GENETIC AND MOLECULAR EVALUATION OF NEWLY SELECTED TOMATO LINES FOR TYLCV RESISTANCE.

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ABSTRACT
Fifteen new lines of tomato were developed from three different hybrids for resistance to tomato leaf curl virus (TYLCV) with desired yield, vegetative, and earliness traits. The 15 lines were evaluated at the 6th generation first under artificial infection conditions and then under field conditions. The evaluated traits were plant length (cm) and number of plants. of branches/plant, first flowering, days to first harvest, no. of fruits\ plant, weight of fruits/plant, and total yield/plant (kg). The results indicated that the genetic variance was positive and higher than the environmental variance for all studied traits. These results were confirmed by the values of heritability, which ranged from 54.42 to 90.26 for No. of branches and Days 1st Flowering, respectively. Line 3 recorded the earliest flowering and days to first harvesting with mean values 17.35±0.88 and 65.0±0.40, respectively.While Line 4 recorded highly mean values for the number of fruits/plant and total yield/ plant, with mean values 31.3±1.24 and 3.28±0.13, respectively.
Among the selected lines, one line was Immune/Highly Resistant (HR), six lines were resistant, and seven lines showed moderate resistance to TYLCV. This finding was confirmed by marker-assisted selection, where Ty-1 and TY3 showed that Lines (7, 8, 9, 10, 11, 12, and 13) were dominant homozygous for ty1; in addition, lines 14 and 15 were dominant homozygous for ty3, and lines 3, 4, and 5 were heterozygous for Ty3. From these results, it could be concluded that the pedigree selection procedure is highly effective in selecting promising resistant tomato lines for TYLCV.

Keywords: Tomato, TYLCV, Genetic parameters, Pedigree selection

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a cornerstone of global agriculture, valued for its nutritional contributions and economic significance. As a primary source of vitamins, antioxidants, and essential nutrients, tomato is integral to diets worldwide, with global production surpassing 189 million metric tons in 2023 (FAO, 2023). However, tomato crops are highly susceptible to biotic stresses, notably Tomato Leaf Curl Virus Disease (TLCVD), caused by the Tomato Leaf Curl Virus (ToLCV), which transmitted by the whitefly (*Bemisiatabaci*), TLCVD causes severe symptoms, including leaf curling, stunted growth, and significant yield losses, with infected fields experiencing up to 90% reduction in productivity (Saikia & Muniyappa, 1989). In tomato production, viruses represent the third biggest obstacle **(Macharia*et al.*,2015)**. Approximately 60 species of the genus Begomovirus damage tomato plants worldwide **(Marie *et al.*, 2012).** According to **Glick *et al.* (2009),** they cause serious infections in tomato crops in the tropics and subtropics, which can result in a yield drop of up to 100%. Tomato yellow leaf curl disease (TYLCV), ageminivirus spread by whiteflies (*Bemisiatabaci* Genn.) is a major threat to tomato production in tropical and subtropical countries worldwide, including Egypt. It has the potential to destroy tomato yield (**Picó *et al.*, 1999).**In the 1960s, the virus was initially discovered and described in Israel (**Diaz-Pendon *et al.*, 2010).** It has spread to various regions of the world, and there are currently around 12 known TYLCV-like viruses **(Marie *et al.*, 2012).**The increasing prevalence of whiteflies, driven by climate change and rising temperatures, exacerbates the spread of TLCVD, making sustainable disease management a priority.
Chemical control of whitefly vectors is often costly, environmentally unsustainable, and insufficient to prevent TLCVD spread. Consequently, breeding for resistance to TLCVD has become a critical strategy for ensuring sustainable tomato production. Breeding programs leveraging genetic resistance

offer a cost-effective and environmentally friendly approach to mitigate disease impact. Among various breeding methods, the pedigree selection program stands out for its effectiveness in developing tomato lines with stable, heritable resistance to TLCVD. Pedigree selection involves the systematic crossing of resistant and elite cultivars, followed by selection of superior progeny based on phenotypic performance and genetic markers over multiple generations (Allard, 1960). This method allows breeders to track inheritance patterns, ensuring the retention of desirable traits such as TLCVD resistance, high yield, and fruit quality. By incorporating molecular markers linked to resistance genes like Ty-1, Ty-2, and Ty-3, pedigree selection enhances precision in identifying resistant genotypes, reducing the time required to develop improved varieties (Hanson et al., 2016).In this respect, there are six Ty genes (Ty-1 to Ty-6) in different tomato varieties responsible for resistance to the TYLCV virus. Ty-1 and Ty-3 are largely dominant genes located on chromosome 6 **(Ji *et al.,* 2007 and Verlaan *et al.,* 2013).** Ty-2, a dominant gene, was located on chromosome 11. **Ji *et al.* (2009)** found that Ty-4, a small QTL on chromosome 3, accounts for just 16% of the variation in symptom intensity. A recessive gene, ty-5, located on chromosome 4, has been identified. Recently, **Hutton and Scott (2014)** discovered a Ty-6 gene on chromosome 10 that provides resistance to the TYLCV. According to **Prasanna *et al.* (2015)**, the Ty-2 and Ty-3 gene-carrying lines may be useful in producing resistant hybrids due to the unique effect of the genes.Despite these advances, challenges remain, including the genetic variability of ToLCV strains and the need for durable resistance. Pedigree selection programs address these challenges by enabling the accumulation of multiple resistance genes, enhancing broad-spectrum resistance. Therefore, this investigation employs a pedigree selection approach to develop tomato lines resistant to TLCVD, combining phenotypic evaluations under field conditions with molecular marker-assisted selection.

