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Research Article
Agro-Morphological Characterization and Bacterial Wilt Screening of Eleven Tomato (*Solanum lycopersicum* L.) Lines for Registration in Benin

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Abstract

Background and Objective: The tomato (*Solanum lycopersicum* L. Mill) is a major crop in Benin, whose productivity is hampered by the lack of high-performance varieties adapted to local conditions. The objective of this study was to evaluate the agro-morphological diversity and bacterial wilt resistance of 11 candidate tomato (*Solanum lycopersicum* L.) lines in Benin through the analysis of their qualitative, quantitative and phenological traits under field conditions across two growing seasons. **Materials and Methods:** Field trials were conducted over two consecutive growing seasons using a completely randomized block design with three replications. Eleven tomato (*Solanum lycopersicum* L.) lines, candidates for registration in Benin, were evaluated. Agro-morphological diversity was assessed through qualitative, quantitative and phenological traits following standard descriptors. Varietal resistance to bacterial wilt was screened under natural field infection conditions and disease incidence was recorded throughout the crop cycle. **Results:** The results reveal significant variability between lines for traits such as plant height, fruit weight and earliness, with remarkable stability in qualitative characteristics. Statistical analyses and hierarchical classification identified high-performing genotypes, such as CLN4398M and CLN4018G, while highlighting season × variety interactions for certain parameters. The lines yielded more than 50 tons/ha, more than eight times the national average (8 tons/ha). The varietal resistance approach showed that the eleven varieties used in the control of bacterial wilt of solanaceous crops have an incidence of between 0 and 5% and can be grouped into the class of highly resistant varieties (CLN2498D, AVTO1955-15, CLN4270F, CLN4398M, CLN4270D, CLN4018G, CLN4270B, CLN4270I, CLN4398D and CLN4270E) and in the resistant variety class (CLN4066G). **Conclusion:** These results offer promising prospects for genetic improvement and the registration of varieties adapted to Beninese conditions, thereby contributing to food security and agricultural development.

Key words: Tomato, phenotypic diversity, inter-seasonal stability, bacterial wilt screening, DUS, Benin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The tomato (*Solanum lycopersicum* L. Mill) is a crucial food and economic resource for many countries, including Benin, where it plays a major role in vegetable production systems. However, national production continues to face major challenges, including a lack of varieties that are adapted and resilient to local biotic and abiotic constraints, which significantly limits yields[1]. To address this issue, genetic improvement and the introduction of new high-performance and resilient varieties are essential strategies for ensuring food security and improving producers' incomes[2,3].

In Benin, the registration of new varieties is governed by strict processes, including the assessment of distinctness, uniformity and stability (DUS), in accordance with National[4] and International Guidelines such as those of UPOV[5], AVRDC[6], IPGRI[7] and Bioversity International[8]. This evaluation is based on a detailed agromorphological characterization of candidate lines, involving the analysis of numerous qualitative, quantitative and phenological traits. Similar studies have been conducted on other crops or tomato varieties to assess their diversity and suitability for breeding programs[9-11]. Lone *et al*.[5] characterized various cherry tomato accessions, demonstrating the importance of morphological descriptors for varietal distinction. Similarly, variability of tomato germplasm for breeding research[12]. Phenological parameters, such as the length of the vegetative or floral cycle, are also crucial because they determine a variety's adaptation to a given environment and its ability to escape certain constraints[13].

This study aims to carry out a complete agromorphological characterization of 11 tomato lines that are candidates for approval in Benin. More specifically, this second part of the study focuses on the combined analysis of qualitative, quantitative and phenological traits to assess phenotypic diversity and group these lines by similarity, incorporating variables related to resistance to bacterial wilt. This approach is crucial for identifying high-performing genotypes and guiding breeding and variety approval programs.

MATERIALS AND METHODS

Study area: The study was conducted at the Vegetable Crops Program (PCM) research station, located at the Agonkanmey Agricultural Research Center (CRA Agonkanmey) of the National Institute for Agricultural Research of Benin (INRAB), in the municipality of Abomey-Calavi (6°24'35" North and 2°19'55" East). This site, located in the Atlantic Department in Southern Benin, served as an experimental setting for two growing seasons. The study was conducted in the big rainy season period in Southern Benin from 21 June to 14 October, 2022 for the first trial and from 28 April to 15 September, 2023 for the second.

Vegetal material: The study focused on 11 tomato lines (*Solanum lycopersicum* L. Mill) selected as candidates for certification, resulting from collaborations between the World Vegetable Center and national research institutions in Benin and two control varieties (PADMA (hybrid, East-West Seed Company) and TLCV15 (variety registered in CaBEV and distributed by INRAB). These lines represent a range of genotypes potentially adapted to Benin's agro-ecological conditions. All lines except PADMA are open-pollinated and are listed in Table 1.

Conducting the experiment: The experimental design consisted of a completely randomized block (CRB) trial with 13 treatments (11 new lines+2 control varieties) and 4 replicates, set up at the Vegetable Crops Program (PCM)/National Institute for Agricultural Research of Benin (INRAB) station in Agonkanmey (Southern Benin) over two seasons (2022 and 2023). Each elementary plot comprised 40 plants in spaced rows (0.8×0.5 m), giving a density of 25,000 plants/ha.

The crop was grown according to recommended farming practices[2], with organic-mineral fertilization, integrated pest and disease management and regular watering, the variables of which are not considered here. Transplanting took place 21 days after sowing.

Parameters measured: The parameters evaluated and the methodology for collecting the relevant data during the two trials were chosen on the basis of three tomato descriptors: The CaBEV certification and registration procedures manual[4], the UPOV descriptor[5] and the IPGRI tomato descriptor[7,8]. Eighteen parameters were evaluated, including nine qualitative and nine quantitative parameters.

Qualitative variables: Observations focused on characteristics of the plant, leaves, flowers and fruit. The qualitative parameters collected are detailed in Table 2. These included inflorescence type, fruit shape, fruit color at maturity and other visual characteristics that distinguish between varieties.

Table 1: List of tomato lines tested

Varieties names	Origin	Observations
CLN2498D	World Vegetable Center	Varieties eligible for registration with CaBEV
AVTO1955-15		
CLN4270F		
CLN4066G		
CLN4398M		
CLN4270D		
CLN4018G		
CLN4270B		
CLN4270I		
CLN4398D		
CLN4270E		
PADMA	East-West Seed Company	Control varieties currently being distributed
TLCV15	INRAB	

Table 2: Qualitative parameters observed

Qualitative parameter	Qualitative parameter specifications
Anthocyanin pigmentation of the stem	Absent/low/medium/high/very high
Leaf density	Low/medium/high
External color of immature fruit	Whitish green/light green/green/dark green/very dark green
Type of inflorescence	Single-pair/two-pair/multi-pair
Pistil scar shape	Point-shaped/star-shaped/linear/irregular
Firmness of the fruit	Soft/medium/firm
Fruit shape in cross section	Round/angular/irregular
Top shape	Depressed/depressed with flattening/flattening/flattening with pointed tip/pointed
Longitudinal section shape	Significantly flattened/flattened/round/oblong/cylindrical/elliptical/heart-shaped/oval/obovate/pear-shaped/obcordate

Quantitative variables: These characteristics were measured accurately on a representative sample of plants. They included variables such as plant height (cm), number of leaves, stem diameter (cm), number of flowers per cluster, number of fruits per cluster, average fruit weight (g), fruit diameter (cm), fruit length (cm), yield per plant (kg) and yield per hectare (ton/ha).

