

Response of piquín chili ecotypes (*Capsicum annuum* var. *aviculare* L.) to different shade coverage levels

Megchún-García, Juan V.²; Lucho-Constantino, Gonzalo G.^{1*}; Cruz-Ángeles R.¹; Natarén-Velázquez J.³; Moreno-Seceña, Juan C.⁴

¹ Tecnológico Nacional de México/Instituto Tecnológico Superior de Jesús Carranza, Carretera transistmica km 127+656.36, Loc. Palo dulce, 96950, Jesús Carranza, Veracruz, México.

² Tecnológico Nacional de México/ Instituto Tecnológico de Boca del Río, Carretera Veracruz-Córdoba Km. 12, 94290, Boca del Río, Veracruz, México.

³ Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Cotaxtla, Carretera Federal Veracruz-Córdoba Km 34.5, 94270, la Esmeralda, Medellín, Veracruz, México.

⁴ Colegio de Postgraduados/Campus Montecillo, Carretera Texcoco Km 36.5, Montecillo, Texcoco, 56264, Estado de México.

* Correspondence: gonzalolucho02@gmail.com

ABSTRACT

Objective: To evaluate the effect of different levels of shade coverage using black mesh on the growth and production of two ecotypes of piquín chili cultivated with plastic mulch.

Design/methodology/approach: 16 g of fruits from wild plants of Ecotypes I and II were collected to analyze their morphological differences. Under greenhouse conditions, germination rate and germination percentage, as well as seedling height and diameter, were evaluated for both ecotypes. A completely randomized block design with eight replications was used. Subsequently, 80-day-old plants were transplanted to the field and established under four shade net treatments: T1 (0% shade), T2 (35%), T3 (50%), and T4 (90%). The variables evaluated in the field included plant height and diameter, and fruit yield.

Results: Two ecotypes of piquín chili peppers were morphologically characterized. Ecotype I showed higher emergence (70%) than Ecotype II. Under greenhouse conditions, both ecotypes exhibited similar development; however, in the field, Ecotype I showed greater vegetative development. Treatment T3 increased fruit production in both ecotypes. Shade levels did not significantly affect plant growth.

Limitations on study/implications: Piquín chili peppers are harvested in agroforestry systems. Given its high demand, shade cover promotes sustainable intensification as an agronomic alternative for its cultivation.

Findings/conclusions: Shade coverage is an intensive and sustainable strategy that helps to genetically conserve chili pepper ecotypes during production.

Keywords: Wild chili, ecotypes, shading nets, plastic padding.

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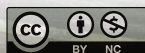
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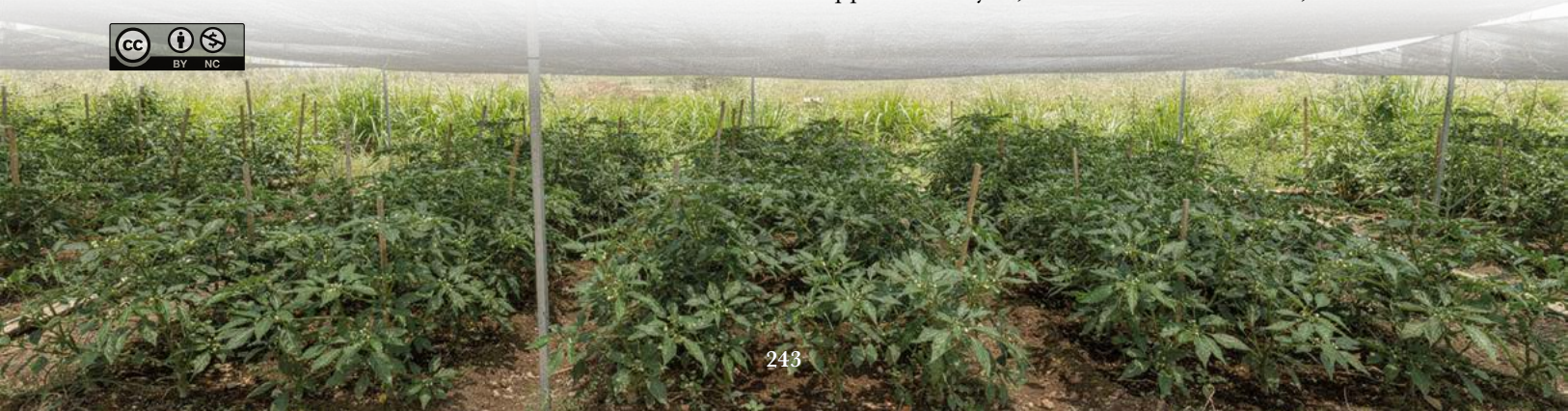
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INTRODUCTION

