crops to increase their yields, reduce water consumption, and increase profits [12]. Traditionally, this control and monitoring have been achieved using robust and well standardized Programmable Logic Circuits (PLC), local control software, and a limited number of sensors. However, due to its high installation and maintenance costs, its use by farms is limited [13].

The technification of tomato crops in emerging economies, such as México, is diverse. According to the United States Department of Agriculture (USDA), 44,814 planted ha of tomato were reported for AY 2020 [14]; the planted area for tomato production was distributed in open-field (66.19%), greenhouse (16.12%), shade mesh

(16.97%) and tunnel (0.72%) technologies. SIAP reported the yields for tomato production for AY 2020 in open-field was 37 tons/ha·-year, greenhouse 185 ton/ha·year, shade mesh 113 ton/ha·year, and tunnel 73 ton/ha·year [8]. These data reflect the impact of tomato production under PA techniques. Nevertheless, the implementation of technology in greenhouses in México as in Latin America is scarce [15,16,17]. This is because the technological transfer between universities and companies is limited, coupled with a poor entrepreneurship culture and the high costs of technification. In this context, a project of medium-tech development such as the one we are presenting here aims to contribute with a system that is both technologically affordable and, at the same time, economically viable for the specific context and needs of producers located in emerging economies such as that of México or Latin America.

1.4. The need of a multidisciplinary approach with cost/benefits considerations

When the multidisciplinary team that carried out the present work was formed, the need for proprietary and original developments was discussed, around the needs of the medium-technical Mexican producers. This was possible due to the plurality of capabilities that this multidisciplinary team possesses in which experts in the areas of agronomy, biotechnology, phytopathology, and process automation were involved (Fig. 1). The participation of FIRA, a financial institution from the Bank of México responsible for technological training and financial services to support Mexican agriculture development, was a critical factor to define technological objectives, economic assessment of the developed technologies, and the possibility of an effective technological transfer. Although there are works dedicated to instrumentation [13,18,19], phytopathological aspects [20,21,22], or biotechnology [23,24,25, 26,27], as far as we know there are no documented experiences of multidisciplinary teams that cover physical, biological, and economic considerations altogether. Furthermore, the evaluation of production costs is rarely considered for projects that involve multiple aspects of crop production and commercialization. We believe that this is a crucial reason which explains why many developments, although technically robust, do not get to the market for solving concrete problems for the producer. The hypothesis of this work was: “Mid-tech greenhouse incorporating biocontrol and biofertilization increase growers’ profits in middle-income countries as México.”.

2. The model of study and main objectives

2.1. Tomato as an experimental model

Tomato production area under PA has grown from 1,078 hectares in 2006 to 15,006 hectares in 2016, which means an average annual increase of 30%; while tomato production under PA increased from 6.5% to 60.7% of the total [28]. In 2020 México produced 3.3 million metric tons and almost 99.7% of the Mexican exports went to the United States. Of these, 40% of tomato produced was grown in greenhouses with only 16% of the total cultivated area (44,814 ha) and an annual average yield of 180 mt/ha *vs* 36.8 mt/ha obtained in open-field [14]. Tomato occupies a third of the entire national infrastructure under PA and constitutes a business where small-scale producers participate from less than one to more than 1,000 ha. The profitability of this crop can be measured by some variables that can determine the stagnation, stability, or business success of the participants. These are, among others: a) the size of the production unit, b) the infrastructure and technology used, c) the technological production plans, and d) the market environment. These factors are of particular importance if the producer has a commercial relationship with a market with stringent quality and quantity demands. To meet this demand with competitive production costs, it is necessary to enhance the production yield making use of mid-tech greenhouse infrastructure and environmental and biocontrol techniques.

In the present project, we used a tomato (*Solanum lycopersicum* L.) variety Frodo 1. We used coconut fiber (30% mixed with 70% of porous red volcanic rock) for the watering system to decrease water and fertilizer consumption. Also, to diminish the inorganic nitrogen consumption, we used *Azospirillum brasilense* as nitrogen-fixing bacteria and *Bacillus velezensis* 83 as the biological control agent (BCA) of *Leveillula taurica* (foliar and substrate

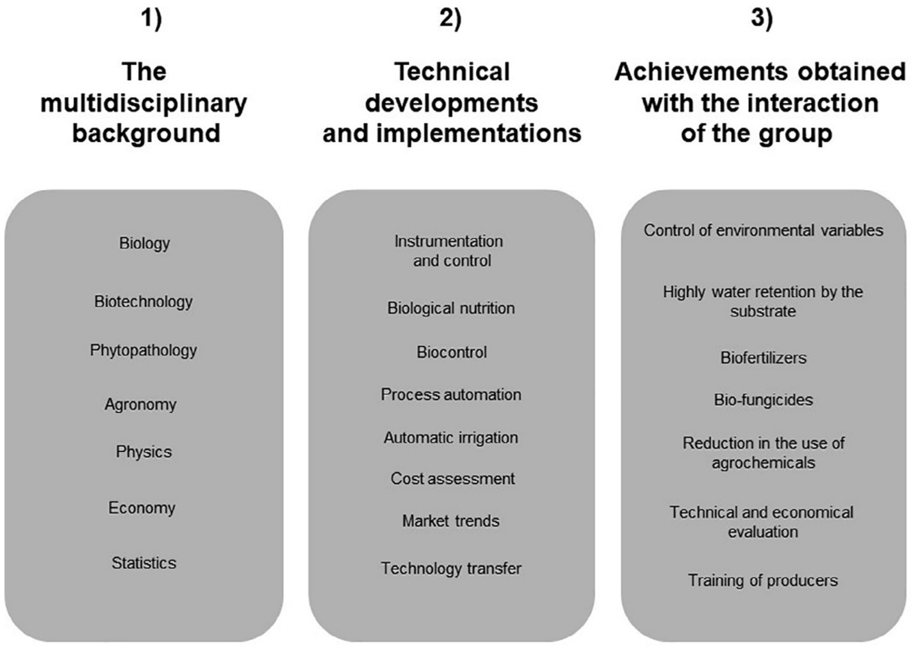


Fig. 1. Scheme of multidisciplinary team approaches and achievements for the technification of intermediate level in tomato crops. 1) The multidisciplinary background; 2) Technical developments and implementations; 3) Achievements obtained with the interaction of the group instrumentation.

applications). The crop was housed in an automated greenhouse with an automatic watering system, shading, and relative humidity monitoring and control.