MATERIALS AND METHODS

This investigation was carried out at the Experimental Genetics Department farm, Faculty of Agriculture, Mansoura University, Egypt, and El-BarmounFarm of Horticulture Research Institute, Mansoura, Egypt, during successive seasons from 2021\2022 to 2024/2025 tobreed resistance lines to tomato leaf curl virus (TYLCV) with better yield, vegetative, and fruit quality traits. Three hybrids supplied by the National Program of Vegetable Seed Production were used as starting plant material for the pedigree selection program. The code, production company, trade names, and number of selected lines after five cycles of selection through three populations are presented in **Table 1.**

Table 1: Code, producing companies, trade names, and number of selected lines.

Code	Producing company	Genotype name	No. of selected lines
54	**Techno green**	**Salma (65010)**	**10 (L1 to L10)**
56	**Pluseeds**	**(209)**	**3 (L11 to L13)**
G1	**Techno green**	**Ty 70\70**	**2 (L14 to L15)**

Pedigree Selection procedure:

Tomato seeds of the three hybrids were sown two times each year in September and March under greenhouse conditions. The growing seedlings were transplanted at the open field after 45 days from planting. Seedlings were transplanted in a randomized block design with four replications.No specific control measures were used against the whitefly vector, and fungicides were applied to manage foliar pathogens. In instances of Lepidoptera insect infestations, specific insecticides were used. six cycles of the selection program were conducted to restore and improve the characteristics of tomato grown under Egyptian conditions. The following **Fig. 1**demonstrates the procedure of the selection program used in thisstudy.

