J. Hortic. Sci.
Vol. 19(2), 2024

https://doi.org/10.24154/jhs.v19i2.1945

Original Research Paper

Development and genetic evaluation of recombinant inbred line population for yield and its attributing traits and marker assisted selection for bacterial wilt resistance in tomato

Chethan Kumar S.[1*], Jawadagi R.S.[1], Fakrudin B.[2], Hanchinamani C.N.[1], Kulkarni M.S.[3], Lakshmidevamma T.N.[2] and Vijayakumar R.[1]

[1]Department of Vegetable Science, [2]Department of Genetics and Plant Breeding, [3]Department of Plant Pathology
University of Horticultural Sciences, Bagalkote 587104, Karnataka, India
*Corresponding author Email : chethu.kumar.1995@gmail.com

ABSTRACT

A total of 147 recombinant inbred lines were developed from the cross Anagha x FBT-41 using single seed descent method to evaluate genetic parameters and identification of superior inbred lines using marker assisted selection. The analysis of variance revealed that there is a significant variation was observed between the lines for all the traits studied. Skewness values ranged from 0.16 (clusters per plant and locules per fruit) to 1.16 (TSS) and kurtosis ranged from 1.73 (clusters plant and locules per fruit) to 4.1 (TSS), which, follows normal distribution suggesting that the involvement of multiple genes with quantitative nature of inheritance. Higher PCV, GCV, h^2_{bs} and GAM was observed in plant height, branches per plant, clusters per plant, fruits per cluster, fruit locules, fruit length, fruit diameter, average fruit weight, fruits per plant and yield per plant indicated additive nature of gene action so there is scope for selection. Out of four bacterial wilt linked marker used, only one SCAR marker *i.e.* SCU176-534 showed polymorphism between parents to use in marker assisted selection to identify superior inbred lines coupled with bacterial wilt resistance. A total seven superior inbred lines were selected coupled with bacterial wilt resistance based on both molecular marker and screening in green house sick plot (phenotypic). This lines can be used in further breeding programme as directly released as variety or use as parents to develop bacterial wilt resistant hybrids.

Keywords: DNA markers, GCV, heritablity, PCV, *Ralstonia solanacearum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is rich in antioxidant properties like lycopene, flavonoids, ascorbic acid and ß-carotene, therefore, considered as protective food. In addition to this, it also valued for its flavour and colour. It belongs to the family *Solanaceae* with the diploid chromosome number 2n= 24 (Jenkins, 1948). All the species of tomato are native to Western South America (Rick, 1976), except the cultivated species *Solanum lycopersicum* (L.), which is native to the Peru-Ecuador region (Rick, 1969).

Achievement of plant breeding depends upon the nature and magnitude of variability present in the genotypes. Likewise, the assessment of heritable and non-heritable components of total variability will have enormous value in the choice of suitable breeding procedures. Genetic resources empower plant breeders to create novel plant gene combinations and select crop varieties more suitable to the needs of diverse agricultural systems (Glaszmann et al., 1992). A systems breeding approach is necessary for the overall improvement of tomato crop. Systematic evaluation of germplasm is of great importance in both agronomic and genetic improvement. Understanding the genetic background and the breeding value of the germplasm accessions intended to be used as essential to initiate systematic breeding programme (Agong et al., 2000). The genetic variance of quantitative trait is can be partitioned in to additive variance (heritable) and non-additive variance, in which non-additive variance again divided into dominance and epitasis type of gene action (non-allelic interaction).

Bacterial wilt is a serious soil borne disease caused by gram negative bacteria, *Ralstonia solanacearum*, which is highly prevalent in hot and humid areas of world. Traditional breeding for bacterial wilt disease has been proven difficult for various reasons, including

time-consuming, low efficiency, the influence of the environment on the development and severity of disease and variation in pathogen populations. The use of marker-assisted selection for the breeding of bacterial wilt resistance will facilitate the retention of resistance loci by eliminating undesirable characters (Yang & Francis, 2007) and also take care of epistatic effects which are otherwise non-detectable phenotypically. Marker-assisted selection is a selection method where selection is essentially based on genotypic value of genotype and it's greatly improves the efficiency of conventional selection and breeding methods. The selection based on genotype requires DNA molecular markers that are tightly linked to trait of interest (Mohan et al., 1997), hence, the present study was carried out to develop the stable homozygous inbred lines of tomato which possess dual traits of high yielding and bacterial wilt resistance.

MATERIAL AND METHODS

The experiment was carried out at Kittur Rani Channamma College of Horticulture, Arabhavi, University of Horticultural Sciences, Bagalkot, Karnataka during November 2019 to December 2021. Two genetically diverse parents which are contrasting for bacterial wilt disease resistance i.e. 'Anagha' (resistant) and 'FBT-41' (susceptible) was used to develop recombinant inbred lines from crossing and followed single seed descent method of generation advancement. A total of 147 recombinant inbred lines were developed and evaluated following augmented randomized block design. In each lines, 20 plants were planted and recommended agronomic package of practices were followed throughout the growing season. Five plants were randomly selected for recording observations on plant height (cm), number of branches per plant, days to first flowering, days to 50% flowering, number of clusters per plant, number of fruit per cluster, average fruit weight (g), number of locules per fruit, fruit length (cm), fruit diameter (cm), number of fruits per plant, total yield per plant (kg), total soluble solids (°Brix), pH in the all 147 lines including parents and checks. Further, the lines which are yielded more than 3 kg of fruits per plant were selected and subjected for quality parameters estimations *viz.*, ascorbic acid (mg/100 g) (Sadasivam & Manickam, 1992), lycopene content (mg/kg fresh weight) (Darshan et al., 2013), fruit firmness (kg/cm^2) was measured using fruit penetrometer and pericarp thickness (mm). The following genetic parameters were calculated using data *viz.*, skewness and kurtosis (Pearson, 1894), ANOVA (Federer & Raghavrao, 1975), components of variance (Lush, 1940), (Chaudhary & Prasad, 1968), heritablity in broad sense (Hanson et al., 1956) and genetic advance (Johnson et al., 1955). The RIPs developed from cross were screened in green house sick plot conditions to ascertain their resistance to bacterial wilt disease. The bacterial wilt sick plot was maintained by growing continuously solanaceous vegetables used for the study. Total number of plants, the number of plants with and without bacterial wilt disease symptoms in each RIPs were counted and observations recorded as per scale given by Winstead & Kelman (1952) (Table 1) and the disease incidence value was calculated.