The Frodo variety is recommended for growing tomatoes for commercial use. It produces medium cylindrical fruit with an intense red color that does not tend to break and, therefore, can be easily transported. Its productive cultivation can be extended for more than six months. This variety is also considered one of the earlier industrial varieties, as it can be harvested as soon as 66–75 d after transplanting the seedlings.

2.2. The pathosystem tomato-powdery mildew, biofertilizers and biofungicides

This project aimed to evaluate the yield, quality, and profitability of tomato crops housed in a mid-tech greenhouse production system using reduced amounts of agrochemicals to control fungal diseases. The results were compared to conventional greenhouse technological production management systems, which are highly dependent on agrochemicals. The pathosystem tomato-powdery mildew was selected as a model to evaluate a biofertilizer (*A. brasilense*) and a biofungicide (*B. velezensis* 83) to control this fungal disease. Powdery mildew is caused by various fungal species which affect leaves, stems, flowers, and fruits of Angiosperms; in the world, about 16 genera (900 species) are known [29,30]. *L. taurica* (Lév.) G. Arnaud is a strict parasite endophytic fungus not cultivable on artificial culture medium, the major pathogen of tomato and other Solanaceae, Alliaceae, and Cucurbitaceae plant families [31,32]. *L. taurica* infections in tomato field crops have been reported to cause yield losses of 52% as well as adverse effects on quality fruit [33].

There are no *L. taurica* resistant cultivars available in the market. The control of this pathogen in tomato crops is conventionally carried out by spraying fungicides such as wettable sulfur, myclobutanil, and azoxystrobin [33,34]. *Bacillus* spp. has been applied to tomato plants to stimulate plant growth and control different phytopathogens. As BCA, strains of *B. subtilis* MBI600 and *B. amyloliquefaciens* SQRT3 cause Induced Systemic Resistance (ISR) in tomato plants grown in greenhouse against soil-borne tomato pathogens (as *Rhizoctonia solani*, *Pythium ultimum,* and *Fusarium oxysporum* f. sp. *radicis-lycopersici-Forl*) and *Ralstonia solanacearum* (tomato bacterial wilt), respectively [35,36]. As for PGPB, the inoculation of *Bacillus fortis* and *Bacillus subtilis* on tomato plants increased the plant's root and shoot biomass and crop productivity [37]. The inoculation of *B. subtilis* in tomato variety Licurich and Moldova also increased tomato productivity [38].

Powdery mildew disease is recurrent in the experimental zone; because of this, *B. velezensis* 83 was applied on the growth substrate and foliar spray. Moreover, to increase productivity in tomato crops and reduce the amount of inorganic nitrogen administered, inoculants of *Azospirillum brasilense* have been used. In fact, inoculation of *Azospirillum* sp. as Plant Growth Promoting Bacteria (PGPB) on tomato varieties reduced transplant stress, increased yields, and diminished chemical fertilizers [39]. The overall aim of this work was to test if a mid-tech greenhouse incorporating biocontrol and biofertilization can increase growers' profits in middle-income countries as México.

3. Methodology

The materials and methods of the project were as described with more detail in a previous work [40]. These involved greenhouse technification, environment control, biofertilization, biological control, production, and considering the substrate that improves the decrease in water use. A summary is included in what follows.

3.1. Materials

The support germination growing media was a commercial Peat Moss-based medium (Sunshine Mix 3, Sun Gro Horticulture, Agawam, MA). The tomato (*Solanum lycopersicum L.*) seeds var. Frodo (Hybrid Tomato, ITSCO, CdMx, México) were sown in pots. Tomato seeds were germinated in the presence of *A. brasilense* at 18–28°C. For biological control *B. velezensis* 83 (accession number LMG S-30921; Fungifree AB™ obtained from Agro&Biotecnia S. de R.L. de C.V.) was used.

3.2. Tomato seed management and treatments with biofertilizer and biological control

Formulations of *B. velezensis* 83 and *A. brasilense* are already available in the Mexican market. Therefore, the *A. brasilense* liquid inoculant was used as a biofertilizer to diminish the nitrogen load and designed to evaluate the effect of nitrogen fixation associated with this product [23]. *A. brasilense* was used during the germination and at the transplantation phases, under a complete nutrition scheme, in which inorganic nitrogen was reduced by 25%. The germination of tomato (Frodo variety) took 21 d when was pre-inoculated with *A. brasilense* (without *A. brasilense* germination took 28 d).

The set of experiments consisted of three production cycles. In each cycle, 14 treatments were evaluated using 34 pots each. The cultivation cycles included between 150 and 160 days, with three harvest months. We used mixed coconut (30%) and porous red volcanic rock (70%) as a highly water-retention substrate. The seedlings were transplanted to 15-liter plastic pots containing the substrate described. Two seedlings were placed per pot, each pot was considered as an experimental unit. Statistical analysis was performed with the average value of production in terms of Kg/pot. The Kg/plant was calculated by dividing the registered value (Kg/pot) by 2. The density of the crop was 2.8 plants/m^2. Two cycles were supposed to calculate the tons/ha year tomato production. For the statistical analysis of the data, Minitab™ 17 Statistical Software (Minitab, LLC, Pennsylvania, USA) was used. The normality test of the data distribution was performed with the Kolmogorov–Smirnov method (α = 0.05) and the test of equality of variances with the Bartlett method (α = 0.05). Since the data showed a normal distribution but there was not equality of variances a Welch's test (α = 0.05) was performed assuming samples without equal variances, followed by the Games-Howell (α = 0.05) as the Post Hoc test.