Wild chili peppers of the genus *Capsicum* L. are widely distributed in Mexico, ranging from sea level to elevations of approximately 2,500 m above sea level, and exhibit



remarkable morphological and sensory biodiversity in terms of fruit shape, size, color, flavor, and pungency. At least 30 species are recognized within the national territory (Hernandez-Verdugo *et al.*, 2019), among which five domesticated species stand out: *Capsicum annuum* L., *C. frutescens* L., *C. pubescens* Ruiz & Pav., *C. chinense* Jacq., and *C. baccatum* L. In the state of Tabasco, several groups of wild species have been collected, the most representative being *C. annuum* var. *glabriusculum* and *C. frutescens* L. (Narez-Jimenez *et al.*, 2014).

Piquin chili (*Capsicum annuum* var. *glabriusculum*) is considered the closest ancestor of cultivated varieties of *C. annuum* and is widely distributed throughout Mexican territory. Its harvest comes primarily from wild populations located in agroforestry systems, representing a significant source of income for rural communities during collection seasons (Ramírez-Novoa *et al.*, 2018). At the international level, India is emerging as Mexico's main competitor, exporting piquin chili to the United States (Balderas-Quezada *et al.*, 2023). In Mexico, most of the production destined for both domestic and international markets originates from natural wild populations (Medina *et al.*, 2010).

Regarding its morphological characteristics, piquin chili fruits are small, upright-growing, and green at the immature stage, turning bright red upon reaching physiological maturity. They may exhibit various shapes and are recognized for their high vitamin A content and antioxidant compounds. Various Indigenous communities have traditionally used these fruits in folk medicine to treat respiratory conditions such as asthma and cough, as well as to relieve sore throat and toothache, among other ailments (Balderas-Quezada *et al.*, 2023).

Piquin chili is commonly associated with thornscrub or submontane shrubland plant communities, in which the Fabaceae family predominates. These species play an important ecological role by providing shade during the early stages of development, thereby contributing to plant establishment and growth (Medina *et al.*, 2010). In the state of Sonora, piquin chili has been reported to occur in close association with *Prosopis glandulosa*, whose shade cover has been linked to the production of higher-quality fruits (Bañuelos *et al.*, 2008).

Among the main factors limiting chili production under open-field conditions are water scarcity and high temperatures. In this context, it has been reported that the combined use of plastic mulch and adequate soil moisture in huacle chili (*C. annuum* L.) grown under greenhouse conditions generated positive effects on plant phenological development, increasing the number of flower buds by 57%, flowers by 83%, fruits by 45%, leaf dry weight by 41.9%, fruit weight by 50.6%, and leaf area and leaf area index by 47% (Sanjuan *et al.*, 2022).

Recent studies have explored alternative production strategies under controlled conditions. In the state of Veracruz, Mexico, for example, the performance of *C. annuum* var. *aviculare* was evaluated under a high-density system (25,000 plants ha⁻¹) with the use of plastic mulch, obtaining yields exceeding 7 t ha⁻¹ (Megchún-García *et al.*, 2024).

Currently, there is a notable lack of specialized technologies for the agronomic management of piquin chili (*Capsicum annuum* var. *glabriusculum*) under intensive production systems (Ramírez-Novoa *et al.*, 2018). Despite this, in Saltillo, Coahuila, the effect of

Raschel-type shade nets with 30% shading and different colors (black, red, blue, and white), with 6×8 mm openings, was evaluated in the cultivation of *C. annuum* var. *glabriusculum*. The results indicated that white and blue nets significantly increased yield, as well as fruit number and size (Paredes-Jácome *et al.*, 2020).