Phenological variables: These parameters were recorded in days after transplanting (DAT). They included the number of days to 50% flowering and the number of days to 50% fruiting. These variables are crucial for assessing the earliness and adaptation of the life cycle of the lines.

Measurements on the plants were taken exclusively on the two central rows of each elementary plot to avoid edge effects. For each plot, 10 plants were selected at random and permanently marked: 5 plants on each central row.

The qualitative and quantitative parameters of the fruit were assessed only once during the experiment, after harvesting. For each variety and in each block, 10 fruits were randomly selected to constitute the analysis sample.

Evaluation of the presence of *R. solanacearum* in the soil at the experimental site: Before setting up the trial, a composite sample of 1 kg of soil was taken from nine samples on 15 m^2 plots at the experimental site. A total of 52 samples were obtained. A quantity of 10 g of each homogenized sample was suspended in 100 mL of sterile water and then left to settle. After half an hour, 45 mL of the supernatant was collected and adjusted to 50 mL in Falcon tubes, then centrifuged at 6000 revolutions per minute (rpm) for 5 min. The supernatant was then discarded and a stock solution was prepared from the pellet and 2 mL of sterile distilled water. From this stock solution, two successive dilutions were made. Petri dishes containing modified SMSA medium were seeded with 100 µL of each dilution. The dishes were incubated at 30°C for 48 hrs before counting the *R. solanacearum* colonies[14] (Fig. 1). The concentration of *R. solanacearum* inoculum was estimated using the following formula:

$$C\ (\text{UFC/g}) = \frac{\text{Number of colony of } R.\ solanacearum}{45\ \text{mL}} \times 100\ \text{mL} \times 10^2$$

Where:
C = Inoculum concentration in the soil
100 mL = Volume of water used for settling
45 mL = Volume of supernatant taken for centrifugation

Assessment of the incidence of bacterial wilt: To assess the incidence of bacterial wilt on the tomato varieties tested, wilted plants were systematically counted across the entire plot. A water test was performed each time to check for signs of bacterial wilt (Fig. 2). The incidence of bacterial wilt (IBW) was calculated using the following formula:

$$P\ (\%) = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

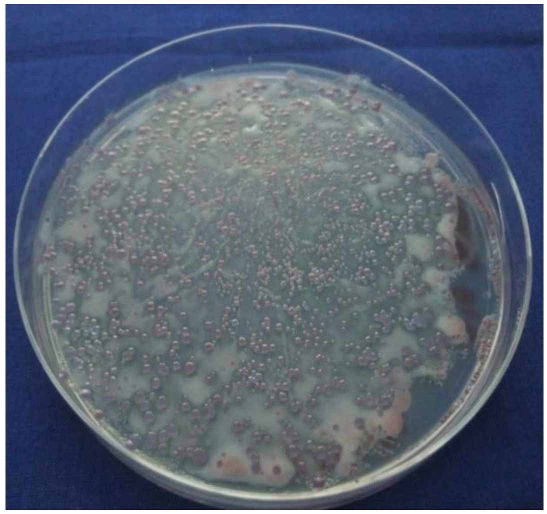

Fig. 1: Colony of *R. solanacearum* after incubation

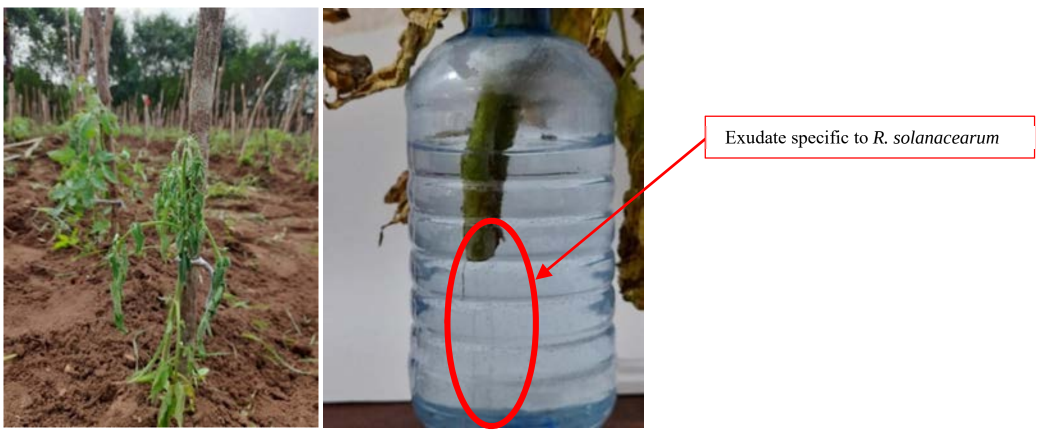

Fig. 2: Water test for detecting exudate flow caused by *Ralstonia solanacearum*

Table 3: Classification scale for the incidence of wilting

Plant behavior	Incidence of bacterial wilt (IBW)
Highly resistant (HR)	0%
Resistant (R)	1-10%
Moderately resistant (MR)	>10-20%
Moderately susceptible (MS)	>20-30%
Susceptible (S)	>30-70%
Highly susceptible (HS)	>70%

Source: Mew and Ho[15]

The resistance levels of tomato varieties to bacterial wilt were assessed according to the classification scale of Mew and Ho[15] (Table 3).

Data processing and analysis: The analysis of qualitative morphological traits was descriptive, aiming to identify variability and distinct patterns within the 13 lines (11 new+2 controls). The effect of season, lines and blocks on the variation in continuous quantitative agromorphological parameters was evaluated using mixed linear models applied to data from the two experimental seasons, considering season and line as fixed factors and block as a random factor. Four model structures were compared: The complete model, the random intercept model, the random slope model and the random intercept and slope model. These analyses were conducted using the "lmerTest" package[16]. The selection of the optimal model was based on minimizing the Akaike Information Criterion (AIC).