3.3. Mid-Tech systematization and greenhouse startup

Two fully automated greenhouses of 360 m^2 were built at FIRA (Tezoyuca, México). The greenhouses were equipped with a wet wall on the northern side, three exhaust fans on the southern side, active ventilation walls (eastern and western sides), and twelve in-house monitoring points, each equipped with sensors of temperature, relative humidity (RH), solar radiation (environmental parameters) as well as pH, electric conductivity, and moisture of the substrate (fertigation). Since the technological level of the greenhouse development intended for this project corresponded to middle technology, no attempt was made to create a microclimate. These would have taken the project out of the economic constraints that the experts in costs indicated would correspond to the level of technology and resources that were characteristic of the local producers. As such, the controllers installed for shading, wet walls, and extraction fans had the purpose of limiting the temperature changes that are experienced from day to night cycles in the geographic region where the project was implemented. To make a quantitative assessment of this control, it is worth comparing data in the greenhouse, as measured in the range

Table 1
Nutritional requirements of the tomato crop (*Solanum lycopersicum L.*) in parts per million (ppm) by phenological stage proposed by FIRA staff*.

Steiner Nutritive solution used by phenological stage (ppm)													
Phenological state	(dS/m)	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo
Transplant	0.5	42	8	68	45	12	28	3	0.5	0.05	0.5	0.025	0.002
Vegetative-Flowering	1.0	84	16	137	90	24	56						
Flowering – start of fruiting	1.5	126	23	205	135	36	84						
	2.0	168	31	273	180	48	112						
fruiting – 1st harvest	2.5	210	39	341	225	60	140						
Harvest	3.0	252	47	410	270	72	168						

(dS/m): electrical conductivity (decisiemens per meter).
*Taken from reference [40].

from 2016 to 2017 with averages provided by a Weather station of the Mexican Comisión Nacional del Agua (CONAGUA) [41]. We registered a range of temperatures from 18.8 to 36°C in August of 2016 and between 14 and 34°C in August 2017. The closest weather station (Alpuyeca, México) reports an average in that region ranging from a minimum of 9°C to a maximum of 40°C in the same month. Although the control was moderate, it was enough to keep the growing of tomatoes within a climate envelope that reduced temperature and humidity stress that leads to disease when extremes are reached.

An independent automated irrigation system per line of pots was designed. The irrigation system acted depending on the soil variables to be controlled (pH, osmolarity, and programmed nutrition). The environmental variable monitoring system consisted of twelve monitoring points to measure the temperature, irradiation, and RH. Each monitoring box transmitted the readings to a central panel to calculate the readings' averages and take the required control action.

3.4. Fertigation system

The fertigation inputs were decided to be pH 6.3–6.4 and output pH 7.5–8.1, and the electrical conductivity between 0.5 and 2.0 dS/m according to the plants' phenological stage and the recommendation of FIRA staff. In some lines, the fertigation system was designed to supply 50% of the nitrogen load to a set of pots, another set with 75% nitrogen, and the rest with the conventional nutritional load. In Table 1 (reproduced from reference [40]) are shown the nutritional requirements of the tomato crop (*Solanum lycopersicum L.*) in parts per million (ppm) by phenological stage proposed by FIRA staff.

3.5. Infection of tomato plants by *L. taurica*

We promoted the infection of tomato plants in the greenhouse. Tomato plants infected with *L. taurica* were placed as inoculum sources inside the greenhouse.

4. The development of the project

4.1. Design, construction, implementation, and improvement of the greenhouse instrumentation

During the project's first year, the first main objective was to construct and automate a 360 m^2 arched greenhouse at FIRA (Tezoyuca, México). In 2017, a second 360 m^2 fully automatized greenhouse was built, with the exact technical specifications of the first greenhouse. The greenhouses were equipped with a wet

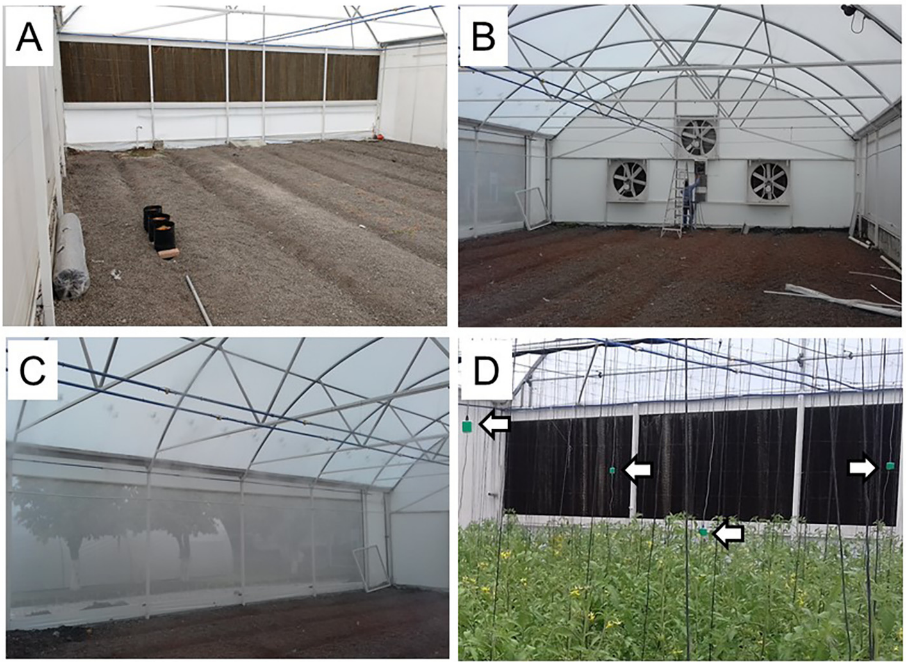


Fig. 2. Construction and automatization of a 360 m^2 arched PVC greenhouse at FIRA (Tezoyuca, México). The greenhouse was equipped with a wet wall on the northern side (A), three exhaust fans on the southern side (B), and active exterior roll walls (over the mesh walls) on the eastern and western sides (C). Twelve environmental monitoring points (temperature, relative humidity, and solar radiation, shown by arrows) were used (D). An automated pull wire mechanism of a horizontal screen was placed to decrease the luminosity and spray humidifiers were installed, both on the inside top of the greenhouses. Also, a particular fertigation system was designed to supply 50% of the nitrogen load to a set of pots, another set with 75% nitrogen, and the rest with the conventional nutritional load recommended by the FIRA experts (Table 1).

wall on the northern side, three exhaust fans on the southern side, active ventilation walls (eastern and western sides), and twelve in-house monitoring points, each equipped with sensors of temperature, relative humidity (RH), solar radiation (environmental parameters) as well as pH, electric conductivity, and moisture of the substrate (fertigation) (Fig. 2). Variables' acquisition was programmed at 15 min intervals, and control of the variables was performed through Proportional-integral-derivative retrofitting algorithms. The validation of the system was carried out in parallel with the experimentation. Some operational and technical problems were raised, and they had to be solved during experimentation. It is important to point out that the time required to validate the instrumentation and the control of the greenhouse must be planned before the operation.