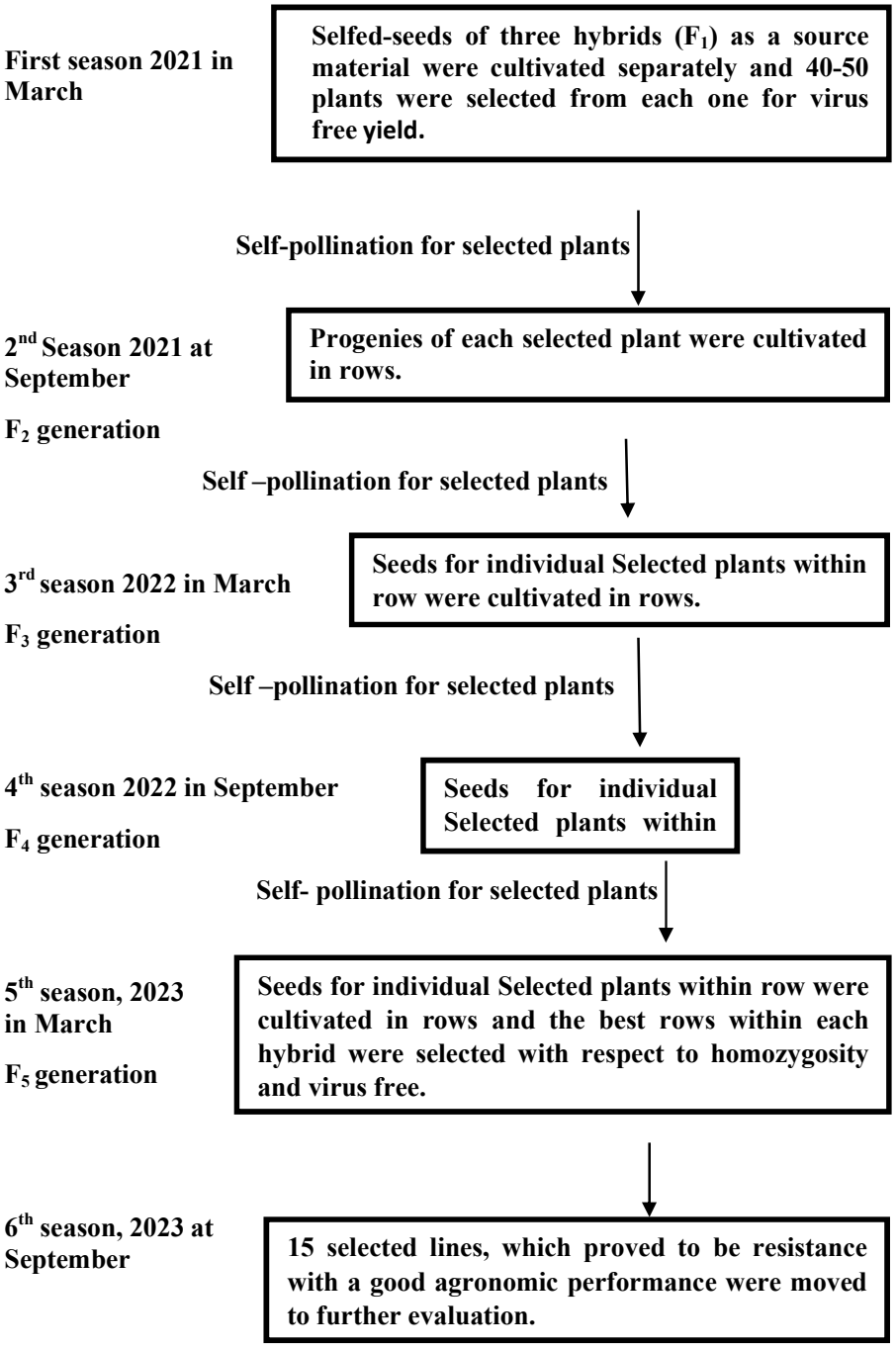

Fig. 1: The fifth season of the selectorial program.

The selected lines at F_5 generation were preliminarily evaluated for virus-free and some morphological, earliness, and yield traits in a randomized block design with four replications. The data were recorded on the following traits: Plant length (cm), No. of branches per plant,days to first flowering, days to first harvest, no. of fruits\ plant, weight of fruits/plant, and total yield/plant (kg). The self-pollinated seeds of selected lines (F6 generation) were divided intotwo parts. The first part was employed for artificial infection tests, and the other part was kept for further evaluation ofpromising lines.

Artificial infection:
The first seed part of 15 selected lines from the 6th generation were evaluated under conditions of artificial infection by whiteflies in an insect–proofgreenhouse at the plant virus identification and diagnosis lab. (PVIDL), plant pathology research institute –agriculture research center. The artificial infection was conducted from Octoberto February during 2024-2025. Fifteen produced lines were evaluated for TYLCV resistance, and the susceptible Alissa inoculated plants were kept under greenhouse conditions until fruiting. Disease severity ratings were recorded according to **Lapidot*et al.*, (2001)**. Disease percentages were recorded using the following formula:
D.I.% = n/N 100
Whereas disease severity percentages were calculated using the following formula (**Yang *et al.*, 1997).**

$$D.S.\% = \frac{(\text{DiseasegradexNo of plant in each grade})}{(\text{Total no of tested plants x highest disease grade})} * 100$$

Statistical analysis
The mean values for each studied trait were calculated and then analyzed using a one-way analysis of variance (ANOVA) in SPSS software (version 2004).

TYLCV detection using PCR:
To detect TYLCV, total DNA was extracted from each selected line using Dellaporta's methods as described by **Dellaporta *et al.*, (1983)**. Primers were designed as described by **Accotto*et al.*, 2000(Table 2).** PCR were performed in 20µl containing 3 µl DNA, 10 µl amaR one Master Mix (Gene Direx, Inc., Taoyuan, Taiwan), 2 µl of 10 pmol of each primer, and 3 µl of sterile water. The amplification condition is as follows: 4 min at 95°C for denaturation and 35 cycles of 30 s at 95°C, 30 s at 60°C, 30 s at 72°C, and 1 extension cycle for 7 min at 72°C. PCR products were analyzed by loading 5 µL of PCR product on a 1% agarose gel in 1% TAE stained with (5 µl/50ml) EZ view stain (Biobatick- Canada)

Table 2: Oligonucleotide primer sequences.

Name	Sequences
Ty-1 (+)	5′-GCC CAT GTA (T/C) C G (A/G) AAG CC-3′
Ty-2 (-)	5′-GG (A/G) TTA GA (A/G) GCA TG (A/C) GTA C-3′

Detection for Ty Genes
Eleven specific primers for Ty genes were used to detect Ty virus genes. The sequences of primers used for Ty gene detection are shown in **Table 3.** PCR were performed in 20µl containing 3 µl DNA, 10 µl amaR one Master mix (Gene Direx, Inc., Taoyuan, Taiwan), 2 µl of 10 pmol of each primer, and 3 µl of sterile water.