Phenological data and discrete quantitative agromorphological parameters were analyzed using a generalized linear model of the Poisson family with mixed effects. Pairwise comparisons of means between the two seasons were performed using Tukey's *post hoc* test at a significance level of 5%. The goodness of fit of the mixed linear models was assessed by calculating the marginal and conditional coefficients of determination R^2, using the r.squaredGLMM() function from the MuMIn package[17], which implements the method of Nakagawa and Schielzeth[18]. The marginal R^2 quantifies the variance explained by fixed effects alone, while the conditional R^2 represents the total variance explained by the entire model (fixed and random effects).

A Mixed Data Factor Analysis (MDFA) was applied to simultaneously explore the quantitative parameters measured at 50% fruiting, quantitative fruit data and categorical growth and fruiting variables for each growing season. Adjusted mean values from mixed linear models were used for quantitative variables.

The observed variability was structured using Ascending Hierarchical Classification (AHC), using Euclidean distances and Ward's aggregation method, applied separately to each season. This approach made it possible to identify and characterize variety groups for each season. A comparative analysis of the dendrograms obtained for the two seasons was conducted to identify similarities and differences in the structuring of variety groups.

All statistical analyses and visualizations were performed using R software version 4.3.0, supplemented by Microsoft Excel 2016 for preliminary data processing.

RESULTS AND DISCUSSION

Diversity of qualitative traits and their stability between seasons according to the tomato line: Comparative analysis of the qualitative morphological traits of 13 tomato lines (11 new+2 controls) over two consecutive seasons reveals both notable diversity between lineages and remarkable stability of traits within each line (Table 4 and 5).

The phenotypic diversity observed between lines is mainly manifested through several distinctive morphological characteristics. The type of inflorescence is the most marked differentiation criterion, with three distinct modalities: The uniparous inflorescence observed in lines AVTO1955-15, CLN4270D, CLN2498D, CLN4398M and TLCV15; the biparous inflorescence characterizing the lines CLN4018G, CLN4270I, CLN4066G and CLN4270B and the rarer multiparous inflorescence found only in CLN4270F and CLN4270E. This variability is accompanied by notable differences in leaf morphology, with three lines (AVTO1955-15, CLN4270E and CLN4270B) distinguished by potato-type leaves, a particular morphological characteristic that could have important implications in terms of pathogen resistance or photosynthetic efficiency, while the other lines have standard foliage.

The shape of the fruit also offers great morphological variability, allowing the lines to be clearly differentiated. Round fruit is observed in CLN4018G, CLN4398D and TLCV15, distinctive heart-shaped fruits in CLN4398M and CLN4270B, various oval to obovate shapes in several other lines and the rare potato shape found in AVTO1955-15 and CLN4270E. This morphological diversity extends to other distinguishing characteristics such as style hairiness, which is absent in CLN4270I, CLN4066G and CLN4270B but present in other varieties, the peduncle attachment with marked depressions in certain lines such as CLN4018G, AVTO1955-15 and CLN4270D and color characteristics varying from light green to whitish green with different intensities depending on the line.

Beyond this inter-line diversity, the analysis reveals exceptional stability in qualitative characteristics for each line between the two seasons, demonstrating the remarkable genetic consistency of the plant material studied (Table 4 and 5). The major morphological characteristics show absolute stability between seasons, particularly the leaf type, which remains perfectly constant with the AVTO1955-15, CLN4270E and CLN4270B retaining their potato-type leaves, while all others maintain their standard foliage. The inflorescence type also shows remarkable stability, with only very slight variations that can be explained more by better characterization in the second season than by actual morphological changes.

The shape of the fruit shows excellent reproducibility between seasons, with round-fruited lines maintaining this characteristic, cordate shapes remaining perfectly consistent and oval to obovate shapes remaining consistent. This stability also extends to fruit firmness, which remains very stable with identical classifications for the vast majority of lines, as well as to color characteristics, where the external color of the immature fruit remains consistent for each line and the intensity of the external color varies slightly but remains within the same ranges for each line.

Table 4: Qualitative morphological characteristics of the different lines during the first season

Character	CLN2498D	CLN4398D	CLN4270E	PADMA	TLCV15	AVTO1955-15	CLN4270F	CLN4066G	CLN4398M	CLN4270D	CLN4018G	CLN4270B	CLN4270I
Anthocyanin pigmentation of the stem	Low	Low	Low	Low	Low	Absent	Low	Absent	Medium	Low	Low	Low	Low
Leaf density	Medium	Medium	Medium	Low	Forte	Faible	Moyenne	Low	Medium	Medium	Medium	Medium	Medium
External color of immature fruit	Light green	Whitish green	Light green	Whitish green	Whitish green	Light green	Light green	Light green	Whitish green	Light green	Whitish green	Light green	Light green
Type of inflorescence	Dot-shaped	Star-shaped	Dot-shaped	Dot-shaped	Dot-shaped	Dot-shaped	Star-shaped	Star-shaped	Irregular	Dot-shaped	Irregular	Star-shaped	Dot-shaped
Pistil scar shape	Single-pair	Two-pair	Multi-pair	Single-pair	Two-pair	Single-pair	Two-pair	Single-pair	Single-pair	Two-pair	Two-pair	Two-pair	Multi-pair
Firmness of the fruit	Medium	Soft	Firm	Firm	Firm	Firm	Firm	Medium	Medium	Firm	Medium	Firm	Firm
Fruit shape in cross section	Round	Round	Round	Round	Angular	Round	Round	Round	Round	Round	Angular	Round	Round
Top shape	Flattened	Flattened	Pointed	Flat to pointed	Flat to pointed	Pointed	Flattened	Pointed	Flattened	Flattened	Depressed to flattened	Flattened	Flattened
Longitudinal section shape	Round	Round	Obovate	Obovate	Oblong	Obovate	Round	Obovate	Oblong	Oblong	Flattened	Round	Round

Table 5: Qualitative morphological characteristics of the different lines during the second season