In bell pepper (*C. annuum* L.) crops, the use of black shade netting promoted increases in relative humidity, plant height, and leaf area (Ayala-Tafoya *et al.*, 2015). This same netting has proven effective in reducing direct solar radiation, thereby helping to mitigate sunscald damage in fruits and to lower ambient temperature. Furthermore, its use recreates conditions similar to the natural habitat of piquin chili, favoring plant physiological development and improving fruit quality compared to open-field cultivation systems (Ayala-Tafoya *et al.*, 2011). Based on the above, the effect of different levels of shade coverage using black netting on the growth and production of two ecotypes of piquin chili cultivated with plastic mulch was evaluated. It was hypothesized that the application of different shading levels positively influences vegetative development and yield performance in at least one of the evaluated ecotypes.

MATERIALS AND METHODS

The study was conducted at the facilities of the Tecnológico Nacional de México/ Instituto Tecnológico Superior de Jesús Carranza, located in the municipality of Jesús Carranza, Veracruz, Mexico.

Fruit collection

Fruits were collected from wild plants exhibiting a healthy appearance and intense green color in the Ejido La Revolución community, San Juan Guichicovi, Oaxaca. Fruits from two piquin chili ecotypes, designated Ecotype I and Ecotype II, were selected at the mature stage. Subsequently, the fruits were exposed to sunlight for 24 h for drying.

Seed germination and seedling production

Seeds from Ecotypes I and II were treated with a growth promoter (HIGH PLANT[®]) at a concentration of 0.8 mL L⁻¹ of water for 24 h. The formulation of the promoter contained the following components: nitrogen (N) 0.01%, phosphorus (P) 0.02%, potassium (K) 0.02%, and gibberellic acid (GA₃) 3.30%. After completion of the treatment, seeds that settled at the bottom of the container were selected for use in the experiment.

A total of n=100 seeds from each ecotype were sown in 200-cell polystyrene trays using a substrate composed of a mixture of sand, sawdust, and vermicompost in a 1:2:2 (v/v) ratio. Seedlings emerged 11 days after sowing (DAS), and when they reached 15 cm in height, they were transplanted into 23×30 cm polyethylene bags.

During the growth period, seedlings were irrigated with water every three days and fertilized every eight days with Multiagro[®] at a dose of 10 mL L⁻¹ of water. For preventive pest management, an organic neem extract (Biocontrol[®]) was applied at a rate of 10 mL L⁻¹ of water. Additionally, during the seedling and transplant stages, Raizal[®] was applied at a dose of 10 g L⁻¹ of water using the drenching method.

Treatments

At 80 days of growth, the plants (32 per ecotype) were transferred to a shade-house system, where raised beds measuring 30×50 cm were established, with a length of 4 m. To conserve substrate moisture, gray plastic mulch (Tacsá[®]) was used and secured with industrial tape (Offiland[®]). Each plant received 500 g of vermicompost. The shade-house system was installed in an open-field area, orienting the shade net from east to west. Each shading level covered an area of 16 m², and the distance between treatments was 4 m.

During transplanting into the shade-house system, a hormonal biostimulant with immunopotentiating properties (X-pledor[®]) and a rooting agent (Raizal[®]) were applied twice, with an eight-day interval between applications. The experimental design was a completely randomized block design with eight replications and four treatments, consisting of different shading percentages: T1 (control, 0% shade net), T2 (35% shade net), T3 (50% shade net), and T4 (90% shade net).

For the morphological characterization of fruits from the mother plants, 16 g of fruits from each ecotype were collected. Fruit length, fruit width, the fruit length-to-width ratio (FL/FW), number of fruits per plant, number of seeds per fruit, and fruit color were measured.

Germination percentage was also evaluated by recording daily the number of seeds showing radicle emergence (2 mm) up to 25 DAS. The Mean Germination Rate (MGR) was calculated using the following formula:

$$MGR = n / t$$

where: *MGR* is the mean germination rate; *n* is the total number of germinated seeds, and *t* is the time from sowing until the last seed germinated.

Emergence percentage was calculated as the number of emerged seedlings divided by the number of seeds sown × 100.

Seedling growth evaluation was initially conducted during the greenhouse stage up to 72 DAS. Subsequently, after transplanting into the shade-house system, monitoring continued until 152 DAS. In both phases, plant height and stem diameter were recorded. Measurements were taken at eight-day intervals, beginning at 16 DAS for seedlings in the greenhouse stage. For plants transplanted into the shade-house system, data recording began at 80 DAS. Measurements were performed using a 3 m measuring tape (Truper[®]) and a caliper (Pretul[®]). Data were recorded in spreadsheets using Microsoft Excel[®] software.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) followed by Tukey's *post hoc* test ($P \leq 0.05$) to determine differences between ecotypes. The statistical software MINITAB, version 17, was used.

