CHILEAN JOURNAL of AGRICULTURAL RESEARCH
RESEARCH ARTICLE

Selection of advanced tomato lines with different calcium concentrations in response to *Alternaria lycopersici* infection

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Received: 28 April 2025; Accepted: 20 August 2025, doi:10.4067/S0718-58392025000600811

ABSTRACT

Several adverse biotic and abiotic factors occur during the development of tomato (*Solanum lycopersicum* L.), reducing yield and fruit quality. This study evaluated the *Alternaria* foliar severity and subsequently the tolerance to Ca deficiency in 90 tomato (*Solanum lycopersicum* L.) genotypes, including 89 advanced S_4 lines and one commercial control. The influence of *Alternaria* on yield and its components as average fruit weight, and total number of fruits per plant was studied using a randomized complete block design with three replicates and four plants per replicate. After plant pathology analysis, *Alternaria lycopersici* was identified as the pathogen responsible for Ca deficiency and yield loss. Despite of the pressure of *A. lycopersici* and Ca deficiency, 14 advanced lines showed a yield like that exhibited by the commercial check 'El Cid'. Among 89 S_4 lines, five tomato lines were resistant to *A. lycopersici* infection (± 0% leaf severity), while 52 lines showed moderate resistance (≤ 25% leaf severity). A total of 24 advanced lines with no symptoms of blossom end rot due to Ca deficiency were identified. Additionally, two outstanding genotypes (48 and 49) from the source LOR95×C were selected for their high total fruit weight per plant and natural resistance to *A. lycopersici*. These lines showed no Ca deficiency, so they can be used as open-pollination varieties to improve the program's elite materials. Finally, susceptible lines to *A. lycopersici* showed Ca absorption capacity decreases in fruit (26.15 µg g^{-1}) and individual fruit weight (59.91 g). In contrast, resistant lines had values of 162.33 µg g^{-1}, and an individual fruit weight of 107.11 g. This suggests that the lower the resistance to *A. lycopersici* in advanced lines, the more fruits with blossom end rot and lower average fruit weight due to calcium deficiency in the plant.

Key words: Blossom end rot, genetic diversity, physiopathology, resistance, *Solanum lycopersicum*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a crop with global significance due to its large production volumes and consumption patterns. As center of tomato domestication and diversification, Mexico is considered a rich source of genes due to the high variation in shape, size, colors, plant and tolerance to biotic and abiotic factors, reported in landraces in previous studies. The importance of these landraces has been demonstrated into public breeding programs where their use increases significantly the yield in experimental hybrids derived from the combining between commercial germplasm and landraces with interesting traits (Martínez-Vázquez et al., 2017). Yield is one complex trait affected by genetic and environmental factors. In tomato, the yield is determined by several specific factors as such: Total biomass production, biomass partitioning, and fruit DM content (Hernández-Bautista et al., 2015). However, there are a high number of biotic and abiotic factors affecting the yield and fruit quality during tomato plant development (Yang et al., 2021). Plant health is

determined by its intrinsic genetic potential and ability to perform normal physiological functions (Schmey et al., 2024). *Alternaria* is the primary fungal genus that reduces tomato crop yield (Rodrigues and Furlong, 2022), resulting in losses of up to 79% (Adhikari et al., 2017).

Alternaria contains at least 693 species (MycoBank, 2024), which includes *A. lycopersici*, a species recently described in China (Gou et al., 2023). This species produces conidia that affects the plant, resulting in foliar lesions with an area of 0.02 ± 1.15 cm (Gou et al., 2023). Despite recent reports from China, this *Alternaria* species is also found in Mexico.

Fruit blossom end rot is a physiological alteration caused by a Ca deficiency in the plant (Topcu et al., 2021), preventing development and reducing its weight. The Ca ion (Ca^{2+}) acts as a signal transducer and interacts with polysaccharides in the fruit cell wall; its slow mobility results in blossom end rot and abnormal fruit development (Hocking et al., 2016; Santos et al., 2023). Although some of the casual agents reducing the yield like *Alternaria* and fruit blossom has been well studied in commercial hybrids, information about their impact of tomato landraces is yet limited. Therefore, the goal of this research was to identify the causal agent of the reduced fruit yield in advanced tomato lines and to select genotypes with different levels of resistance to *A. lycopersici* while avoiding symptoms of blossom end rot caused by Ca deficiency.

MATERIALS AND METHODS

Experiment 1. Selection of resistant and moderately resistant lines to *Alternaria* and blossom end rot caused by Ca deficiency

Genetic material. Foliar severity (FS) was assessed under natural infection conditions on yield variables of 90 tomato (*Solanum lycopersicum* L.) genotypes, including 89 advanced S_4 lines and the commercial hybrid 'El Cid', which was used as the control. All genotypes exhibited an indeterminate growth habit. The lines originated from crosses between S_9 lines of commercial hybrids of the saladette type, characterized by an elongated fruit shape, designated as 'C' (C), 'L' (L), and 'R' (R), and S_9 lines of the native tomato type "Chino criollo", which are bears flattened and ribbed fruits (LOR-79, LOR-81, LOR-82, LOR-84, LOR-85, LOR-91, LOR-95, LOR-97, LOR-103, and LOR-111) from Tehuacán, Puebla, Mexico.