Table 3:Sequences of primers for Ty Gene marker.

Gene's Name	Primer's Name	Sequence (5' ------ 3')	Exp. Size (bp)	PCR File	Reference
Ty-1	SSR-47 f	TCCTCAAGAAATGAAGCTCTGA	191(Resistant)/ 180(Susceptible)	94°C for 5 min followed by 35 cycles of 94°C for 30 s, 45°C for 30 s, 72°C for 2 min with a final extension at 72°C for 10 min.	**Nogueira *et al.*, (2011)**
	SSR-47 r	CCTTGGAGATAACAACCACAA			
Ty-1	SSR-48 f	ATCTCCTTGGCCTCCTGTTT	191(Resistant)/ 180(Susceptible)		
Ty-1	SSR-48 r	GTCATGGCCACATGAATACG			
Ty-2	TG0302 f	TGGCTCATCCTGAAGCTGATAGCGC	900 (Resistant)/ 800(Susceptible)	94°C for 4 min followed by 35 cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 1.5 min with a final extension at 72°C for 10 min.	**Garcia *et al.*, (2001)**
Ty-2	TG0302 r	AGTGTACATCCTTGCCATTGACT			
Ty-2	TY2 R1	TGAT(T/G)TGATGTTCTC(T/A)TCTCT(C/A)GCCTG	+ TG0302 F 450 and/or 600		
Ty-3	FLUW25 f	CAAGTGTGCATATACTTCATA(T/G)TCACC	640 and/or 475	94°C for 4 min followed by 35 cycles of 94°C for 30 s, 53°C for 1 min, 72°C for 1 min with a final extension at 72°C for 10 min.	**Yuanfu *et al.*, (2009)**
Ty-3	FLUW25 r	CCATATATAACCTCTGTTTCTATTTCGAC			
Ty-3	P6-25-f2	GGTAGTGGAAATGATGCTGCTC	450 and/or 320		
Ty-3	P6-25-r5	GCTCTGCCTATTGTCCCATATATAACC			

Results & Discussion
Analysis of Variances and Genetic Parameters:
The analysis of variances and mean squares for all studied traits was presented in **Table 4**. The results indicated that the highly significant differences ($p < 0.01$) between the evaluated lines for all the studied traits, including plant length, number of branches/plant, days to first flowering, days to first harvesting, number of fruits per plant, fruit weight per plant, and total yield (kg). Furthermore, the mean squares of all studied traits between lines were larger than the corresponding values within

lines. These indicated a strong genetic variability between tested lines, and the smallest mean square values within lines suggest less environmental influence, which supports thehigh validity of the detected genetic variations. The large genetic variation is essential for effective selection because it forms the basis for genetic improvement, according to **Singh and Chaudhary (1985).** This is consistent with the findings of **Allard (1999),** who found that large differences in ANOVA imply that heritable genetic variables, rather than environmental fluctuations, drive the trait.

Table 4: The analysis of variances and mean squares for all studied traits.

S.O. V	Df	Plant length	No. of branches	Days 1st Flowering	Days to first harvesting	No. fruits/Plant	weight of Fruits/Plant(gm)	Total yield (kg)
Between Lines	14	1457.34**	3.53**	98.69**	103.60**	78.21**	615.48**	0.6733**
Within Lines	45	68.61	0.618	2.59	4.79	6.03	93.53	0.104

Where, ** significant at 1% level of probability.

The genetic variance, environmental variance, phenotypic variance, heritability in a broad sense, and genetic advancement were calculated for the 15 selected lines and the resultsfor all studied traits were presented in **Table 5**. The results indicated that the genetic variance (σ^2g) was positive and higher than the environmental variance (σ^2e) for all studied traits.These results were confirmed by the values of heritability, which ranged from 54.42 to 90.26 for No. of branches and Days 1st Flowering, respectively.The heritability of all evaluated traits was found to be high, indicating a predominance of additive gene activity. The evaluations of heritability benefit the plant breeder in the selection of genotypes from diverse genetic populations. Therefore, high heritability helps in effective selection for a trait. **Samima *et al*., (2023)** found thathigh heritability values in the broad sense were observed for most of studied traits, including plant height (99.47%), average fruit weight (98.91%), number o fruits per plant (98.46%), fruit yield per plant (98.34%), days to 50% flowering (97.33%), days to first flowering(95.67%), number of branches per plant (94.63%) and days to first fruit harvest (94.40%).