The instrumentation was divided into a subsystem on environmental monitoring (RH, temperature, and solar irradiation), a web-server subsystem (capable of monitoring and sending information remotely, developed in Java), and a subsystem of control that action the wet wall in the northern side, spray humidifiers on the roof, up to three exhaust fans at the southern side, active ventilation walls on eastern and western sides and open/close the automated shading (to control the environmental parameters). We also designed an independent subsystem for automatic irrigation, which acted depending on the soil variables to be controlled (pH, osmolarity, and programmed nutrition).

The environmental variable monitoring system was designed and built consisting of twelve monitoring points with four sensors each, which measured temperature, irradiation, and air RH. Each monitoring box transmitted the readings to a central panel to calculate the readings' averages and take the required control action. A day/night cyclical tendency of the temperature, RH and luminosity data was expected, and the control was programmed to avoid abrupt departures on extremely hot or cold days. Thus, it was possible to maintain statistically similar intervals during seasonal changes. The environmental conditions to produce tomatoes were between 14 and 34°C, RH in the range of 28–85%, and maximum light near 3,300 footcandles (~35,000 lux) (Fig. 3), following also the FIRA staff recommendations. Overall, the control systems allowed for maintaining the greenhouse within the above-mentioned recommended intervals.

4.2. Design and evaluation of the fertigation system

A particular fertigation system was designed to supply 50% of the nitrogen load to a set of pots, another set with 75% nitrogen, and the rest with the conventional nutritional load recommended by the FIRA experts (Table 1). The nitrogen load reduction was designed to evaluate the effect of nitrogen fixation associated with the liquid inoculant formulation of the *A. brasilense* [23]. It has been reported that the reduction in inorganic nitrogen in the formulation of fertigation improves the fixation capacity of *Azospirillum* in crops [25,42]. The fertigation inputs were decided to be pH 6.3–6.4 and output pH 7.5–8.1, and the electrical conductivity between 0.5 and 2.0 dS/m according to the plants' phenological stage and the recommendation FIRA staff. The cultivation cycles were between 150 and 160 days in this production system, with three harvest months. The use of coconut fiber, in combination with the monitoring of the conductivity in the substrate, allowed to reduce water consumption (among 44–60%) from 60 L of water per plant using 100% porous red volcanic rock to 34 L of water per plant using the mixed coconut (30%) and porous red volcanic rock (70%) as substrate in 15 L plastic pots. A reduction of ~28% in the tomato cost unit of production was achieved.

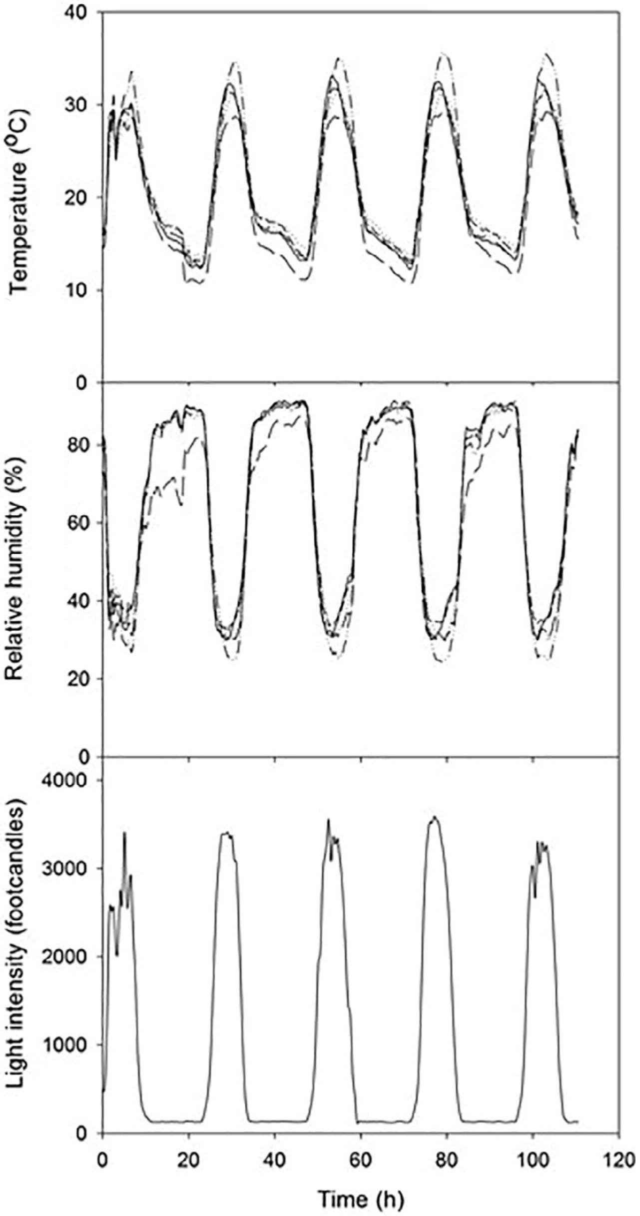


Fig. 3. As expected, a day/night cyclical tendency of the temperature, humidity, and luminosity data was obtained. The controllability was programmed to avoid abrupt departures on extremely hot or cold days. The controlled greenhouse was between 14 and 34°C (five sensors widely distributed), relative humidity between 25% and 85% (five sensors widely distributed), and a maximum light of 3,300 footcandles (the sensors were located inside the greenhouse, below the automated pull wire mechanism of the horizontal screen). The temperature and humidity sensor that deviates from the other four is the one that is close to the exhaust fans.