RESULTS AND DISCUSSION

Table 1 presents the morphological results of the fruits from the studied ecotypes. Fruits of Ecotype I had an average length of 1.61 ± 0.02 cm, whereas fruits of Ecotype II were longer, reaching an average of 2.51 ± 0.04 cm. The FL/FW ratio showed that fruits of Ecotype II were more elongated or narrower compared to those of Ecotype I.

Regarding width, fruits of Ecotype I were slightly wider and, in addition, this ecotype produced a greater number of fruits per plant compared to Ecotype II. Significant differences ($P \leq 0.05$) were recorded between the two ecotypes in terms of the number of seeds. At maturity, both ecotypes exhibited red-orange coloration. However, the most distinctive characteristic was the shape of the fruit apex: Ecotype I was characterized by an oval-shaped tip, whereas Ecotype II exhibited a pointed tip.

These morphological differences may be associated with structural factors derived from the adaptation of the ecotypes to different local microclimates. Hernández-Verdugo *et al.* (2012) indicate that climatic factors such as temperature, water availability, and human intervention directly influence the phenotype of wild chili populations. Likewise, Zhiglia *et al.* (2014) report that the genus *Capsicum* is characterized by wide variability in fruit type, color, shape, flavor, and phytochemical content. In the case of wild chili (*Capsicum annuum* var. *glabriusculum*), traits such as the FL/FW ratio, fruit shape, and apex shape have been identified as key characters for distinguishing among populations (Alcalá-Rico *et al.*, 2022).

Emergence percentage

ANOVA showed significant differences ($P \leq 0.05$) in emergence percentage between Ecotype I and Ecotype II. In both cases, emergence began at 10 DAS, with the appearance of two seedlings in Ecotype I and three in Ecotype II. By 14 DAS, Ecotype I reached 70% emergence, whereas Ecotype II reached 55%. These percentages remained constant until 20 DAS (Figure 1).

Mean germination rate was higher in Ecotype I, with a value of 3.5, compared to Ecotype II, which recorded a rate of 2.75. These results suggest that Ecotype I has greater germinative capacity under the evaluated experimental conditions.

Similar findings have been reported in previous studies. Research on seeds of *Capsicum annuum* L. var. *glabriusculum* (chiltepín) treated with gibberellic acid showed 97% germination at eight days and an average seedling height of 6.5 cm at 30 days under greenhouse

Table 1. Morphological characteristics of fruits from Ecotype I and Ecotype II of *Capsicum annuum* var. *aviculare* collected from mother plants in the Ejido La Revolución, San Juan Guichicovi.

Characteristics of the fruit	Ecotype I	Ecotype II
Fruit length (cm)	1.61 ± 0.02	2.51 ± 0.04
Fruit width (cm)	0.76 ± 0.02	0.69 ± 0.01
FL/FW	2.14 ± 0.07	3.65 ± 0.07
Number of fruits per plant	204	138
Number of seeds per fruit	20.6 ± 1.19	22.7 ± 2.49
Fruit color at maturity	Red-orange	Red-orange

FL/FW: ratio of the length and width of the fruit.

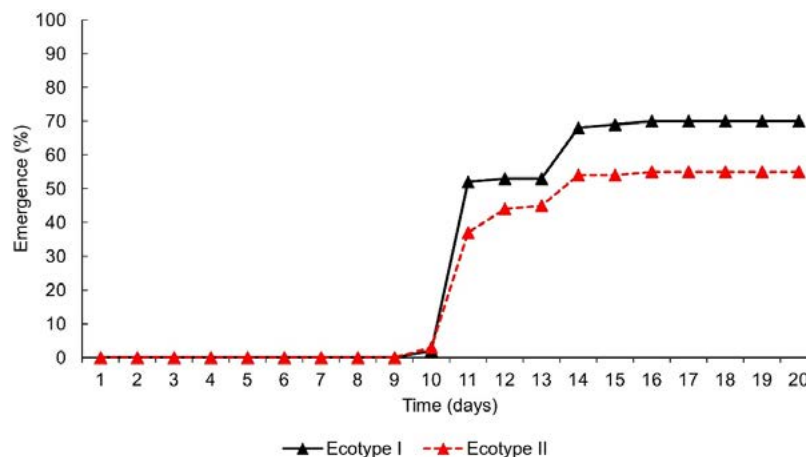


Figure 1. Emergence percentage of Ecotype I and Ecotype II of *Capsicum annuum* var. *aviculare*.