Experimental design and agronomic management. The experiment was carried out under greenhouse hydroponic conditions during the spring-summer 2020 season in Montecillo, Texcoco (19°30' N, 98°53' W; 2250 m a.s.l.), Mexico. The genotypes were evaluated in a randomized complete block experimental design with three replicates and four plants per replicate.

Sowing occurred on 23 May 2020, followed by transplantation 30 d later in polyethylene bags (40 × 40 cm) containing red tezontle as a substrate, with a spacing of 0.4 m between plants and 1.5 m between rows. The Steiner (1961) nutrient solution was used throughout the crop cycle. In the vegetative stage, 50% concentration was used, while in the flowering and fruiting stages, it increased to 100%. The electrical conductivity (EC) of the nutrient solution was maintained at 2.0 ± 0.2 dS m^{-1} during the vegetative stage and at 2.8 ± 0.2 dS m^{-1} for the flowering and fruiting periods. The pH of the solution was maintained between 5.5 and 6.0. The plants were tutored and managed from a single stem. To control whiteflies (*Bemisia tabaci*; Hemiptera: Aleyrodidae), the insecticides chlorantraniliprole + lambda-cyhalothrin (Ampligo Syngenta Agro S.A. de C.V., Ciudad de México, Mexico) under a concentration of 0.4 L ha^{-1}, and imidacloprid (Confidor, Bayer de México S.A. de C.V., Ecatepec, Estado de México, Mexico) under a concentration of 0.5 L ha^{-1}, were applied.

Variables. At the fruit development stage, FS was measured on a scale (Figure 1). The effect of *Alternaria* on Ca absorption in plants exhibiting natural infection of blossom end rot due to Ca deficiency in fruit (CDE) was determined by recognizing the presence or absence of blossom end rot in the fruit. The genotypes were classified into nine categories: 1 = resistant (0% FS), 4 = moderately resistant (≤ 25% FS), 7 = moderately susceptible (± 65% FS), and 8 = susceptible (≥ 80% FS).

Fruit harvest per plant was carried out manually in two cuts at 143 d after transplantation, when genotypes exhibited 6-7 mature clusters, and 170 d corresponding to full maturity with around 9 mature clusters. To assess the impact of *Alternaria* infection on yield variables, total fruit weight per plant (TFW, kg) was calculated by

adding the weight values obtained from both cuts. The total number of fruits per plant (TNF) was registered, and the average fruit weight (AFW, g) was determined by dividing TFW by TNF.

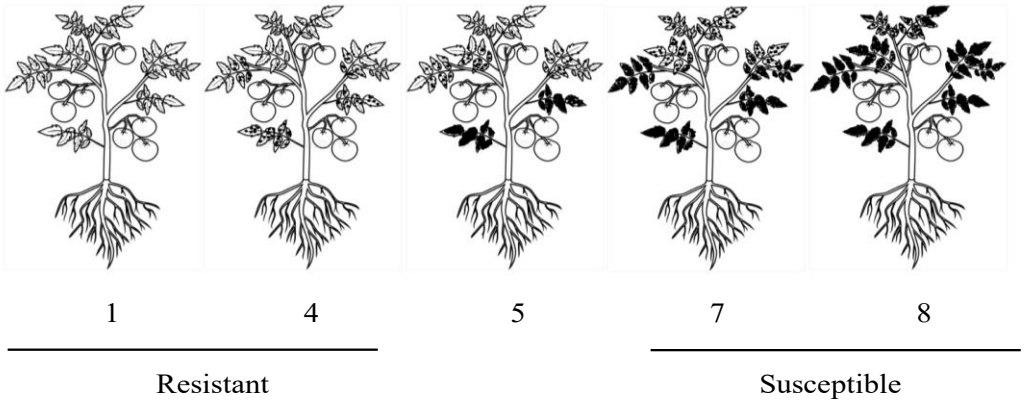

Figure 1. *Alternaria lycopersici* foliar severity (FS) assessment scale in tomato plants (*Solanum lycopersicum*). 1 = resistant (± 0% FS), 4 = moderately resistant (≤ 25% FS), 7 = moderately susceptible (± 65% FS), and > 8 = susceptible (≥ 80% FS).

Statistical analysis. An individual ANOVA was performed for each trait. The Shapiro-Wilk test ($p < 0.05$) validated the assumption of normal distribution. The Tukey test ($p < 0.05$) was used to compare the means of TFW, TNF, and AFW. Because the FS and CDE variables did not follow a normal distribution, FS was analyzed using nonparametric statistics with multiple range combinations (Friedman, 1937). Considering that the CDE variable is binary, Poisson regression was used. The Bonferroni criterion was applied to contrast linear hypotheses when comparing means. Spearman's correlation was used to assess the relationship between all variables. Statistical analyses were conducted using R version 4.1.0 (R Core Team, 2020).

Identification of *A. lycopersici* as the causal agent in S_4 lines

Isolation of the causal agent. In April 2023, three lines susceptible to the pathogen (scores 1 and 2, Figure 1) causing necrotic spots were selected and planted in greenhouse conditions. To identify the causal agent, symptomatic leaves were collected. To assess symptom progression, 0.5 cm^2 leaf tissue were selected and disinfected with a 1% sodium hypochlorite solution (v/v). The tissue was then rinsed three times with sterile distilled water to remove excess moisture. The disinfested tissue portions were then placed on Petri dishes containing potato dextrose agar (PDA) medium; after 72 h, mycelium growth was observed, and the culture was transferred to new dishes until conidia were produced. After 8 d, serial dilutions from 10^{-1} to 10^{-5} were used to obtain monoconidial cultures.