4.3. Design, implementation, and results of the experimental system

The experiments were designed to integrate and economically evaluate existing technologies to produce tomato variety (Frodo 1, Maviri). It is important to point out that formulations of *B. velezensis* 83 and *A. brasilense* are already available in the Mexican market. *B. velezensis* 83 is commercialized under the label of Fungifree AB™, a biofungicide effective against several fungal plant pathogens (*L. taurica*, among others) attacking more than 20 different crops [40,43]. A liquid formulation containing two combined *A.*

brasilense strains (Maxifer™) was used as a nitrogen fixation inoculant, successfully used for biofertilization of several crops in México [23]. We initially carried out two production cycles, and twenty-two treatments were evaluated (480 pots), where production from eight to twelve tomato harvests in each cycle was evaluated. Unfortunately, high variability within treatments avoided obtaining conclusive results. Indeed, 20 pots per treatment were not enough to get significant differences between treatments which were one of the main lessons obtained from this first set of experiments. Farmers frequently rely on total productivity to evaluate new products or technologies as the leading indicator. This is probably an adequate parameter when large experimental set-ups are available, but not in the case where only a few experimental units are available. Considering this first set of experiments, two additional production cycles were done, and reliable results were obtained. In each cycle, 14 treatments (34 pots each) were evaluated. *A. brasilense* was used at the germination and at the transplantation phases under a complete nutrition scheme, in which inorganic nitrogen was reduced by 25%.

4.4. Biological control of *L. taurica*

In the project, we promoted the infection of tomato plants in the greenhouse. Tomato plants infected with *L. taurica* were placed as inoculum sources inside the greenhouse, achieving infection two days after their introduction. Nevertheless, the incidence of powdery mildew was highly variable, and the experiment was not reproducible. No powdery mildew was detected in our conditions in any treatment (chemical or biological). So, it was not possible to evaluate the biological control of *L. taurica* by *B. velezensis* 83. However, the results showed that the use *A. brasilense* and *B. velezensis* 83 had a positive effect on total yield (~39% higher) and fruit quality (~55% more production of first quality) of tomato. In our experience, using this biofertilizer and biofungicide on tomato greenhouse production systems may be a viable alternative to obtain a higher yield and quality of tomato fruit without spraying synthetic fungicides to control powdery mildew. Our findings may be significant for some regions of México, where traditionally 13–18 applications of fungicides are made to prevent or control powdery mildew in intensive production systems in undetermined growth tomato varieties [34].

4.5. Tomato growth evaluations using biological treatments

The most relevant results of the last two consecutive tomato production cycles are reported here since the treatments included in the experimental design were refined according to each cycle experimented. The germination of tomato (Frodo variety) took 21 days and was pre-inoculated with *A. brasilense* (without *A. brasilense*, germination took 28 d). The seedlings were transplanted into 15 L plastic pots. Two seedlings were placed per pot, and the density of the crop was 2.8 plants/m^2.

For biological treatments, the commercial product Fungifree AB™ was used to evaluate the effect on the growth and yield of the tomato plants when applying *B. velezensis* 83 on the foliage as biofungicide and to the substrate as plant growth-promoting bacteria. Three biological treatments and non-inoculated plants control were evaluated. For each treatment with Fungifree AB™, ten applications were done to the foliage and six to the substrate. To evaluate the effect of the treatments on the quality, the harvested fruits were classified according to their weight in first (≥100 g/fruit), second (≤99-60 g/fruit), and third (≤59 g/fruit) quality.

In terms of plant growth, there were no significant differences between the treatments evaluated and the control. It was also found that there were no significant differences in tomato production when the plants received *Bv* 83 foliar or *Bv* 83 foliar + substrate low treatment with respect to control plants (3.3 Kg/plant), since these plants produced 3.5 and 3.8 Kg/plant, respectively. In contrast, in the *Bv* 83 foliar + substrate high treatment the plants produced 4.5 Kg/plant (Table 2). The estimated tomato yield with the *Bv* 83 foliar + substrate high treatment was 254 ton/ha·year, which represented almost 43% more than the average yield of a crop in greenhouse agriculture technology in México, which between 2007 and 2017 was of ~177 ton/ha·year [28]. Under the conditions in which tomato cultivation was developed in the greenhouse, this treatment increased the total tomato yield by 19% of first quality tomato, in contrast, to control plants (184 ton/ha·year). It is known that production yield always varies depending on the technologies used, from open-field cultivation to production in highly instrumented greenhouses with automated irrigation, nutrition, and phytosanitary control systems. As a referent, in México it is considered that the tomato yield production in low-tech greenhouses has yields of 120 ton/ha·year, in medium technology from 200 to 250 ton/ha, and in the high technology up to 600 ton/ha [28].

4.6. Economic analysis

For profitability estimation, the unit cost of production (UCP) of the greenhouse-grown tomato was calculated considering the average tomato yield (ton/ha·year) estimated in each case (Table 2 and Table 3). The cost of production involves the variable and the fixed costs. The variable costs were constituted by the cost of inputs (seed, agrochemicals, fertilization) and the direct labor cost. The fixed expenses were included by the price of accessories and tools for cultural activities, services (greenhouse rent, amortization of fixed initial investment), and technical assistance for tomato crop management. The cost of the fixed initial investment included the price of a plastic wall, irrigation equipment, and the structure of the greenhouse, the total capital needed was estimated at USD 125,000.00. The amortization was calculated considering a financing interest of 12%, paid for ten years. A sale price of 0.5 USD/Kg was considered [44]. The treatment with the highest profitability was the *Bv* 83 foliar + substrate high, while the one with the lowest profitability was the control (Table 3). The technical–economic study of the treatments showed that the UCP was 38% higher in control plants compared to the best biological treatment applied. Due to the higher yields obtained with the *Bv* 83 foliar + substrate

Table 2
Greenhouse tomato production with different *B. velezensis* 83 biological treatment.