The genetic advance is combined with heritability; it leads to more accurate predictions of genetic gain under selection. The highest genetic advancement as a percentage of mean (ΔG)was recorded for plant length (35.07), followed by weight of fruits/plant (17.94) and days to 1st flowering (9. 59).Whereastotal yield and no. of showedthe lowest estimate of genetic advance. **Samima *et al*., (2023)** recorded that**the** highest estimate of genetic advance was recorded in plant height, whereas average fruit weight, days to first fruit harvest, showed moderate genetic advance,and number of fruits/plants, days to 50% flowering, days to first flowering, number of branches per plant, and fruit. **Rai *et al*., (2016)** found powerful heritability with high genetic gain for fruit yield/plant, average fruit weight, and number of fruits/plants.

Sivasubramanyan and Madhavamenon (1973) established a grading system for GCV and PCV, defining values less than 10% as low, those between 10% and 20% as moderate, and those exceeding 20% as high. Thus, the genotypic and phenotypic coefficients of variation (GCV and PCV) for the traits studied showed a wide range. The GCV values varied from a low of 7.02% for days to first harvest to a high of 20.61% for days to first flowering. Similarly, the PCV values ranged from 7.86% to 21.70% for the same two traits, respectively. The highest values for both GCV (20.61%) and PCV (21.70%) were recorded for the number of days to first flowering. Several other traits exhibited moderate GCV and PCV values, including the number of fruits per plant (16.38% GCV, 18.9% PCV), plant length (15.15% GCV, 16.77% PCV), total yield (14.95% GCV, 19.63% PCV), number of branches (13.7% GCV, 18.69% PCV), and weight of fruit per plant (11.85% GCV, 15.56% PCV). The lowest GCV (7.02%) and PCV (7.68%) were found for the number of days to first harvest.These results do not agree with the results obtained by **Samima *et al*., (2023)** who found highgenotypic variance were recorded for both fruit yield/plant and fruityield, plant height, number of branches/plant, number of fruits/plant, average fruit weight, whereas days to first flowering, days to 50% flowering, days to first fruit harvest showed moderate genotypic variation. **Dar and Sharma (2011)** observed high GCV and PCV values for fruit number per plant and overall yield.

Table 5: Variance components and genetic parameters for the studied traits.

Genetic parameter	Plant length	No.-of branches	Days-1st Flowering	Days to first harvest	No.-of fruits/Plant	Weight-of Fruits/Plant (gm)	Total yield (kg)
σ^2g	347.09	0.738	24.02	24.70	18.05	130.48	0.142
σ^2e	68.61	0.618	2.59	4.79	6.03	93.53	0.104
σ^2ph	415.70	1.36	26.61	29.49	24.07	224.53	0.246
H_b %	83.49	54.42	90.26	83.75	74.96	58.11	57.75
ΔG	35.07	1.30	9. 59	9.37	7.58	17.94	0.59
G.C.V%	15.15	13.7	20.61	7.02	16.38	11.85	14.95
E.C.V%	6.73	12.60	6.77	3.09	9.46	10.03	12.79
P.C.V%	16.77	18.69	21.70	7.68	18.9	15.56	19.63

The mean performance for all studied traits of the 15 selected lines after six cycles of the selection program wasdetermined, and the results are presented in **Tables 6 and 7**. The results showed that the mean values of plant length ranged from 93.6±6.51 for line 2 to 155.2±6.13 for line 13.For the number of branches/plants, it ranged from 4.12 ± 0.07for line 6to 8.00 ± 0.37 for line 13. The best lines for the number of branches were L13,followed by L1,with mean values 8.00±0.37 and 7.08±0.47, respectively. Regarding the days of the first flowers, the selected lines'mean values ranged from 17.35±0.88 to 31.88 ± 0.98. Line 3 recorded the earliest flowering with a mean value 17.35 ± 0.88, followed by line 4 with a mean value 18.68 ± 0.40. In contrast, line 14exhibited the latest flowering with a mean value 31.88 ± 0.98. For days to first harvest, the selected lines' mean values ranged from 65.0±0.40 to 80.8±2.20, respectively. Lines 3, 4,6,and 15 recorded the earliest one for days to first harvesting with means values65.0±0.40, 66.5±0.28, 66.5±0.64, and 66.5±0.28,respectively.

For plant height**Mahmoud andOsman (2023). Studied 12**F 9 for phenotypic TYLCV tolerance, breeding lines. They wereselected by a bulk selection. And found plant height ranged from 0.73 ± 0.02 to 1.43 ± 0.07\ m

Babu*et a*l., (2018)showed number of branches/plants ranged from 3.37 to 7.20and days to first harvesting ranged from 66.00 to 76.33. **El-Morsy *et a*l., (2021)** found that the number of branches/plantsranged from 5.0 to 12.67.