conditions (Araiza *et al.*, 2011). On the other hand, González *et al.* (2015) evaluated the effect of the biostimulant Biozyme TF (gibberellic acid 31 ppm + indoleacetic acid 31 ppm + zeatin 83 ppm) at 1.6%, applied by seed immersion for 24 h. In their study with *Capsicum annuum* L. var. *glabriusculum*, they achieved 86% germination at 12 days after sowing, indicating a positive effect of the hormonal treatment.

Seedling height and stem diameter under greenhouse conditions

No significant differences were observed in seedling height between Ecotypes I and II (Figure 2a). Ecotype I showed a height range from 3.4 ± 0.03 cm to 22.5 ± 0.16 cm, whereas Ecotype II ranged from 3.4 ± 0.00 cm to 21.0 ± 0.00 cm. From 48 DAS onward, seedlings of Ecotype I were slightly taller than those of Ecotype II. Regarding stem diameter, a trend similar to that observed for seedling height was recorded. Values for Ecotype I ranged from 0.10 ± 0.00 cm to 0.31 ± 0.00 cm, while Ecotype II ranged from 0.10 ± 0.00 cm to 0.30 ± 0.00 cm. A slight increase in stem thickness was observed in both ecotypes from 56 DAS to 72 DAS. The absence of differences in height and stem diameter between Ecotypes I and II indicates that, despite possible genetic variation, both exhibited similar vegetative development under controlled growing conditions. The slight variations observed during the seedling stage may be attributed to differences in cell elongation rate and differential assimilation of nutrients contained in the growth promoter used. Previous studies support these findings. Cano *et al.* (2015) evaluated 16 accessions of *Capsicum annuum* var. *glabriusculum* treated with gibberellic acid, potassium nitrate, and hydrogen peroxide. In that study, gibberellic acid was the treatment that most strongly promoted germination and growth rate, particularly in accessions from Acajoneta and Tuxpan, which reached germination rates of up to 70%. Furthermore, low germination in these accessions was mainly attributed to physiological dormancy.

Plant height under black shade net conditions

In the evaluation of the different shading levels applied in the shade-net system, no statistically significant differences ($p \leq 0.05$) were found among treatments for Ecotypes I