Treatment	Tomato		
	Kg/plant (±SD)	Kg/m^2/cycle	Ton/ha·year
Bv 83 foliar ($6.7\times10^7 < 1.3\times10^8$ CFU/plant)	3.5^b (±0.9)	9.8	196.5
Bv 83 foliar + substrate low ($6.7\times10^7 < 1.3\times10^8$ CFU/plant + 1×10^6 CFU/plant)	3.8^b (±0.9)	10.5	210.6
Bv 83 foliar + substrate high ($6.7\times10^7 < 1.3\times10^8$ CFU/plant + 1×10^8 CFU/plant)	4.5^a (±1.4)	12.7	254.4
Control (non-inoculated plants)	3.3^b (±0.8)	9.1	183.6

Different letters mean significant differences according to Welch's (α = 0.05) and Games-Howell (α = 0.05) test.

Table 3
Profitability of greenhouse tomato grown with different *B. velezensis* 83 biological treatments.

Treatments	*Bv* 83 foliar	*Bv* 83 foliar + substrate low	*Bv* 83 foliar + substrate high	Control (non-inoculated)
Tomato (Kg/plant)	**3.5^{b}**	**3.7^{b}**	**4.5^{a}**	**3.3^{b}**
UCP (USD/Kg of tomato)	**0.33**	**0.37**	**0.30**	**0.42**
VARIABLE COSTS	***0.1881***	***0.2349***	***0.1995***	***0.2640***
SEED (Tomate saladette)	**0.0310**	**0.0289**	**0.0240**	**0.0332**
AGROCHEMICALS	**0.0388**	**0.0363**	**0.0350**	**0.0363**
Insecticide	**0.0049**	**0.0045**	**0.0038**	**0.0052**
Conventional	0.0043	0.0040	0.0033	0.0046
Neonicotinoides	0.0003	0.0003	0.0002	0.0003
Pyririproxyfen	0.0015	0.0014	0.0012	0.0016
Flupyradifurone	0.0025	0.0023	0.0019	0.0027
Organic	0.0006	0.0005	0.0004	0.0006
Soybean oil	0.0004	0.0003	0.0003	0.0004
Argemonine and berberine extracts	0.0001	0.0001	0.0001	0.0001
Soap	0.0001	0.0001	0.0001	0.0001
Fungicide	**0.0057**	**0.0053**	**0.0095**	**0.0009**
Conventional	0.0002	0.0002	0.0001	0.0009
Carbamates (Previcur energy™)	0.0002	0.0002	0.0001	0.0002
Sulfur (Velsul 725™)	-	-	-	0.0007
Biological	0.0055	0.0052	0.0093	-
B. velezensis 83 (Fungifree A&B ™)	0.0055	0.0052	0.0093	-
Bactericide	**0.0003**	**0.0003**	**0.0002**	**0.0003**
Conventional	0.0003	0.0003	0.0002	0.0003
Quaternary ammonium salts	0.0003	0.0003	0.0002	0.0003
Biostimulant	**0.0197**	**0.0184**	**0.0152**	**0.0211**
Root	0.0021	0.0019	0.0016	0.0022
1-Naphthylacetic acid (ANA) + Indole 3-butyric acid (IBA)	0.0006	0.0006	0.0005	0.0006
Cytokinins + Auxins	0.0002	0.0002	0.0002	0.0002
Indolebutyric Acid	0.0003	0.0002	0.0002	0.0003
N-P-K + Amino acids	0.0004	0.0004	0.0003	0.0005
Trace elements	0.0005	0.0005	0.0004	0.0006
Foliage	0.0037	0.0034	0.0028	0.0039
N-P-K (20–30-10)	0.0008	0.0007	0.0006	0.0008
N-K-C org	0.0013	0.0012	0.0010	0.0014
N-K-C org + Fe	0.0014	0.0013	0.0011	0.0015
B + Cu + Fe	0.0000	0.0000	0.0000	0.0000
Chelating agents	0.0002	0.0001	0.0001	0.0002
Fruit	0.0140	0.0130	0.0108	0.0150
Ca + B + amino acids	0.0007	0.0006	0.0005	0.0007
Calcium	0.0003	0.0002	0.0002	0.0003
Free amino acids	0.0018	0.0016	0.0014	0.0019
Boron	0.0001	0.0001	0.0001	0.0001
N-P-K (5–15-45)	0.0007	0.0006	0.0005	0.0007
Cyt + Gibb + Aux + Vitamins	0.0040	0.0037	0.0031	0.0043
Cytokinins	0.0065	0.0061	0.0051	0.0070
Nutrients assimilation	**0.0038**	**0.0035**	**0.0029**	**0.0040**
Organic complexes	0.0038	0.0035	0.0029	0.0040
Fulvic acid	0.0038	0.0035	0.0029	0.0040
Acidifyant	**0.0045**	**0.0042**	**0.0035**	**0.0048**
Inorganic acid	0.0045	0.0042	0.0035	0.0048
FERTILIZATION	**0.1116**	**0.1042**	**0.0862**	**0.1195**
Conventional	0.1116	0.1042	0.0862	0.1195
Nitrogenous	0.0538	0.0502	0.0416	0.0576
$Ca(NO_3)_2$	0.0216	0.0201	0.0167	0.0231
KNO_3	0.0323	0.0301	0.0249	0.0346
Phosphate	0.0123	0.0114	0.0095	0.0131
KH_2PO_4	0.0123	0.0114	0.0095	0.0131
Potassium	0.0060	0.0056	0.0046	0.0064
K_2SO_4	0.0060	0.0056	0.0046	0.0064
Complexes	0.0113	0.0106	0.0088	0.0121
Trace elements	0.0113	0.0106	0.0088	0.0121
Other compounds	0.0282	0.0263	0.0218	0.0302
$MgSO_4$	0.0043	0.0040	0.0033	0.0046
Fe	0.0091	0.0085	0.0071	0.0098
B	0.0011	0.0011	0.0009	0.0012
H_3PO_4	0.0131	0.0123	0.0101	0.0141
H_2SO_4	0.0005	0.0004	0.0004	0.0005
Other	**0.0006**	**0.0005**	**0.0004**	**0.0004**
Pest monitoring material	0.0004	0.0004	0.0003	0.0003
Plastic glue	0.0001	0.0001	0.0001	0.0001
Chromatic traps	0.0004	0.0003	0.0003	0.0003
Combustible	0.0001	0.0001	0.0001	0.0001