Character	CLN4018G	CLN4398D	CLN4270I	AVTO1955-15	PADMA	CLN4270D	CLN2498D	CLN4066G	CLN4270F	CLN4398M	TLCV15	CLN4270E	CLN4270B
PA	Present	Present	Present	Absent	Present	Present	Absent	Absent	Present	Present	Present	Present	Present
IPA	Low	Low	Low	Absent	Low	Low	Low	Absent	Low	Medium	Low	Low	Low
PH	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
TF	Standard	Standard	Standard	Pomme de terre	Standard	Standard	Standard	Standard	Standard	Standard	Standard	Pomme de terre	Pomme de terre
PS	Present	Present	Absent	Present	Present	Absent	Present	Absent	Absent	Present	Absente	Absent	Absent
PF	Half drop	Half drop	Half drop	Half drop	Half drop	Half drop	Half drop	Half drop	Half drop	Half drop	Half drop	Half drop	Half drop
TI	Two-pair	Two-pair	Multi-pair	Single-pair	Single-pair	Two-pair	Single-pair	Single-pair	Two-pair	Single-pair	Two-pair	Multi-pair	Two-pair
FSL	Round	Round	Obovate	Obovate	Obovate	Obovate	Oval	Obovate	Cordiforme	Round	Obovate	Oval	Cordiforme
DAP	Low	Absent or very weak	Absent or very weak	Low	Absent or very weak	Absent or very weak	Low	Absent or very weak	Low	Absent or very weak	Low	Absent or very weak	Absent or very weak
FS	Depressed to flattened	Flat to pointed	Flat to pointed	Flat to pointed	Flat to pointed	Flat to pointed	Flattened	Flat to pointed	Flat to pointed	Flattened	Flat to pointed	Flattened	Flat to pointed
FCT	Angular	Round	Round	Round	Round	Round	Round	Round	Round	Round	Angular	Round	Round
FCP	Irregular	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed	Pointed
CAP	Low	Absent or very weak	Absent or very weak	Absent or very weak	Low	Absent or very weak	Faible	Absent or very weak	Low	Absent or very weak	Absent or very weak	Absent or very weak	Absent or very weak
CEF	Whitish green	Whitish green	Light green	Light green	Whitish green	Light green	Light green	Light green	Light green	Whitish green	Whitish green	Light green	Light green
ICE	Medium	Low	Medium	Strong	Strong	Medium	Strong	Low	Forte	Medium	Strong	Medium	Medium
FF	Medium	Soft	Firm	Firm	Firm	Firm	Medium	Medium	Firm	Medium	Firm	Firm	Firm

ICP: Intensity of hypocotyl color, PH: Pubescence of hypocotyl, DF: Leaf density, TF: Leaf type, PF: Leaf habit, PS: Style pubescence, TI: Inflorescence type, FSL: Longitudinal section shape, DAP: Depression of the peduncle attachment, FS: Shape at the top of the fruit, FCT: Cross-sectional shape, FCP: Shape of the pistil scar, CAP: Ribbing at the peduncle attachment, CEF: External color of the fruit, ICE: Intensity of the external color of the fruit and FF: Firmness of the fruit

Table 6: Effect of factors on growth parameters

Variable	Factor	Num DF	Den DF	F value	Pr(>F)	Marginal R^2	Conditional R^2
Height (cm)	Season	1	40	430.72	<2.2e-16***	0.5121	0.5850
	Variety	12	37	3.75	0.00096***		
	Season: Variety	12	39	1.45	0.1859		
Diameter at the collar (mm)	Season	1	936	0.01	0.9078	0.0230	0.0230
	Variety	12	936	0.87	0.5828		
	Season: Variety	12	936	1.09	0.362		
Pedicel length (mm)	Season	1	3	76.35	0.00331**	0.1764	0.1863
	Variety	12	72	4.04	8.85e-05***		
	Season: Variety	12	72	1.78	0.0683		
Length of pedicel from the abscission zone (mm)	Season	1	3	3.42	0.1607	0.2507	0.4420
	Variety	12	34	6.83	4.3e-06***		
	Saison: Variety	12	34	1.50	0.172		
Length between nodes at 50% fruiting (mm)	Season	1	3	5.27	0.10718	0.1228	0.3693
	Variety	12	34	2.28	0.02897*		
	Season: Variety	12	32	0.65	0.78495		
		DF		Chi^2	$Pr(>Chi^2)$		
Number of branches	Season	1	229.15	0.172	0.3561	0.3569	
	Variety	12	208.29	<2.2e-16***			
	Season: Variety	12	111.62	0.227			

The results of this study reveal marked phenotypic diversity among the 11 tomato lines candidate for registration in Benin, in terms of both qualitative and quantitative phenological characteristics. This variability is essential for responding to local agronomic challenges, particularly adaptation to biotic and abiotic constraints[19]. The stability of qualitative traits between the two seasons, such as inflorescence type and fruit shape, confirms the genetic robustness of the lines, a key criterion for variety approval[8]. These observations are consistent with the work of Figàs *et al.*[20], which highlights the importance of morphological stability for varietal distinctness.

Agromorphological characterization of tomato lines: Inter-seasonal comparison: Analysis of quantitative traits also revealed significant phenotypic variability among the 13 tomato lines.

Variability and stability of growth characteristics: The highly significant variety effect (F = 3.75, p = 0.00096) reveals significant genetic variability between lines in terms of plant height (Table 6). The PADMA had the greatest height (71.16 cm), followed by CLN4270E (67.85 cm) and CLN4270B (65.55 cm), while TLCV15 had the lowest height (49.12 cm). This variation range of 22.04 cm indicates substantial selection potential for this architectural trait (Fig. 3a). The insignificant season×variety interaction (p = 0.1859) indicates high phenotypic stability. The variety hierarchy is maintained between seasons, ensuring the predictability of performance for this architectural trait.

The absence of a significant varietal effect (F = 0.87, p = 0.5828) indicates low genetic variability between lines for collar diameter. Values range from 9.03 mm (CLN4066G) to 10.85 mm (CLN4398M), suggesting relative genetic homogeneity for this trait. The non-significant season×variety interaction (p = 0.362) confirms excellent phenotypic stability, making this trait highly predictable between environments.

The highly significant varietal effect (Chi^2 = 208.29, p<2.2e-16) demonstrates marked genetic variability in the number of branches (Table 6). The CLN4398M has the highest number of branches (8), followed by CLN4398D and TLCV15 (7 each), while CLN4270D and CLN4270E have the lowest number (4) (Fig. 3b). The non-significant season×variety interaction (p = 0.227) ensures high architectural stability, maintaining the varietal hierarchy between seasons.

The length of the pedicel shows a highly significant varietal effect (F = 4.04, p<0.0001). The PADMA has the greatest length (2.81 mm), followed by TLCV15 (2.75 mm), while AVTO1955-15 and CLN4398D have the lowest values (2.27 mm) (Fig. 3c). The season×variety interaction tends toward significance (p = 0.068), suggesting moderate stability with possible differential sensitivity of genotypes to environmental conditions. For pedicel length from the abscission zone, the highly significant variety effect (F = 6.83, p<0.000001) reveals high genetic variability. The TLCV15 and CLN4066G had the highest values (1.32 and 1.31 mm, respectively), compared to 0.79 mm for AVTO1955-15 (Fig. 3d). The non-significant season×variety interaction (p = 0.172) guarantees high phenotypic stability, allowing for reliable predictions of variety ranking.

The significant variety effect (F = 2.28, p = 0.02897) indicates moderate genetic variability for internode length at 50% fruiting. The PADMA has the maximum value (4.53 mm), while TLCV15 has the minimum value (3.31 mm) (Fig. 3e). The non-significant season×variety interaction (p = 0.785) confirms exceptional phenotypic stability for this architectural trait.