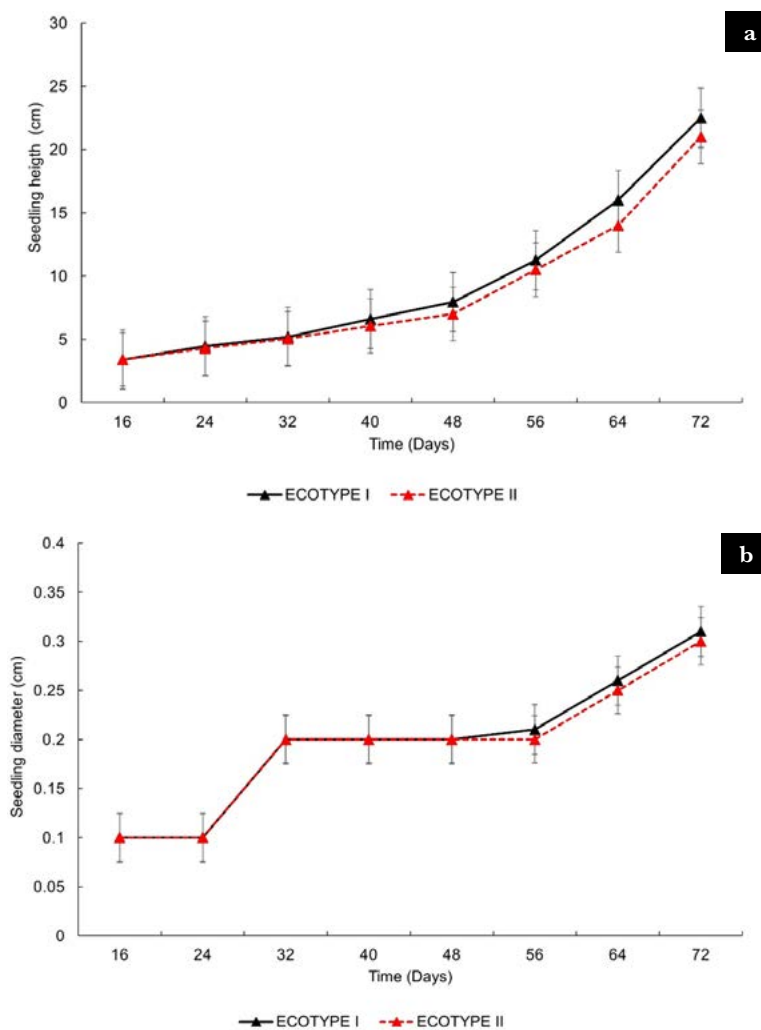


Figure 2. Height (a) and stem diameter (b) of seedlings of Ecotypes I and II of *Capsicum annuum* var. *aviculare* grown under greenhouse conditions.

and II (Figure 3a and 3b). It is important to note that, during the productive stage, plants of Ecotype I were generally taller than those of Ecotype II. Plants of Ecotype I exposed to 90% shading (T4) were the tallest among treatments, reaching an average height of 77 ± 0.32 cm at 152 DAS, compared with plants grown without shading (T1), which averaged 62.5 ± 3.0 cm (Figure 3a).

In contrast, Ecotype II exhibited a different growth pattern compared to Ecotype I. Plants grown under the different treatments showed similar height, except for those under T2, which were slightly shorter during the productive stage (Figure 3b). Slower growth was observed in plants under T2 and T3 between 96 and 112 DAS; however, during the productive stage, no significant differences were recorded compared to the other treatments. Plants of chile piquín (*Capsicum annuum* var. *aviculare*) under T4 (90% shade) reached similar sizes by 104 days of age, exceeding 65 cm in height. Plants of chile piquín (*C. annuum* var. *aviculare*) grown under plastic cover have shown positive effects on growth under open-field conditions, reaching 68 cm at 97 days (Megchun *et al.*, 2024). Our results indicate that the