Table 3 (*continued*)

Treatments	*Bv* 83 foliar	*Bv* 83 foliar + substrate low	*Bv* 83 foliar + substrate high	Control (non-inoculated)
Gasolin	0.0001	0.0001	0.0001	0.0001
Gasolin additive	0.0000	0.0000	0.0000	0.0000
LABOR	**0.0061**	**0.0650**	**0.0538**	**0.0746**
Laborer	0.0061	0.0650	0.0538	0.0746
FIXED COSTS	***0.1440***	***0.1344***	***0.1036***	***0.1543***
ACCESSORIES AND TOOLS	**0.0228**	**0.0213**	**0.0099**	**0.0245**
Plant tutoring accessories	0.0037	0.0035	0.0029	0.0040
Tomato rings	0.0005	0.0004	0.0004	0.0005
Wire hook	0.0023	0.0021	0.0018	0.0025
Raffia	0.0010	0.0009	0.0008	0.0011
Material for cultural work	0.0004	0.0004	0.0003	0.0004
Pruning tasks	0.0004	0.0004	0.0003	0.0004
Material for harvest	0.0005	0.0004	0.0004	0.0006
Plastic agricultural crates	0.0004	0.0004	0.0003	0.0005
Bucket of water	0.0001	0.0001	0.0001	0.0001
Material for monitoring	0.0002	0.0002	-	0.0003
Digital thermo-hygrometer	0.0000	0.0000	0.0000	0.0000
Digital pH and conductivity portable meter	0.0002	0.0002	0.0002	0.0002
Material for measurement	0.0000	0.0000	0.0000	0.0000
Graduated cylinder (100 mL)	0.0000	0.0000	0.0000	0.0000
Plastic measuring beaker (500 mL)	0.0000	0.0000	0.0000	0.0000
Material for fumigation	0.0008	0.0007	0.0006	0.0008
Manual spray pump	0.0001	0.0001	0.0001	0.0001
Motorized spray pump	0.0006	0.0006	0.0005	0.0007
Plant pot	0.0043	0.0040	0.0033	0.0046
Polyethylene black grow bags (40*40)	0.0043	0.0040	0.0033	0.0046
Material for substrate	0.0129	0.0120	0.0025	0.0138
Tezontle	0.0043	0.0040	0.0008	0.0046
Coconut fiber	0.0086	0.0080	0.0017	0.0092
SERVICES	**0.1212**	**0.1131**	**0.0936**	**0.1298**
Greenhouse rent	0.0025	0.0024	0.0020	0.0027
Amortization of fixed investment	0.1126	0.1050	0.0870	0.1205
Technical assistance	0.0061	0.0057	0.0047	0.0065
SALE PRICE	**0.5**	**0.5**	**0.5**	**0.5**
Income/sales	98,273.51	105,312	127,208	91,794
Revenue/Ha	33,002	27,541	50,107	14,992
PROFITABILITY (%/Ha)	**51%**	**35%**	**65%**	**20%**

high treatment, the profitability was 2 times higher than that of the control.

4.7. Critical analysis of the case study

This project started integrating a multidisciplinary team to tackle, in an integral way, an important problem in México: food supply security. One of the main problems we faced was the high biological variability of tomato production, an issue that has been reported previously [45,46]. That forced us to increase the number of replicas in the experiments and thus limiting the number of experiments. Notably, the use of commercial seeds, currently used by producers, allowed us to confront the technology in an actual situation and obtain realistic results. Although to increase the reproducibility, the use of high-quality seeds (phenotypically) can be considered for future works.

The project involved testing two commercial biological products developed by Mexican companies, which have worked closely with research institutions in México. This was a significant contribution because the producers can use products already available in the market, tested, and registered by the Mexican agencies [23,43]. One critical aspect was the initial homemade instruments used to control temperature and moisture since they were not robust enough to resist environmental conditions inside the greenhouse. We had to use commercial instruments to fix this, allowing us to control the main environmental variables properly.