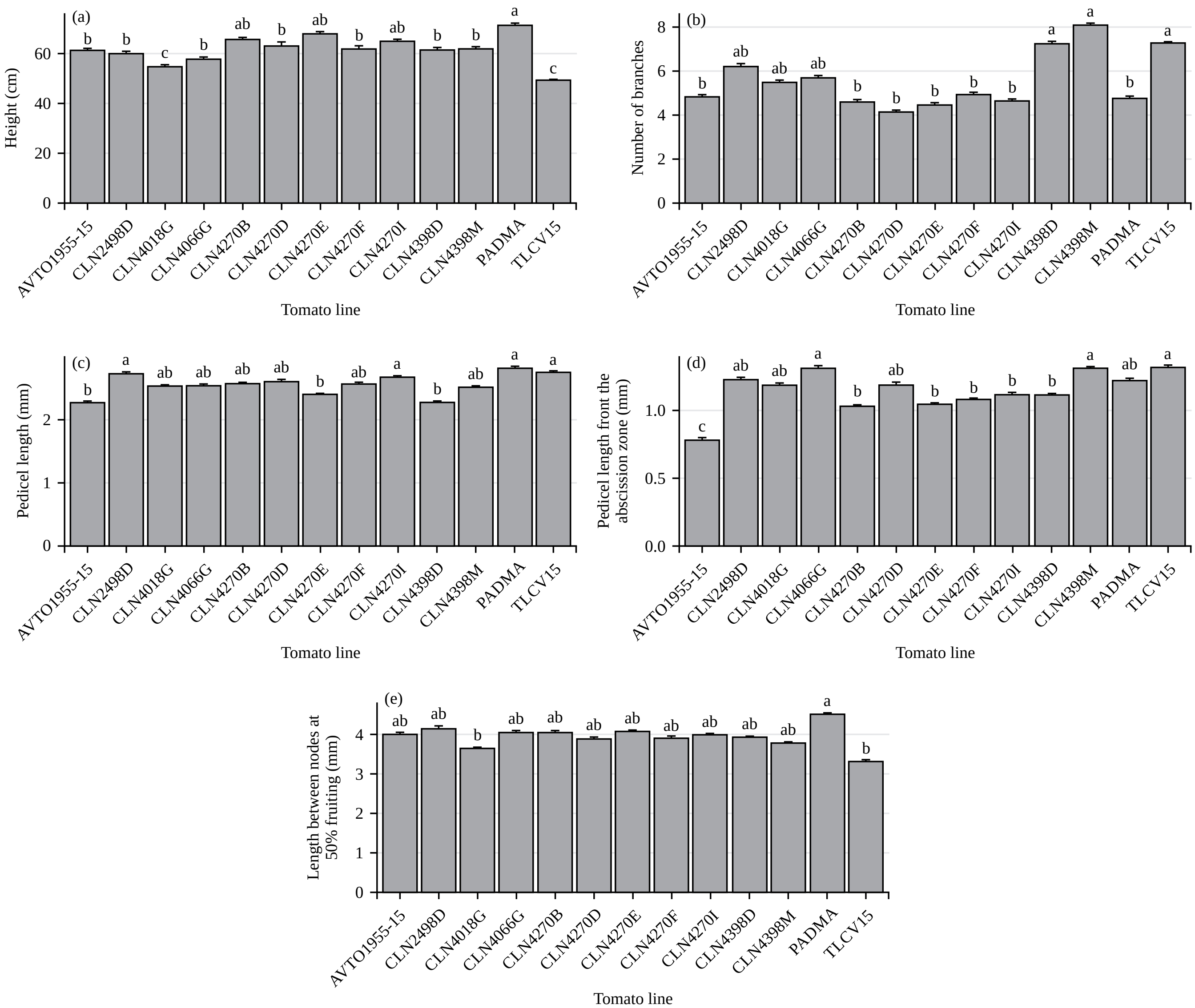

Fig. 3(a-e): Variation in plants growth parameters according to tomato line, (a) Mean values of plant height according to tomato line, (b) Mean values of number of branches according to tomato line, (c) Mean values of pedicel length according to tomato line, (d) Mean values of length of pedicel from the abscission zone according to tomato line and (e) Mean values of length between nodes at 50% fruiting according to tomato line

Bars with the same letter are not significantly different at the 5% threshold according to Tukey's test

Quantitative traits, such as plant height and fruit weight, show significant variability, offering potential for the selection of high-performing genotypes. For example, the CLN4398M and CLN4018G lines, with high fruit weight (approximately 100 g), could be favored for breeding programs aimed at increasing yields. However, the significant season×variety interaction for certain traits, such as fruit length, indicates environmental sensitivity, requiring multi-location evaluations to confirm their adaptation[25].

Variability and stability of production characteristics: The highly significant varietal effect ($F = 14.65$, $p<2.2e-14$) reveals high genetic variability for fruit length (Table 7). The CLN4066G has the maximum length (64.89 mm), followed by PADMA (59.73 mm), while CLN4398D has the minimum length (48.96 mm) (Fig. 4a). However, the highly significant season×variety interaction ($p = 2.13e-05$) indicates low phenotypic stability. Variety ranking can be significantly altered depending on seasonal conditions, requiring multi-environment evaluations.