Table 6: Mean performances of selected lines for some morphological and earliness traits

Selected plants		Plant length			No. of branches/plant			Days 1st Flowering			Days 1st Harvesting		
		Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
L1		128.3	138.6	133.9±2.27	6.5	8.5	7.08±0.47	21.0	22.2	21.53±0.25	67.0	69.0	68.0±0.57
L2		77.5	105.0	93.6±6.51	5.5	6.1	5.70±0.13	18.3	20.5	19.38±0.47	64.0	70.0	67.8±1.30
L3		86.0	110.0	97.7±4.90	4.6	5.8	5.20±0.25	15.0	19.0	17.35±0.88	64.0	66.0	65.0±0.40
L4		101.6	121.2	114.5±4.59	5.0	8.2	6.80±0.77	17.7	19.7	18.68±0.40	66.0	67.0	66.5±0.28
L5		98.5	107.5	101.7±1.99	4.6	5.8	5.23±0.24	18.0	19.5	18.83 ±0.34	66.0	68.0	67. 0±0.40
L6		95.0	107.8	100.5±2.71	4.0	4.3	4.12±0.07	18.0	19.4	18.78±0.36	65.0	68.0	66.5±0.64
L7		124.2	133.3	128.2±2.19	4.6	6.4	5.82±0.41	19.0	25.1	21.65±1.32	66.0	68.0	66.8±0.47
L8		110.0	122.6	116.6±2.61	5.8	7.0	6.34±.028	29.0	30.0	29.30±0.23	67.0	74.0	71.0±1.70
L9		104.0	137.0	120.6±6.93	5.0	7.0	5.88±0.42	20.1	25.1	22.05±1.08	68.0	74.0	70.5±1.50
L10		120.8	143.6	127.8±5.38	5.4	7.6	6.60±0.58	21.0	27.0	24.03±1.34	73.0	75.0	74.0±0.40
L11		130.0	138.3	134.6±1.71	6.0	7.2	6.70±0.26	29.0	33.4	30.58±0.96	74.0	80.0	76.8 ±1.30
L12		143.6	160.0	150.5±3.84	6.0	8.0	6.71±0.47	23.5	27.8	25.83±0.97	74.0	76.0	75.0±0.57
L13		140.0	170.0	155.2±6.13	7.2	9.0	8.00±0.37	28.5	30.0	29.25±0.32	75.0	81.0	78.8±1.30
L14		140.0	144.8	143.2±1.11	6.0	7.6	6.75±0.35	29.8	34.5	31.88±0.98	74.0	84.0	80.8±2.20
L15		118.0	130.0	126.3±2.79	6.5	6.8	6.65±0.65	26.8	29.8	27.90±0.68	66.0	67.0	66.5±0.28
L.S.D	0.10	9.69			0.801			1.75			2.389		
	0.05	6.992			0.577			1.84			1.716		

As shown in **Table 7**, Line 4 had the highest number of fruits per plant, with a mean of 31.3±1.24. This was closely followed by Line 12 (31.3±0.47), and then Lines 1 and 15, which had identical mean values of 30.7±1.40 and 30.7±1.36, respectively. In contrast, Line 2 had the lowest number of fruits per plant, with a mean value of 17.9±0.77. For average fruit weight per plant, Lines 2, 5, and 6 recorded the highest values (114.9±2.02, 113.3±8.60, and 109.9±3.80, respectively). Meanwhile, Line 11 had the lowest average fruit weight, with a mean of 72.8±1.80.**Vijeth*et al*., (2018) and**

Mahmoud and Osman(2023) and found that the average weight of fruit per plant ranged from (70.0 g to 119.33 g.) and (70.82 ± 0.61 to 126.02 ± 5.3) respectively. Total yield is of primary importance in any selection program. In this study, the mean total yield per plant ranged from 1.86±0.05 kg for Line 14 to 3.28±0.13 kg for Line 4. **Kumar et al *et al*., (2021)** found that total yield/plant ranged from d from 0.48 to 4.30 kg.

Table 7: Mean performances of selected lines for yield traits.