DNA extraction and PCR. The DNA was extracted from 7-d-old monoconidial cultures using the 2% cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990) using 3 M sodium acetate. The quality of DNA was evaluated using spectrophotometry in a NanoDrop 2000 UV-Vis (Thermo Scientific, Waltham, Massachusetts, USA). The DNA was considered of high quality when the absorbance ratios 260/280 and 260/230 nm were between 1.8 and 2.2.

The PCR was performed with primers ITS5 (forward, 5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (reverse, 5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990), which amplify a fragment of approximately 550 base pairs from the internal transcribed spacer (ITS) region of rDNA. Additionally, the partial amplification of the *ALTA1* gene (forward, 5′-ATGCADTTCACCACCATCGC-3′; reverse, 5′-ACGAGGGTGAYGTAGGCGTC-3′), which encodes the Alt a 1 allergenic protein, was performed (Hong et al., 2005).

The PCR reaction mixture was prepared in a final volume of 15 µL using 7.86 µL HPLC water, 3 µL 5× PCR reaction buffer, 0.6 µL dNTPs solution (20 µM each), 0.18 µL each primer (10 µM), 3 µL genomic DNA (60 ng),

and 0.18 µL (2 U) GoTaq DNA polymerase (Promega, Madison, Wisconsin, USA). The PCR reaction was performed in a C1000 Touch thermal cycler (Bio-Rad, Hercules, California, USA) and consisted of an initial denaturation cycle at 95 °C for 5 min, followed by 30 cycles at 95 °C for 1 min, 58 °C for 1 min, and 72 °C for 2 min, followed by a 10 min extension cycle at 72 °C.

To verify the amplification of the DNA fragments, 3 µL PCR product was mixed with 1 µL GelRed (Biotium, Fremont, California, USA), which was loaded on a 2% SeaKem LE agarose gel (Lonza, Rockland, Maine, USA) and run on a horizontal electrophoresis system (Bio-Rad, USA) at 80 V for 1.5 h. The amplicons were visualized using an Infinity-3026 WL/LC/26MX transilluminator (Vilver Lourmat, Collégien, France). The amplified PCR products were cleaned using the ExoSAP-IT enzymatic method (Affymetrix, Santa Clara, California, USA) according to the manufacturer's instructions. The DNA strands (forward and reverse) were then sequenced using the Sanger Sequencing service (Psomagen, New York, New York, USA).

Molecular identification. The resulting strands corresponding to the ITS region and the *ALTA1* gene were assembled using BioEdit v7.2.5 (Hall, 1999). The closest sequences were determined by comparison using the basic local alignment search tools nucleotide (BLASTn) option of the National Center for Biotechnology Information (Altschul et al., 1990). For phylogenetic analysis, the *Alternaria* section reference sequences deposited in the GenBank database were used (Table 1). The ITS and *ALTA1* sequences were concatenated with Mesquite v.3.81 (Maddison and Maddison, 2023), and the nucleotide substitution model was determined using the jModelTest program (Darriba et al., 2012).

Table 1. *Alternaria* species used in the multilocus phylogenetic analysis and their GenBank accession numbers. Isolates and accession numbers generated in the current study are indicated in bold letters. ITS: Internal transcribed spacer. Accession numbers in the process of assignment. T = Type.

		GenBank accession number	
Species	Strain	ITS	*ALTA1*
A. alternantherae	CBS 124392^T	KC584179	KP123846
A. alternata	EGS 34-016^T	AF347031	AY563301
	CBS 119399	KP124361	KP123910
	CBS 102602	KP124332	KP123881
	CBS 102596	KP124328	KP123877
	CBS 121547	KP124372	KP123920
A. arborescens	CBS 102605^T	AF347033	AY563303
	CBS 124281	KP124414	KP123961
A. infectoriae	CBS 210.86^T	DQ323697	FJ266502
A. lycopersici	YZU 221185	OQ519795	OQ473633
	YZU 221186^T	OQ519794	OQ473632
	CPO 27		
	CPO 28		
A. mali	CBS 106.24^T	KP124298	KP123847
A. orogonensis	CBS 542.94^T	FJ266478	AY563283
A. solani	CBS 116442	KJ718240	KJ718747
	CBS 109157	KJ718238	KJ718238
A. solanicola	YZU 221189	OQ534548	OQ473631
	YZU 221190^T	OQ519793	OQ473630
A. tomaticola	CBS 118814^T	KP124357	KP123906
A. tomato	CBS 114.35	KP124446	KP123992
	CBS 103.30	KP124445	KP123991
A. tenuissima (=*A. alternata*)	CBS 918.96	AF347032	AY563302

Phylogenetic reconstruction was carried out using Bayesian inference and maximum likelihood probabilistic methods, which take into account discrete character optimization criteria. MrBayes v.3.2.1 (Huelsenbeck and Ronquist, 2001; Ronquist et al., 2012) was used for Bayesian inference, with four interchangeable chains: One cold chain and three hot chains. The analysis was stopped when the standard deviation of the splits fell below 0.01, and the trees were sampled every 1000 generations. With the 'burn in-phase' option, 25% of the total number of trees generated were discarded, and the posterior probability was calculated for the remaining trees. FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualize and edit the tree.