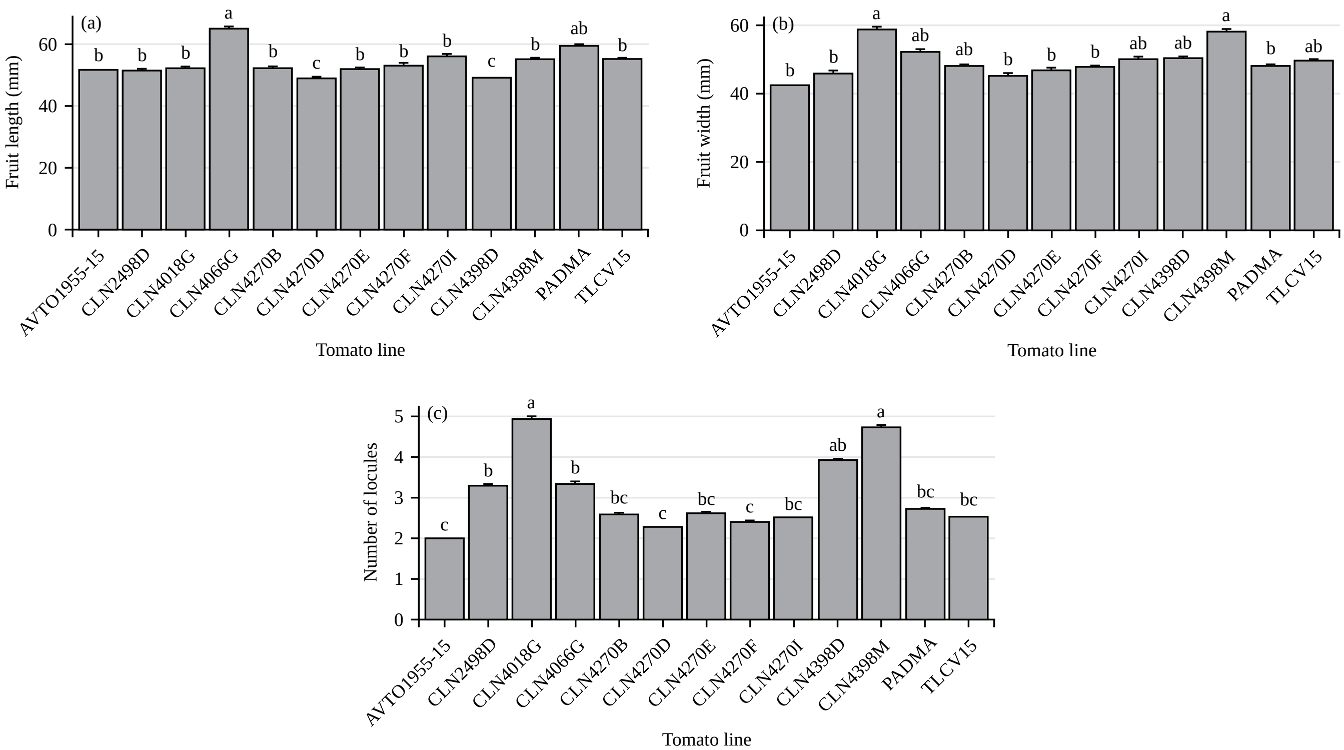

Fig. 4(a-c): Variation in average length, average width and average number of locules of fruits according to tomato line, (a) Mean values of fruit length according to tomato line, (b) Mean values of fruit width according to tomato line and (c) Mean values of number of locules according to tomato line

Bars with the same letter are not significantly different at the 5% threshold according to Tukey's test

Table 7: Effect of factors on yield parameters

Variable	Factor	Num DF	Den DF	F value	Pr(>F)	Marginal R^2	Conditional R^2
Fruit length (mm)	Season	1	6	233.50	5.32e-06***	0.6585	0.7149
	Variety	12	70	14.65	1.03e-14***		
	Season: Variety	12	70	4.56	2.13e-05***		
Fruit width (mm)	Season	1	6	279.24	2.91e-06***	0.6725	0.7254
	Variety	12	71	15.08	4.25e-15***		
	Season: Variety	12	71	2.44	0.01004*		
Fruit weight (g)	Season	1	6	711.89	0.172	0.7721	0.7895
	Variety	12	71	35.11	<2.2e-16***		
	Season: Variety	12	71	14.87	0.595		
Potential yield (ton/ha)	Season	1	3	233.53	0.00061***	0.9089	0.9589
	Variety	12	3	4.98	0.0144*		
	Season: Variety	12	69	3.31	0.1008		
		DF		Chi^2	$Pr(>Chi^2)$		
Number of locules	Season	1	1.85	0.1737	0.1892	0.1892	
	Variety	12	248.86	<2e-16***			
	Season: Variety	12	9.25	0.6817			

The highly significant varietal effect ($F = 15.08$, $p<2.2e-15$) demonstrates excellent genetic variability for fruit width. CLN4018G dominates (58.96 mm), followed by CLN4398M (58.35 mm), while AVTO1955-15 has the minimum width (42.26 mm) (Fig. 4b). The significant season×variety interaction ($p = 0.01004$*) reveals moderate stability with differential sensitivity of genotypes to environmental variations.

The highly significant varietal effect ($Chi^2 = 248.86$, $p<2e-16$) reveals considerable genetic variability in the number of locules. CLN4018G and CLN4398M have the maximum (5 locules), followed by CLN4398D (4 locules), while several lines (AVTO1955-15, CLN4270D, CLN4270F, CLN4270I) have the minimum (2 locules) (Fig. 4c). The insignificant season×variety interaction ($p = 0.682$) confirms the high stability of this architectural trait between environments.

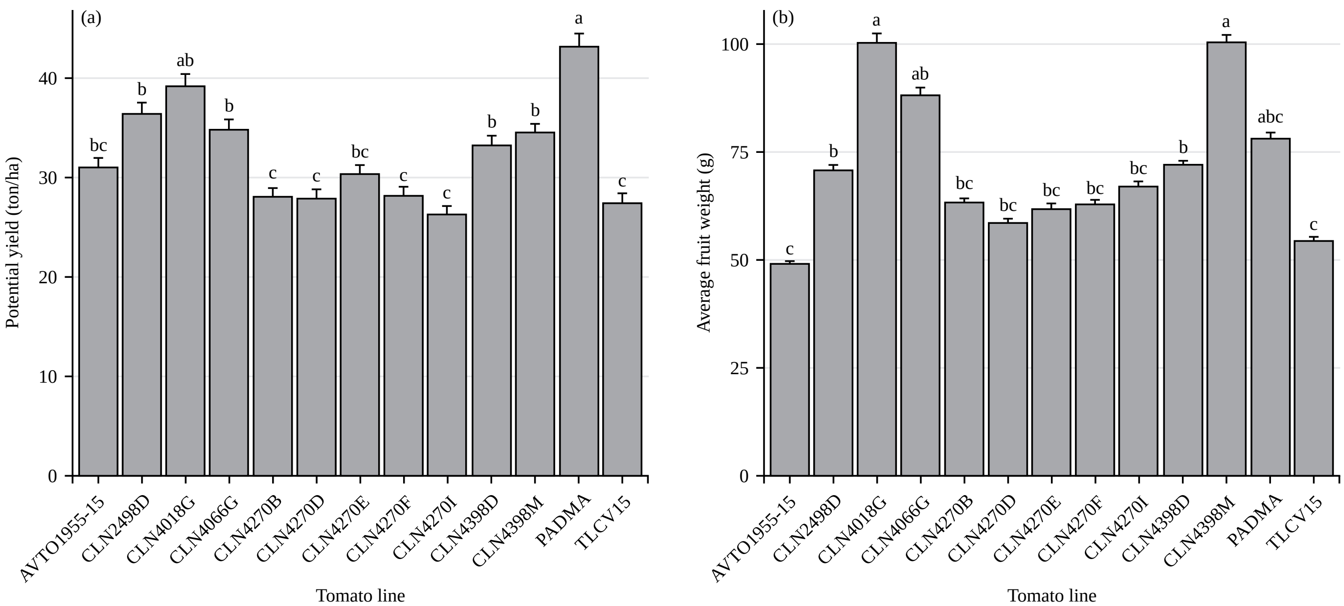

Fig. 5(a-b): Variation in potential yield and average fruit weight according to tomato line, (a) Mean values of potential yield according to tomato line and (b) Mean values of fruit weight according to tomato line
Bars with the same letter are not significantly different at the 5% threshold according to Tukey's test

The significant varietal effect (F = 4.98, p = 0.0144) reveals exploitable genetic variability for potential yield. The PADMA dominates (43.21 ton/ha), followed by CLN4018G (39.14 ton/ha), while CLN4270I has the lowest yield (26.31 ton/ha) (Fig. 5a). The non-significant season×variety interaction (p = 0.1008) ensures satisfactory stability in the variety ranking despite significant seasonal variations.