Selected plants		No. of fruits /Plant			Average weight of Fruit/Plant (gm)			Total weight of Fruits/plant (Kg)		
		Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
L1		27.00	34.00	30.7 ±1.4	90.50	109.00	102.0±4.10	3.31	2.20	2.90±0.24
L2		16.00	19.80	17.9±0.77	110.50	120.10	114.9±2.00	2.37	1.85	2.04±0.114
L3		24.00	28.00	25.9±0.88	95.00	111.98	101.9±3.60	3.20	2.34	2.86±0.18
L4		28.00	34.00	31.3±1.24	89.10	108.10	97.4±4.30	3.67	3.08	3.28±0.13
L5		25.00	29.00	27.0±0.91	102.00	139.10	113.3±8.60	3.48	2.79	3.17±0.14
L6		18.50	23.00	20.1±1.00	102.00	118.90	109.9±3.80	2.65	2.16	2.38±0.10
L7		26.00	33.00	29.2±1.60	81.70	97.68	91.1±3.50	2.97	2.54	2.69±0.10
L8		23.00	26.00	24.2±0.75	98.00	109.80	104.1±2.90	2.71	2.47	2.62±0.05
L9		19.00	26.60	21.3±1.78	84.00	115.10	97.8±7.60	2.95	1.79	2.38±0.29
L10		21.00	29.00	25.5±1.93	90.13	106.20	95.8±3.60	3.10	2.21	2.53±0.21
L11		24.00	31.30	27.3±1.50	70.10	78.12	72.8±1.80	2.44	1.76	2.24±0.16
L12		30.00	32.00	31.3±0.47	74.50	80.61	77.9±1.42	2.65	2.50	2.59±0.03
L13		25.00	28.00	26.5±0.64	76.55	115.40	87.0±9.40	2.25	1.97	2.12±0.05
L14		18.20	22.00	20.1±0.77	87.82	104.50	97.9±3.60	1.95	1.71	1.86±0.05
L15		26.80	33.00	30.7±1.36	78.25	89.80	82.0±2.60	2.60	1.77	2.19±0.23
L.S.D	0.01	3.868			10.785			0.338		
	0.05	2.649			7.788			0.244		

Disease severity for TYLCV in selected lines after six cycles of selection:

During the study period, significant variations in the intensity of TYLCV were observed among the tomato lines. In terms of disease incidence percentage, line 11 showed the highest incidence at 74.5%, indicating its susceptibility to the virus.Among the selected lines, line 4 was Immune/Highly Resistant (HR) with a 0% disease incidence. On the other hand, the lowest disease incidence was found in six lines, which wereline 7 (15%), line 8 (18%), line 3 (20%), line 9 (21.5%), line 15 (22%), andline 14 (25%).The other seven lines showed moderate resistance to TYLCV,which were line 2 (42%), line 6 (45%), line 10 (45.5%), line 12 (46.5%), line 1 (47.5%), line 1 (50%) and line 13 (50%).These findings are corroborated by previous studies by **Ravindra *et al.*, (2018)** theyfound thatthe disease incidence ranged from 3.33 to 76.67 % while **Siam Joy*et al*., (2025)** found thatthe disease incidence ranged from 33 to 59 at 60 days.

Table 8: Variabilityin *Lycopersicon ESculantum* lines Reactions to Tomato Leaf Curl Virus

No	Total number	DS%	Level of resistance/susceptibility
1	10	47.5	Moderately resistant (MR)
2	10	42	Moderately resistant (MR)
3	10	20	Resistant (R)
4	10	0	Highly resistant (HR)
5	10	50	Moderately resistant (MR)
6	10	45	Moderately resistant (MR)
7	10	15	Resistant (R)
8	10	18	Resistant (R)
9	10	21.5	Resistant (R)
10	10	45.5	Moderately resistant (MR)
11	10	74.5	Moderately susceptible
12	10	46.5	Moderately resistant
13	10	50	Moderately resistant
14	10	25	Resistant (R)
15	10	22	Resistant (R)

Where: DS%= Disease Severity

Detection of TYLCV using PCR techniques:
The gel analysis showed that all fifteen samples of the selected lines had positive PCR results for TYLCV, displaying a strong band at 540 bp **(Fig. 4)**. This confirmed that the virus was present in every one of the fifteen lines.The absence of bands (540 bp) in the gel confirmed that the virus wasn't present in control negative samples.These findings were supported by **Samarakoon *et al*., (2012) and Ibne Siam *et al*., (2025)**, they discovered that PCR techniques are an excellent tool for rapidly and thoroughly diagnosing samples infected with TYLCV.