The maximum likelihood phylogenetic tree was constructed using raxmlGUI 2.0 (Edler et al., 2020), and the support of each node was calculated using a fast-bootstrapping analysis with 1000 iterations using the parameters set by the program (general time-reversible model with a GTR + G gamma distribution). The resulting tree was visualized using the MEGA 7 program (Kumar et al., 2016).

Experiment 2. Influence of *A. lycopersici* on Ca absorption in fruit and yield of advanced S_4 lines

Genetic material. To assess and compare the impact on yield and Ca content in the fruit, three *A. lycopersici* tolerant lines and three susceptible lines identified during the 2020 agricultural cycle were selected. All genotypes had an indeterminate growth habit. The advanced S_4 lines were originated from crosses between S_9 lines of commercial hybrids of the saladette type 'C' (C) and 'L' (L), characterized by an elongated fruit shape, and S_9 lines of the native tomato type "Chino criollo", characterized by flattened and ribbed fruits (LOR-79, LOR-81, LOR-95, and LOR-111) from Tehuacán, Puebla, Mexico. The evaluation took place under greenhouse hydroponics conditions during the spring-summer 2023 season in Montecillo, Texcoco, Mexico.

Experimental design and agronomic management. The genotypes were assigned using a randomized complete block design, with three replicates and four plants per replicate. Sowing took place on 24 June 2023, and transplantation 30 d later. Crop management was similar to that described in the previous section. Imidacloprid (Confidor, Bayer de México S.A. de C.V., Ecatepec, Estado de México, Mexico) was applied at a dose of 0.5 L ha^{-1}, and chlorantraniliprole + lambda-cyhalothrin (Ampligo, Syngenta Agro S.A. de C.V., Ciudad de México, Mexico) at a dose of 0.4 L ha^{-1}, both as preventive measures against whitefly (*Bemisia tabaci*).

Variables. Fruits were manually harvested from each plant in two cuts at 114 d after transplantation, when genotypes exhibited 4-5 mature clusters, and 141 d with around 6-7 mature clusters. During the first and second harvests, fresh juice was extracted from two fruits per plant per plot to determine calcium concentration (Ca^{2+}, µg g^{-1}). The measurements were worked using a portable ion meter (Horiba, LAQUAtwin Ca-11, Kyoto, Japan), following the methodology described by (Cooke, 1975; Himelrick and Ingle, 1980). Furthermore, the average fruit weight (AFW, g), total fruit weight per plant (TFW, kg), and total number of fruits per plant (TNF) were calculated by adding the values obtained from both cuts. The total number of fruits with blossom end rot per plant was also registered.

Statistical analysis. A regression analysis was carried out to determine the relationship between yield variables (TFW, AFW, and TNF), Ca concentration in fruit (CaF), and genotype susceptibility to *A. lycopersici* (SALy). The Shapiro-Wilk test ($p < 0.05$) confirmed the assumption of a normal distribution. Spearman correlation was used to assess the relationship between the variables. Statistical analyses were conducted using the R programming language and environment (R Core Team, 2020).

RESULTS

Identification of resistant and moderately resistant lines to *Alternaria* and blossom end rot caused by Ca deficiency

In the current study, five S_4 lines were found to be resistant to the pathogen (± 0% foliar severity [FS]), while 52 lines showed moderate resistance (≤ 25% FS) (Figure 2).

In this study, 24 advanced lines without blossom end rot caused by Ca deficiency in fruit (CDE). Blossom end rot was observed in one fruit in at least 1 out of 12 plants evaluated per genotype in 22 S_4 lines; 23 lines showed blossom end rot in one fruit in at least 2 out of 12 plants evaluated per genotype, while 21 lines exhibited blossom end rot in at least one fruit in all plants of the genotypes evaluated (Figure 3).