The extremely significant varietal effect (F = 35.11, p<2.2e-16) indicates exceptional genetic variability for fruit weight. The CLN4398M (100.35 g) and CLN4018G (100.31 g) had the highest weights, while AVTO1955-15 had the lowest weight (49.02 g) (Fig. 5b). This range of 51.33 g offers remarkable potential for improvement. The insignificant season×variety interaction (p = 0.595) guarantees exceptional phenotypic stability, ensuring the commercial predictability of this economically crucial trait.

Phenological diversity: Phenological observations revealed significant differences in the duration of developmental stages between the lines studied.

- **Days to 50% flowering:** The time to 50% flowering ranged from 23 days (CLN4398M) to 32 days (CLN2498D) after transplanting, a difference of 9 days between the earliest and latest lines. This variability in flowering precocity indicates significant genetic differences in the initiation of the reproductive phase. The lines CLN4398M (23 days) and CLN4270B (24 days) stand out as the earliest, while CLN2498D (32 days after transplanting) has the latest flowering (Table 8)
- **Days to 50% fruiting:** The period to 50% ripe fruit showed variability from 36 days (PADMA) to 49 days (CLN4270F) after transplanting, representing a difference of 13 days (Table 8). This range reveals contrasting production cycles, with PADMA having the shortest cycle (36 days) and the CLN4270F (49 days) and CLN4270E (48 days after transplanting) lines having the longest cycles. It is noteworthy that despite differences in floral precocity, several lines converge towards similar fruiting times (43-46 days for the majority)

Early-maturing varieties such as CLN4398M and PADMA could be preferred for short growing seasons or challenging climatic conditions, while longer-maturing varieties could offer advantages in terms of yield or fruit quality.

The observed phenological variability, particularly the differences in the number of days to 50% flowering and fruiting, suggests that certain lines (such as CLN4398M and PADMA) could be suited to short growing cycles, a valuable characteristic in environments subject to climatic stress[26]. In contrast, longer-cycle lines may be suitable for more intensive production systems.

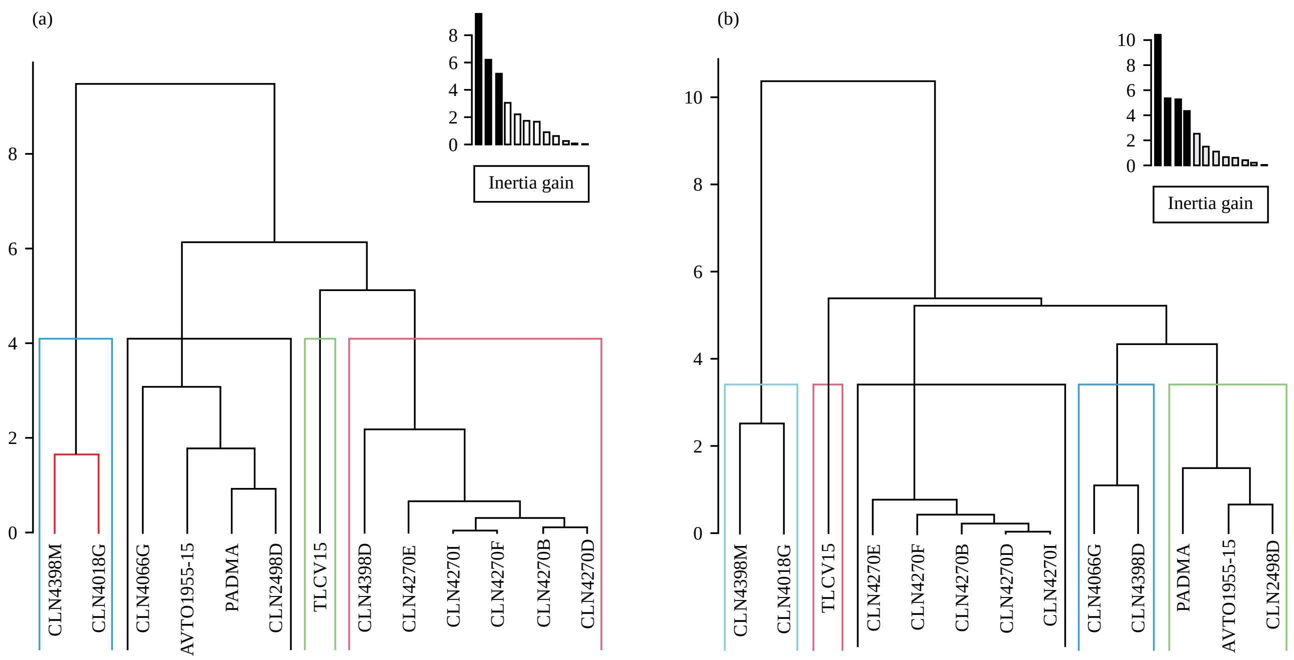

Fig. 6(a-b): Groups of tomato lines by season, (a) Hierarchical classification tree for season 1 and (b) Hierarchical classification tree for season 2

Table 8: Variability of phenological traits among tomato lines

Line	Average number of days before 50% flowering (DAT)	Average number of days before 50% fruiting (DAT)
AVTO1955-15	28	43
CLN2498D	32	43
CLN4018G	25	43
CLN4066G	26	43
CLN4270B	24	46
CLN4270D	25	47
CLN4270E	26	48
CLN4270F	25	49
CLN4270I	27	48
CLN4398D	26	46
CLN4398M	23	45
PADMA	28	36
TLCV15	26	44
Pr(>Chi^2)	0.07387	0.02176

Hierarchical structuring of tomato lines

Stability between seasons: Figure 6 illustrates the hierarchical classification of tomato lines for two distinct seasons in order to assess the stability of their groupings. Analysis of the clusters delineated by the colored rectangles reveals notable stability for certain lines, while others show sensitivity to seasonal variations.

Perfect stability is observed for the group of two lines, CLN4399M and CLN4018G, which remain associated in both seasons. In addition, several lines consistently rank separately, notably CLN4056G, TLCV15 and CLN2498D, suggesting that their profiles are intrinsically distinct and robust to environmental conditions.