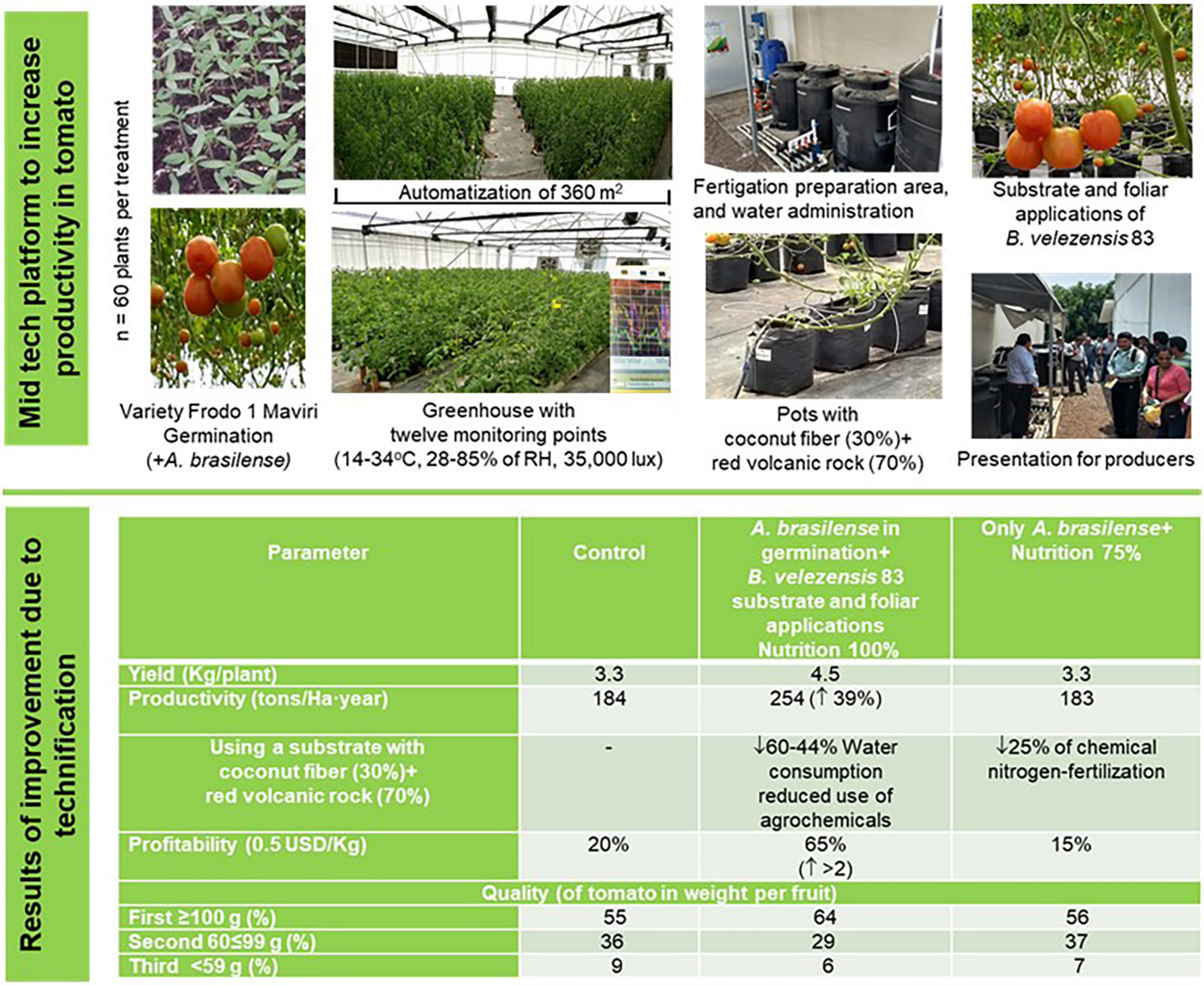
As a result, we received several producers' requests interested in the technologies. We also performed demonstrations to producers, which concluded that the technology developed can be implemented in modules, depending on their needs and financial/technological capabilities. Even though the greenhouse could be partially instrumented, the automatic irrigation is necessary. Despite the aspects commented (biological variability, reliable instrumentation, large number of tests), we were able to develop a set of technologies that could be named "intermediate technology" that producers can implement and that can represent an increase in profits of 45%, as compared to the conventional greenhouse technology (Fig. 4) **[Supplementary video]**. In the present work, we reduced the use of agrochemicals (pesticides) and almost 25% of chemical nitrogen-fertilization.

4.8. The lessons of the project

The multidisciplinary challenge, time, and cost constraints had secondary effects from which lessons learned and experienced, mistakes and goals achieved can be deduced. Here are some of these lessons and potential difficulties for multidisciplinary teams in developing innovative systems for agriculture.

Positive experiences:

- A multidisciplinary group allows to define more comprehensive and challenging aims and scopes on a development project as compared to those produced by a single group. In this project, the design criteria of the sensors and control had to meet both the technical requirements and the upper limits in cost, as the project is meant to be economically viable for Mexican producers.



Parameter	Control	*A. brasilense* in germination+ *B. velezensis* 83 substrate and foliar applications Nutrition 100%	Only *A. brasilense*+ Nutrition 75%
Yield (Kg/plant)	3.3	4.5	3.3
Productivity (tons/Ha·year)	184	254 (↑ 39%)	183
Using a substrate with coconut fiber (30%)+ red volcanic rock (70%)	-	↓60-44% Water consumption reduced use of agrochemicals	↓25% of chemical nitrogen-fertilization
Profitability (0.5 USD/Kg)	20%	65% (↑ >2)	15%
Quality (of tomato in weight per fruit)			
First ≥100 g (%)	55	64	56
Second 60≤99 g (%)	36	29	37
Third <59 g (%)	9	6	7

Fig. 4. Summary of the main achievements of multidisciplinary approaches for sustainable agriculture applied to tomato greenhouse production. In the results improvement table summarizing the results, the arrows indicate increase or decrease with respect to control.

- Training producers and forming specialized human resources with a wider view of the problem-solving strategies is one of the significant outputs of a research project when a multidisciplinary team is available.
- On the technical side, the comprehensive data collection, as obtained from the continuous monitoring of the greenhouse, provides data for future evaluation of artificial intelligence models and neural networks since it provides accurate environmental and physiological plant responses to real environmental inputs.
- Due to the presence of Bank of México Staff (FIRA), with expertise in economics and experience with technical transference to farmers, the project was carried out with a philosophy of lean development, low costs, and transfer viability from the beginning, unlike academic projects, which are developed with fewer constraints, making them less practical at the technology transfer stage.

Negative experiences:

- The biological variability of the plants was not considered at the beginning, this lead to difficulties in relating the cause-effect of environmental control and crop production. Hence, choosing seeds with low genetic variability is essential to monitor the effects closely. However, this study exemplifies the reality a producer faces with access to seeds of different qualities.
- The initial implementation of the instrumentation and control software in conjunction with the current biological control and water-saving experiments required time and training. This was also corrected in later stages and included commercial controllers and sensors as a parallel backup that assisted the systems developed by the group.

5. Conclusions

The developed technology (including the integral use of environmental control of the greenhouse, fertigation, the use of a highly water-retention substrate, a biofertilizer, and a biofungicide) yielded up to 254 ton/ha·year of tomato (Frodo variety), achieving reductions of 44–60% in water consumption and 28% in the cost unit of production increasing the profits for the producer in about 45% about Mexican conventional greenhouses management. In addition, it was possible to reduce the use of agrochemicals (pesticides) and almost 25% of chemical nitrogen-fertilization.

This case study has shown that it is possible to significantly increase profits in mid-tech greenhouse tomato production in middle-income countries like México by increasing productivity and crop quality and decreasing the use of water and agrochemicals using greenhouse automatization, crop management, and beneficial bacteria applied to the crop.

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Conflict of interest

The authors declare no conflict of interest.

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Supplementary material

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