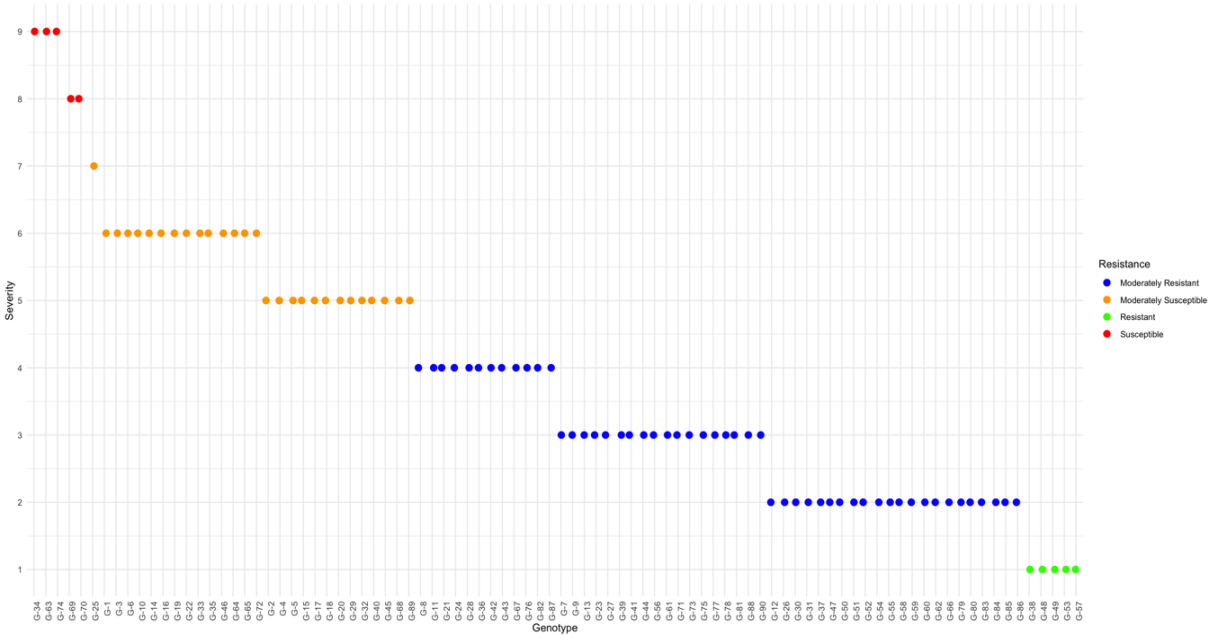

Figure 2. *Alternaria lycopersici* foliar severity assessed in 89 advanced tomato lines (*Solanum lycopersicum*) and one commercial control.

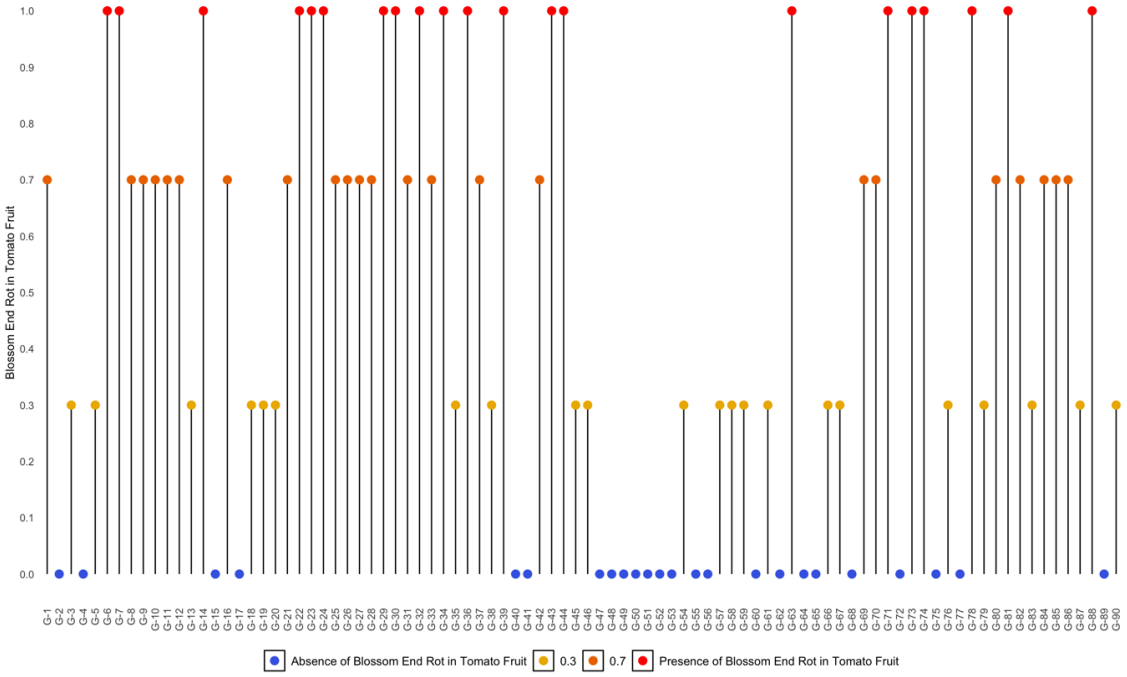

Figure 3. Blossom end rot in fruit caused by Ca deficiency in 89 advanced tomato lines (*Solanum lycopersicum*) and commercial control.

Spearman correlation

Results show weak but significant correlations between yield variables, plant susceptibility to *A. lycopersici*, and Ca deficiency. This demonstrates the impact this pathogen has on yield reduction and Ca absorption (Roy-García et al., 2019). For the CDE variable, a highly significant and negative correlation was observed with total fruit weight per plant (TFW) and average fruit weight (AFW) (r = -0.16*** and r = -0.21***, respectively) (Table 2), indicating that the presence of blossom end rot in the fruit reduces its development and weight, affecting the overall plant potential weight to a low or intermediate extent.

The FS variable showed a significant and positive correlation with TFW and AFW (r = 0.15*** and r = 0.13**, respectively). This indicates that as the FS score increases, the genotype exhibits greater resistance to *A. lycopersici* (Figure 1) while presenting a normal Ca absorption in the fruit, resulting in an increase in yield variables (TFW and AFW). The greater the plant susceptibility as indicated by the FS scale rating, the lower the evaluation score of the genotypes (Figure 1), so the plant is unable to absorb the available Ca, resulting in a decrease in potential yield in advanced tomato lines due to a reduction in TFW and AFW (Table 2). The TNF exhibited neither negative nor positive affectation given that the number of fruits is determined by the number of leaves, which are the source of assimilates based on their phyllotaxy (Quintana-Baquero et al., 2010).