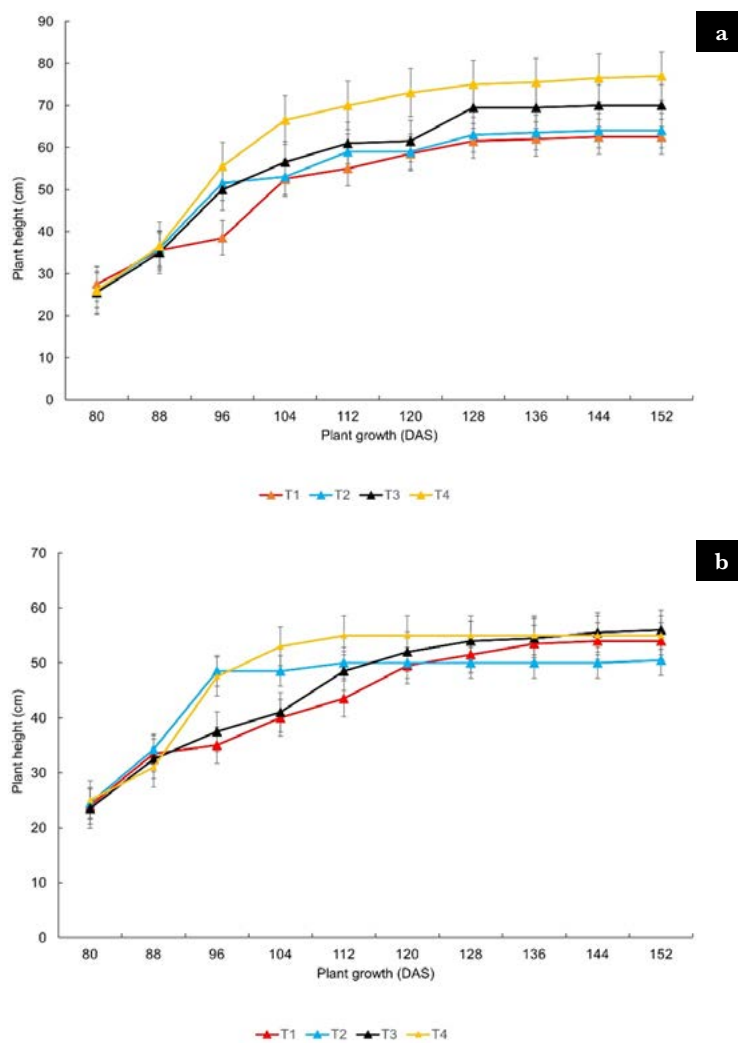


Figure 3. Plant height at 80 DAS of Ecotype I (a) and Ecotype II (b) grown under a shade-net system with different shading levels. T1: no shade net; T2: 35% shade net; T3: 50% shade net; and T4: 90% shade net. Means \pm standard error for each treatment. DAS: days after sowing.

combined use of plastic mulch and 90% shade net under open-field conditions enhances the development of chile piquín plants, as plant height increases in response to reduced light availability.

Stem diameter of plants grown under black shade net conditions

Under the shade-net system, plants of Ecotype I evaluated at 80 DAS under treatment T3 exhibited a significantly greater stem diameter during the first two weeks compared to the other treatments (Figure 4a). However, between 104 and 152 DAS, no significant differences in stem diameter were observed among the shading treatments and the control plants (T1).

For Ecotype II, plants under T4 showed a slight increase in stem diameter at 88 DAS. In contrast, plants under T1 were thinner between 96 and 120 DAS, which was

associated with increased plant height (Figure 3). At 152 DAS, no significant differences in stem diameter were recorded between shaded plants and the control group (Figure 4b). Overall, no significant differences in stem thickness were observed between Ecotypes I and II under the shade-house system (Figure 4). These results indicate that both ecotypes follow a similar growth pattern with respect to stem diameter. Additionally, a possible relationship between increased stem thickness and greater plant height was observed, suggesting that both traits may be developmentally coordinated and potentially regulated by genetic mechanisms. It is worth noting that these results differ from those reported by Paredes-Jacóme *et al.* (2019), who observed that chile piquín grown in macrotunnels with 30% white Raschel shade net exhibited an average basal stem diameter of 6.29 mm, whereas plants grown under black net reached 4.15 mm.