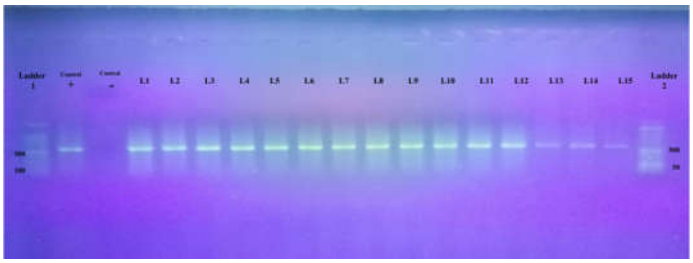

Fig. 4: PCR amplification for detecting tomato yellow leaf curl

Screening Markers Linked to Ty-1, Ty-2, Ty-3, Ty-Resistance:
Based on **Figure 5 (A & B)**, the results indicate that seven lines (7, 8, 9, 10, 11, 12, and 13) were dominant homozygous for the Ty-1 markers. Only lines 1 and 14 showed a dominant heterozygous genotype for Ty-2 **(Figure 6 A & B)**. The detection of Ty-3 revealed that lines 7, 8, 9, 10, 11, 12, 12, 13, 14, and 15 were dominant homozygous **(Figure 7 A & B)**, while line 1 were heterozygous for Ty-3 **(Figure 7 A & B)**. Lines 3,4 and 5wereheterozygousfor Ty-3 in **Figure 7B**.The results confirmed that the 15 selected lines contain only Ty-1, Ty-2, and Ty-3 resistance genes, and the population of each line was homogeneous.The disease severity results we calculated were consistent with the Ty genes results, showing that lines with Ty-1, Ty-2 and Ty-3 genes are resistant or tolerant to Tomato yellow leaf curl virus (TYLCV).Tomatoes, including both hybrids and varieties, have been developed to include **Ty-1** through **Ty-6** resistance genes, which were introgressed from related species **(Prasad *et al*., 2020; Yan *et al*., 2021 and Mori *et al*., 2022)**. Most commercial hybrids currently contain the **Ty1** and **Ty3** resistance genes **(Koeda and Kitawaki 2024)**.

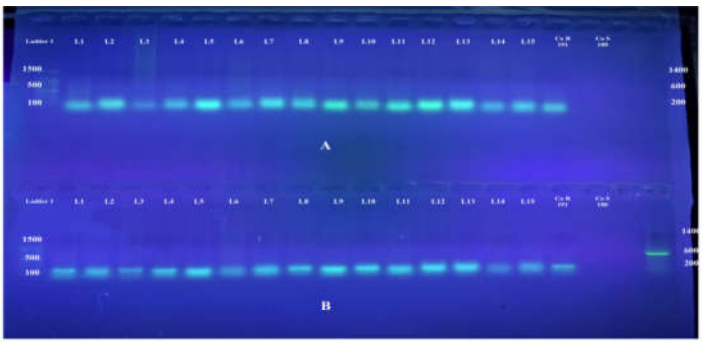

Fig. 5 (A &B): Detection for Ty-1 resistant gene using primer SSR-47 f and SSR-47 r (A) and Ty-1 resistant gene using primer SSR-48 f and SSR-48 r (B) for L1 to L15 samples of selected lines. Where: ConR&ConS are resistant and susceptible controls, respectively.

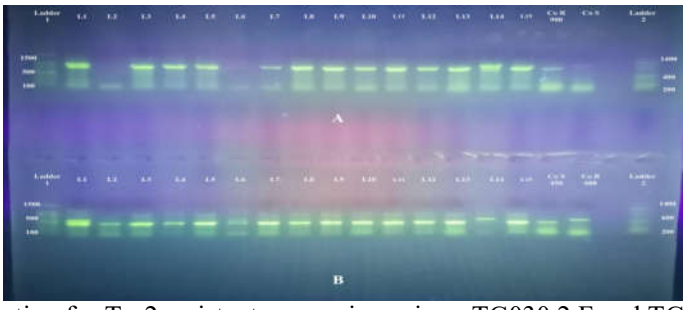

Fig. 6 (A &B): Detection for Ty-2 resistant gene using primer TG030 2 F and TG0302 R (A) and Ty-2 resistant gene using primer TG030 2 F and TY2 R1 (B) for L1 to L15 samples of selected lines. Where: ConR&ConS are resistant and susceptible controls, respectively.

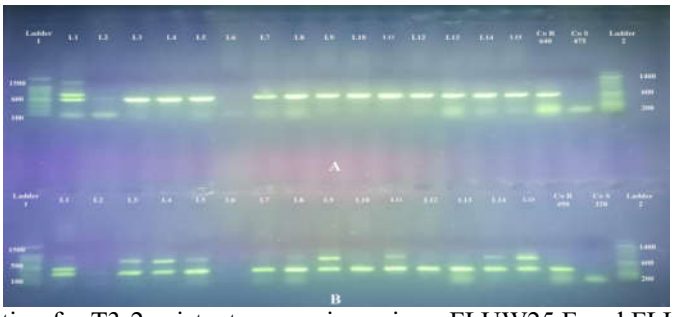

Fig 7 (A &B): Detection for T3-2resistant gene using primer FLUW25 F and FLUW25 R (A) and Ty-3 resistant gene using primer P6-25-F2 F and P6-25-R5 (B) for L1 to L15 samples of selected lines. Where: ConR&ConS are resistant and susceptible controls, respectively.

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