Table 2. Spearman correlation coefficient between plant susceptibility to *Alternaria lycopersici* and yield variables in saladette tomato (*Solanum lycopersicum*) genotypes with a broad genetic base and a commercial control. TNF: Total number of fruits per plant; TFW: total weight of the fruits per plant; AFW: average fruit weight; FS: foliar severity caused by plant susceptibility to *A. lycopersici*; CDE: Ca deficiency in the fruit; *, **, and ***: statistical significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively; ^{ns}: nonsignificant.

Yield variables	FS	CDE
TNF	0.03^{ns}	0.04^{ns}
TFW	0.15***	-0.16***
AFW	0.13**	-0.21***

Agronomic behavior in the presence of *Alternaria* in advanced S_4 tomato lines

ANOVA revealed significant differences ($p \leq 0.05$) in the variables TFW, TNF, and AFW among genotypes, indicating significant genotypic variation within the line populations and the control (Table 3).

The present study found at least 14 advanced lines that statistically matched the fruit yield of the commercial control hybrid 'El Cid' (Table 4), which could be used as a source of germplasm or open-pollinated varieties. Similarly, some of these lines produced more and heavier fruits than the control.

Table 3. Mean squares of the yield variables evaluated in saladette tomato (*Solanum lycopersicum*) genotypes with a broad genetic base and commercial control. DF: Degrees of freedom; TFW: total weight of the fruits per plant; TNF: total number of fruits per plant; AFW: average weight of the fruit; CV: coefficient of variation; *, **, and ***: statistical significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Source of variation	DF	TFW	TNF	AFW
Repetition	2	30.55***	1101.94***	4906.45***
Genotypes	89	0.73***	165.95***	421.26***
Error	91	0.21	32.40	78.77
CV, %		13	12	13

Table 4. Average values of the three agronomic variables evaluated among the 15 highest-yielding saladette tomato (*Solanum lycopersicum*) genotypes studied. Genotypes with the same letter in each column are statistically equivalent (Tukey, $p \leq 0.05$). C: Hybrid 1; L: hybrid 2; R: hybrid 3; HSD: honestly significant difference.

Genotype	Origin	Total fruit weight	Total number of fruits per plant	Average fruit weight
		kg plant^{-1}	nr plant^{-1}	g
38	LOR85×C	4.53^{a}	47^{a-n}	132.15^{a-j}
90	EL CID	4.47^{a}	57^{a-k}	114.26^{a-m}
56	LOR81×C	4.24^{ab}	55^{a-l}	121.63^{a-l}
48	LOR95×C	4.19^{a-c}	60^{a-g}	108.01^{a-j}
67	LOR82×C	4.11^{a-d}	52^{a-n}	124.64^{a-k}
58	LOR81×C	4.09^{a-d}	61^{a-d}	128.99^{a-k}
05	LOR85×L	3.98^{a-d}	60^{a-h}	108.14^{d-m}
82	LOR103×R	3.97^{a-d}	54^{a-m}	133.90^{a-j}
57	LOR81×C	3.92^{a-d}	55^{a-m}	128.99^{a-k}
49	LOR95×C	3.92^{a-d}	53^{a-n}	131.21^{a-j}
72	LOR111×C	3.92^{a-d}	49^{a-n}	118.56^{a-m}
68	LOR82×C	3.86^{a-d}	39^{j-n}	141.79^{a-h}
50	LOR95×C	3.85^{a-d}	49^{a-n}	135.81^{a-i}
40	LOR85×C	3.83^{a-d}	45^{a-n}	125.79^{a-k}
02	LOR85×L	3.81^{a-d}	39^{i-n}	152.27^{a-e}
HSD		1.61	20.19	31.48

Alternaria lycopersici identification as a pathogen that causes yield loss in S_4 lines

Phylogenetic analysis allows the accurate and correct identification of pathogen taxonomies (Gou et al., 2023). The concatenated sequences of the ITS region and the *ALTA1* gene from isolates CPO 27 and CPO 28 were used to build the phylogenetic tree. The final matrix consisted of 955 characters (Table 5). The Bayesian analysis was run for 10 million generations with a final standard deviation of 0.003, producing 20 002 trees, of which 15 002 were used to calculate the posterior probability.

Table 5. Characteristics of the concatenated matrix used to construct the *Alternaria* phylogenetic tree. ITS: Internal transcribed spacer; *ALTA1*: main allergen-encoding gene for *Alternaria*; bp: base pairs.

Gene/region	Number of characters (pb)	Nucleotide substitution model
ITS	416	GTR + I
ALTA1	539	HKY +I

Strains CPO 27 and CPO 28 were grouped in the same clade as the type of strain of *A. lycopersici* (Figure 4). In the pathogenicity test, *A. lycopersici* was described as a species with small conidia that causes lesions in the leaf with an area of 11.50 ± 0.20 mm (Gou et al., 2023).