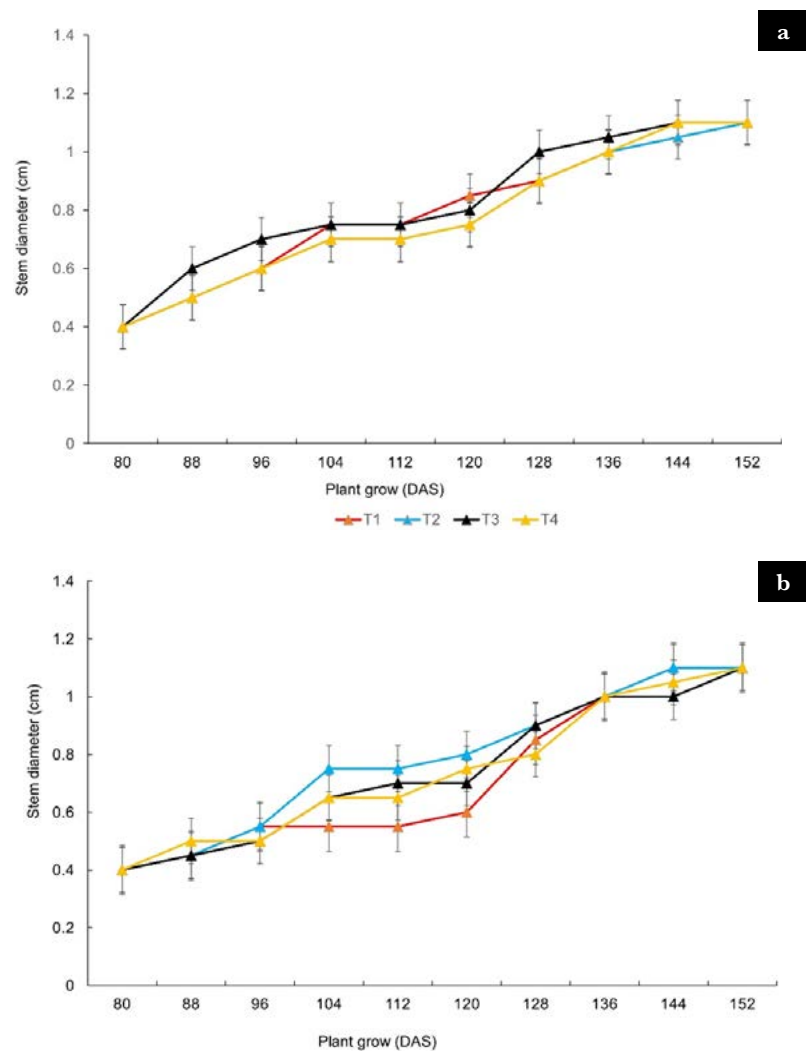


Figure 4. Stem diameter of plants at 80 DAS of Ecotype I (a) and Ecotype II (b) grown under a shade-net system with different shading levels. T1: no shade net; T2: 35% shade net; T3: 50% shade net; and T4: 90% shade net. Means \pm standard error for each treatment. DAS: days after sowing.

Fruit production under black shade net conditions

Significant differences in fruit production were found between the evaluated ecotypes ($p \leq 0.05$), with Ecotype I exhibiting higher productivity than Ecotype II across the different shading levels (Table 2). Within Ecotype I, treatment T3 had the greatest positive effect on fruit production, with an average of 310 ± 4.48 fruits per plant. For Ecotype II, treatments T3, T4, and T2 produced a higher number of fruits compared to the non-shaded treatment (T1), with T3 being the most productive, averaging 185 ± 1.41 fruits per plant.

Table 2. Number of fruits obtained under the shade-net system with different shading levels.

Treatment	Ecotype I	Ecotype II
T1	235 ± 8.07 b	153 ± 4.48 b
T2	249 ± 8.37 b	168 ± 5.13 a
T3	310 ± 4.48 a	185 ± 1.41 a
T4	256 ± 1.45 b	174 ± 1.69 a

T1: no shade net; T2: 35% shade net; T3: 50% shade net; and T4: 90% shade net. Means followed by different letters are significantly different (Tukey, $p \leq 0.05$) within each ecotype.

Studies conducted in Sonora, Mexico reported that the dry fruit yield of chiltepín (*Capsicum annuum* var. *glabriusculum*), harvested every three days, was 1.45 times higher under greenhouse conditions compared to plants grown under mesquite (*Prosopis* sp.) stands. Furthermore, recent research has indicated that chiltepín production can be significantly improved when cultivated under colored shade nets or structures with anti-aphid mesh walls (Caughey *et al.*, 2020). The use of white and blue shade nets increases yield, fruit number, and fruit size (Paredes-Jacóme *et al.*, 2019). Chiltepín is considered a fruit of high nutritional relevance. In this context, Reyes *et al.* (2018) demonstrated that the use of a blue shade net with 30% shading had a positive effect on vitamin C content and antioxidant properties of the fruits compared to plants grown under open-field conditions with a 30% black shade net. On the other hand, the use of plastic mulch has shown additional benefits in the production of *Capsicum annuum*, such as huacle chili. According to Sanjuan *et al.* (2022), this strategy improved soil moisture content and resulted in an 85% increase in flower bud formation, an 89% increase in open flowers, and a 65% increase in fruit production under greenhouse conditions. Black shade nets help reduce temperature and protect fruits from sun damage; therefore, their effect may vary depending on shade density (Ayala-Tafoya *et al.*, 2015).

CONCLUSIONS

Seedlings of Ecotypes I and II grown under greenhouse conditions at 72 DAS showed no differences in height or stem diameter. However, after transplanting to the field, plants of Ecotype I were taller than those of Ecotype II during the productive stage at 152 DAS. The different shading levels did not have a significant impact on plant growth. Nevertheless, the 50% shading treatment (T3) enhanced fruit production in both ecotypes, with Ecotype

I producing 1.68 times more fruits during the productive stage compared to Ecotype II. Therefore, Ecotype I of *Capsicum annum* var. *aviculare* represents a viable alternative for establishment and production under 50% shade-net conditions (T3).

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