However, other groupings show significant instability. The CLN4270 lineage group (E, I, F, B and D), which is associated with the CLN4399D lineage in season 1 (Fig. 6a), separates from the latter in season 2, where CLN4399D becomes an isolated group (Fig. 6b). This separation indicates that the similarity relationships between these lineages are not fixed and are influenced by seasonal factors, which alters their classification.

Thus, the hierarchical classification of tomato lines is partially stable from one season to the next. While some lines maintain consistent similarity profiles, others exhibit phenotypic plasticity that is reflected in variable group composition[21].

Table 9: Concentration of *R. solanacearum* inoculum in the soil at the experimental site

	Block 1	Block 2	Block 3	Block 4
Average concentration of *R. solanacearum* inoculum (CFU/g)	37,022.22	48,622.22	27,977.78	39,066.67

Table 10: Effect of bacterial wilt incidence on tomato varieties

Variety	Incidence of bacterial wilt (%)	Classification according to Mew and Ho[18]
TLCV15	16.88[a]	MR
CLN4066G	0.63[b]	R
CLN4398D	0.00[b]	HR
CLN4270B	0.00[b]	HR
CLN4018G	0.00[b]	HR
CLN4270D	0.00[b]	HR
CLN4398M	0.00[b]	HR
CLN4270F	0.00[b]	HR
AVTO1955-15	0.00[b]	HR
PADMA	0.00[b]	HR
CLN4270E	0.00[b]	HR
CLN4398D	0.00[b]	HR
CLN2498D	0.00[b]	HR
Probability (Pr)	<0.001	-

In the same column, averages marked with the same letter are not significantly different at the 5% threshold according to Tukey's test, HR: Highly resistant, R: Resistant and MR: Moderately resistant

The hierarchical classification showed partial stability in groupings between seasons, reflecting both the genetic consistency of certain lines and the phenotypic plasticity of others. This observation is consistent with the conclusions of Kouam *et al.*[22], who emphasize the importance of considering genotype × environment interactions in breeding programs.

Results of screening new tomato varieties for bacterial wilt

Evaluation of the presence of *R. solanacearum* at the experimental site: The isolation of *R. solanacearum* in the laboratory showed that the average inoculum concentration in the soil at the experimental site varies between blocks. The inoculum concentration is highest in block 2 with 48,622.22 CFU/g of soil, followed by block 4 with 39,066.67 CFU/g of soil and blocks 1 and 3 with average concentrations of 37,022.22 CFU/g of soil and 27,977.78 CFU/g of soil, respectively (Table 9). These results confirm the presence of the bacterium in all four (04) blocks.

Effect of bacterial wilt on tomato varieties: The results of the evaluation of the effect of bacterial wilt on the varieties tested are presented in Table 10. Overall, the incidence of bacterial wilt varied between 0 and 16.88% for all 13 varieties tested. Analysis of variance showed that there is a significant difference ($p<0.001$) between the 11 new tomato varieties currently being introduced and the susceptible control variety TLCV15. In fact, the rate of wilting of plants due to *R. solanacearum* varied from 0 to 0.63% for the new varieties, while it was 16.88% for the TLCV15 variety. Furthermore, no difference was recorded between the resistant control PADMA and the new tomato varieties (Table 10).

Based on the classification of Mew and Ho[15] the results showed that the majority of the 11 varieties tested were highly resistant to bacterial wilt, as was the resistant control variety (PADMA). Only the CLN4066G variety was resistant, with a significantly equal incidence rate of 1% (Pr <0.001).

Evaluation of the performance of tomato varieties developed by WorldVeg revealed that under the conditions of the experiment, of the eleven varieties tested, varieties CLN2498D, AVTO1955-15, CLN4270F, CLN4398M, CLN4270D, CLN4018G, CLN4270B, CLN4270I, CLN4398D and CLN4270E, as well as the PADMA control, showed no signs of wilting, while the CLN4066G variety and the TLCV15 control did wilt. However, the distribution of IFB frequencies reveals no significant difference between the eleven varieties and the resistant PADMA control, with bacterial wilt incidence varying between 0 and 5%. The wilting observed in the CLN4066G variety could be genetic in nature. Indeed, the level of resistance to the disease varies depending on whether or not the variety possesses one or both of the main bacterial wilt resistance genes Bwr-12 and Bwr-6[23,24].

The results obtained made it possible to distinguish two classes of varieties (highly resistant varieties and resistant varieties) among the 11 tested. Similar results were obtained by Zohoungbogbo *et al.*[25], who identified during experiments conducted in controlled environments and in the field that several F5 lines (AVTO and CLN) developed by WorldVeg showed high levels of resistance to bacterial wilt with good yields. This resistance to bacterial wilt observed in the eleven varieties tested (including 10 highly resistant individuals) is also comparable to the results of Ganiyu *et al.*[26], who observed individuals highly resistant to bacterial wilt among the AVTO tomato lines. This result also confirms the work of Laeshita and Arwiyanto[27], according to which the AVTO and CLN lines developed by WorldVeg are derived from the F1 hybrid variety "Servo", which is recognized in Indonesia for its high resistance to bacterial wilt caused by *R. solanacearum*.

The results of this study confirm the ability of the PADMA variety to resist *R. solanacearum* in soil[28,29] when screening tomato varieties to identify cultivars resistant to bacterial wilt. Similarly, Burnham *et al.*[17] also observed that the PADMA variety maintained its resistance 28 days after inoculation with *R. solanacearum* in a controlled environment, with zero incidence. The TLCV15 variety, known for its susceptibility to wilt caused by *R. solanacearum*, showed moderate resistance. This behavior could be explained by the action of environmental factors, particularly temperature. Indeed, comparing the results of the inoculation test carried out under conditions similar to those of our study (28.3°C) and the results of a natural infestation at 31.2°C, Dossoumou *et al.*[14] concluded that the virulence of the bacterium decreases with temperature. Similarly, Caruso *et al.*[30] reported that *R. solanacearum* loses its virulence at low temperatures.

CONCLUSION

This study provided an in-depth characterization of 11 tomato lines that are candidates for approval in Benin, highlighting their phenotypic diversity and inter-seasonal stability. The results show that several lines, such as CLN4398M and CLN4018G, have promising agronomic traits, including high fruit weight and good phenotypic stability. However, the environmental sensitivity of certain traits highlights the need for further evaluation in various agroclimatic contexts. The lines identified as early-maturing or high-yielding could help improve tomato productivity in Benin, thereby meeting farmers' needs and market demands. The eleven varieties developed by WorldVeg would be very useful for market gardeners in the major tomato-producing areas of Southern Benin, particularly in the Ouémé Valley, where tomato production has been abandoned due to bacterial wilt. This work provides a solid basis for breeding and certification programs, while highlighting the importance of an integrated approach combining morphological evaluation, genetic stability and environmental adaptation.

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