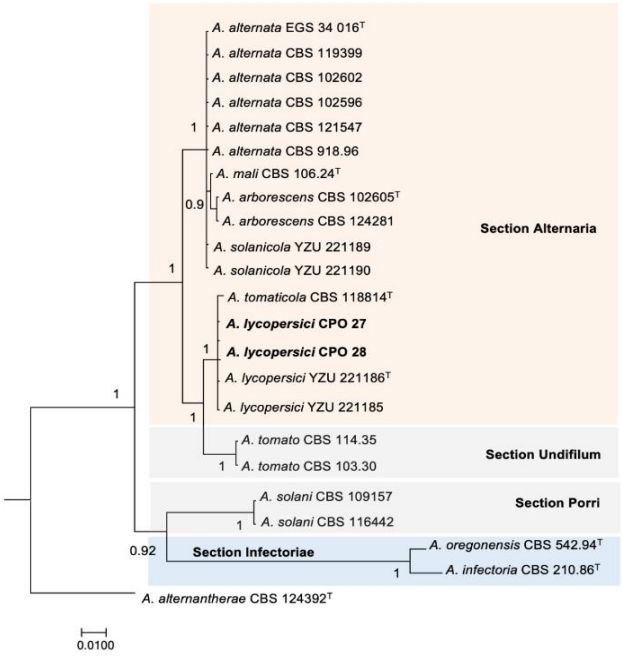

Figure 4. Phylogenetic tree for *Alternaria* spp. built using the Bayesian inference method and maximum likelihood for concatenated sequences of the ITS region and *ALTA1* gene.

Influence of *A. lycopersici* on Ca absorption in fruit and yield of advanced S_4 lines

The susceptibility to *A. lycopersici* (SALy) variable showed a positive correlation with the variables AFW and TFW ($r = 0.93^{***}$ and $r = 0.97^{***}$, respectively) (Table 6), indicating that the greater the susceptibility to *A. lycopersici*, the higher the level of affectation in the average weight of the fruit and the total weight of fruits per plant. Plant susceptibility to *A. lycopersici* is negatively correlated with the total number of fruits with blossom end rot per plant ($r = -0.96^{***}$).

Table 6. Spearman correlation coefficient between susceptibility to *Alternaria lycopersici*, fruit Ca absorption, and yield variables in advanced saladette tomato (*Solanum lycopersicum*) lines. TFW: Total fruit weight; AFW: average fruit weight; TNF: total number of fruits per plant; SALy: genotype susceptibility to *A. lycopersici*; CaF: Ca concentration in fruit; TNF with AN: total number of fruits with blossom end rot per plant; *, **, and ***: statistical significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively; ^{ns}: nonsignificant.

Yield components	SALy	CaF	TNF with AN
AFW	0.93^{***}	0.97^{***}	-0.91^{***}
TFW	0.97^{***}	0.97^{***}	-0.97^{***}
TNF	0.55^{*}	0.43^{ns}	-0.52^{*}
TNF with AN	-0.96^{***}	-0.92^{***}	

The variable Ca in the fruit showed a positive correlation with both AFW and TFW ($r = 0.97^{***}$). This means that the more Ca in the fruit, the heavier the individual fruit is and the higher the overall crop yield. Similarly, there is a negative relationship between CaF and TNF with blossom end rot ($r = -0.92^{***}$), indicating that the lower the amount of Ca in the fruit, the more fruits with blossom end rot per plant. The variable total number of fruits with blossom end rot per plant had negative correlations with AFW, TFW, and TNF ($r = -0.91^{***}$, $r = -$

0.97***, and r = -0.52***, respectively), which indicates that the greater the number of fruits with blossom end rot, the lower the average weight, total fruit weight, and number of fruits per plant produced by the crop; thus, Ca reduction in the fruit affects the potential yield in advanced S_4 lines.

During the spring-summer 2023 season, the genotype SALy effect on the Ca absorption capacity in fruit and yield of advanced S4 lines was evaluated. When comparing the advanced lines Rd-03 (resistant) and Nf-05 (susceptible) in terms of the variables CaF and TFW, a Ca absorption capacity in fruit of 7.38% and a loss of potential yield of 25.36% were reported in Nf-05 in relation to the Rd-03 genotype (Table 7).

Table 7. Average values for five agronomic traits of six tomato (*Solanum lycopersicum* L.) genotypes identified during the spring-summer 2020 agricultural cycle. Genotypes with the same letter in each column are statistically equivalent (Tukey, $p \leq 0.05$). Rd: yield; Nf: necrosis in fruit; TFW: total fruit weight per plant; TNF: total number of fruits per plant; AFW: average fruit weight; SALy: genotype susceptibility to *Alternaria lycopersici;* CaF: Ca concentration in fruit. HSD: honest significant difference.

Genotype	Origin	TFW	TNF	AFW	SALy	CaF
		kg plant^{-1}	nr plant^{-1}	g		µg g^{-1}
Rd-01	LOR95×C	4.00^{a}	67^{a}	107.11^{b}	1^{a}	162.33^{b}
Rd-02	LOR95×C	4.06^{a}	56^{a-b}	129.11^{a-b}	1^{a}	192.33^{a}
Rd-03	LOR95×C	4.11^{a}	50^{b}	137.22^{a}	1^{a}	203.00^{a}
Nf-04	LOR81×L	1.86^{b}	52^{b}	68.30^{c}	6^{b}	35.00^{c}
Nf-05	LOR79×C	1.04^{b}	48^{b}	43.76^{c}	8^{c}	15.00^{c}
Nf-06	LOR111×C	1.52^{b}	51^{b}	59.91^{c}	7^{c}	24.00^{c}
HSD		0.92	11.53	25.82	1.27	26.15

DISCUSSION

Exploring the genetic potential in lines with resistance to *A. lycopersici* and Ca deficiency

To express tomato genetic potential, it is necessary, to the greatest extent possible, to reduce adverse environmental effects such as pathogen-caused diseases (Thomazella et al., 2021) or physiological effects (Bai et al., 2018). *Alternaria* is a widely distributed pathogen in tomato crops, and under favorable conditions of fungus proliferation, it reduces crop yield capacity and, in extreme cases, results in total production loss (Zhao et al., 2022).

Calcium is a key macronutrient in tomato crop nutrition. Calcium deficiency, which results from decreased absorption and transport in the plant (Karlsons et al., 2023), leads to blossom end rot and abnormal fruit development (Hocking et al., 2016), and is classified as a physiological disease (Topcu et al., 2021). Necrosis typically occurs 15 d after flowering, when the growth rate of the fruit increases (Cardona et al., 2005; Ruiz et al., 2008). According to Foolad (2007), wild tomato germplasm is an important source of genes conferring resistance to biotic and abiotic factors, as well as the ability to improve the characteristics of commercial materials.

In this research, genotypes 48, 49, and 53 were identified for displaying no leaf lesions and no blossom end rot in fruit, which is indicative of *Alternaria* and Ca deficiency. The heterotic richness provided by the expanded genetic baseline lines has the ability to prevent blossom end rot caused by Ca deficiency in the plant, which in extreme cases results in a 50% loss in total crop production (Djangsou et al., 2019), as well as possessing attributes that allow for greater crop health.

Effect of *A. lycopersici* on the yield of S4 genotypes

Genotypes 48 and 49 of LOR95×C origin (Table 4) had statistically comparable yields to the commercial control, natural resistance to *Alternaria* (Figure 2), and no Ca deficiency (Figure 3). This means that these advanced lines, resulting from crosses between native germplasm and lines derived from commercial "saladette" type hybrids, can increase yield potential to levels like the commercial control.

Alternaria lycopersici under natural infestation conditions in S_4 lines

It is necessary to identify the present *Alternaria* species to avoid crop yield losses. *Alternaria lycopersici* was identified as the causal agent for Ca deficiency and yield loss in isolates CPO 27 and CPO 28 using phylogenetic analysis. Gou et al. (2023) provided the first description of *A. lycopersici* for tomato cultivation in the world. However, because Mexico is the center of tomato domestication and diversity (Salgado-Meraz et al., 2018), it is more susceptible to the presence of phytopathogenic fungi. Despite its recent discovery in China, this species can be found in Mexico, causing leaf damage and, as a result, crop yield loss.

Negative impact of *A. lycopersici* on S_4 lines

During the spring-summer 2023 season, despite using the Steiner (1961) nutrient solution throughout the crop cycle, genotypes susceptible to *A. lycopersici* showed Ca absorption capacity decreases in fruit, with readings of 26.15 µg g^{-1} and individual fruit weight of 59.91 g. In contrast, resistant materials had readings of 162.33 µg g^{-1}, which is within the normal parameters of Ca requirements in fruit (Hocking et al., 2016), with an individual fruit weight of 107.11 g. This suggests that the lower the resistance to *A. lycopersici* in advanced lines, the more fruits with blossom end rot and lower average fruit weight due to calcium deficiency in the plant (Table 6).

Alternaria lycopersici may produce leaf lesions (Gou et al., 2023), which prevents the plant from properly absorbing Ca, which is essential to reach the normal size and weight of the tomato fruit. The cell wall of the fruit is a source of oligogalacturonides, such as cytosolic Ca, which are responsible for the signaling mechanisms that occur during the defense response, causing an alteration in the Ca supply in the fruit (Hocking et al., 2016). This process manifests itself as blossom end rot, which affects development (Santos et al., 2023).

CONCLUSIONS

Alternaria lycopersici was identified as the pathogen responsible for Ca deficiency in tomato fruit and yield loss. Five resistant lines (± 0% foliar severity) were identified, while 52 lines showed moderate resistance (≤ 25% foliar severity) to *A. lycopersici*. Genotypes 48 and 49 of LOR95×C origin outperformed the control for total fruit weight, with yields of 4.19 and 3.92 kg $plant^{-1}$, respectively. They were also naturally resistant to *A. lycopersici* and did not exhibit Ca deficiency. As a result, they were selected in order to estimate the yield loss of materials susceptible and resistant to *A. lycopersici*. Plant Ca deficiency tests resulted in the identification of 24 advanced lines without blossom end rot.

When comparing the advanced lines Rd-03 (resistant) and Nf-05 (susceptible) in the variables of Ca concentration in fruit and total fruit weight per plant, a Ca absorption capacity in fruit of only 7.38% and a loss of potential yield of 25.36% were reported in Nf-05 compared to Rd-03. This is attributed to the presence and degree of susceptibility to *A. lycopersici* in the evaluated genotypes. Advanced lines with high total fruit weight per plant and resistance to *A. lycopersici* can be used as open-pollinated varieties or as germplasm for improving elite materials in the program. The main contributions of these lines will be reflected in a reduction in agrochemical use to prevent yield loss through host resistance.

Author contribution

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Acknowledgements

The principal author, Alba Melissa Castañeda-Villa, would like to thank the Consejo Nacional de Humanidades, Ciencias y Tecnologías (Conahcyt) for providing the academic scholarship to pursue a Doctorate in Science in Genetic Resources and Productivity at the Colegio de Postgraduados, Campus Montecillo.

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