Table 1 : Disease rating scale for bacterial wilt of tomato caused by *R. solanacearum* (Winstead & Kelman, 1952)

Reaction observed	Rating
No wilting	0
Less than 10% wilted plants	1
11–25% wilted plants	2
26–50% plants wilted	3
51–75% plants wilted	4
>75% plants wilted	5

Accordingly, they were classified based on the per cent disease incidence and grouped into the following standard scale (Aslam et al., 2017) (Table 2). For maker assisted selection, the genomic DNA was prepared following method given by Gawel & Jarret (1991). Four bacterial wilt disease linked SSR and SCAR marker were utilized (Table 3) and the final PCR products were subjected to gel electrophoresis on a 2.5% agarose gel to visualize the polymorphic bands.

Table 2 : Scale based on per cent disease index for the categorization of tomato germplasm (Aslam et al., 2017)

Reaction observed	PDI
Highly resistant	00-20
Resistant	21-30
Moderately resistant	31-40
Moderately susceptible	41-50
Susceptible	51-60
Highly susceptible	61-90
Extremely susceptible	91-100

$$\text{Per cent disease index} = \frac{\{(\text{No. of plants at disease rating score ``1'' } \times 1) + (\text{No. of plants at disease rating score ``2'' } \times 2) + (\text{No. of plants at disease rating score ``3'' } \times 3) + (\text{No. of plants at disease rating score ``4'' } \times 4) + (\text{No. of plants at disease rating score ``5'' } \times 5)\}}{(\text{Total number of plants observed} \times 5)} \times 100$$

Table 3 : List of bacterial wilt resistance linked primers and their nucleotide sequence

Primername	Marker type	Forward sequence (5'-3')	Reverse sequence (5'-3')	Annealing temp (°C)	Source
SCU176-534	SCAR	TTGAACCAAGAATCTATTCG	GAACTTGAATGCCTACCAAA	45.6	Truong et al. (2015)
TSCAR AAG/CAT	SCAR	AGAAGGTCACGGCGAGA	TGAGTCCTGAGTAACTGG	48.1	Miao et al. (2009)
TG564	SSR	TGAGGTGCAAATGGGGTAGTG	GCAATGAAGGCCTACAGATGAC	52.0	Sujeet Kumar et al. 2018)
SSR 20	SSR	GAGGACGACAACAACAACGA	GACATGCCACTTAGATCCACCA	58.9	Sol Genome Project

RESULT AND DISCUSSION

The analysis of variance revealed significant variation between the lines for all the traits studied, indicating presence of sufficient amount of genetic variability among the lines (Table 4).

The study of distribution using skewness and kurtosis provides information about the nature of gene action and number of genes controlling the traits. Further, the skewness ranged from 0.16 (clusters per plant and locules per fruit) to 1.16 (TSS) and kurtosis ranged from 1.73 (clusters per plant and locules per fruit) to 4.1 (TSS). All the traits studied recorded skewness value near zero and kurtosis value near three, suggesting that the involvement of multiple genes with quantitative nature of inheritance. It concludes that these traits will be effective if the selection is carried out intensively from the early generation to the advanced generation (Jayaramachandran et al., 2010) (Table 6).

Analysis of variance by itself is not enough and conclusive to explain all the inherent genotypic variances in the lines. One of the ways by which variability present in the germplasm could be assessed, is through a simple approach of examining the range of co efficient of variation. Results revealed that ample range of genetic variability was observed in the lines derived from cross Anagha × FBT-41 (Table 5).

Genetic parameters revealed that phenotypic coefficient of variation were higher than the genotypic coefficient of variation for all the traits observed, revealing the existing variation is due to the genetic constitution of genotypes and added with environmental variance. This will help in further selection of promising types. The estimates of phenotypic and genotypic coefficient of variation showed that for all of the characters studied, the PCV was higher than the GCV and there was a small difference observed between them. Suggesting that recombinant inbred line populations of the crosses played a greater role in character expression than the environment. Higher range of GCV and PCV were observed for the traits *viz*., plant height, number of cluster per plant, number of fruits per cluster, number of locules per fruit, average fruit weight, number of fruits per plant and yield per plant indicated that the presence of wide range of variation for these characters, which reflected that these traits were governed by additive gene action. So, there is scope for improvement of these traits through selection and it would be rewarding. High heritability along with high genetic advance over mean were recorded for the traits plant height, branches per plant, clusters per plant, fruits per cluster, locules per fruit, fruit length, fruit diameter, average fruit weight, fruits per plant and yield per plant. This results indicates that higher response to selection observed for this traits as these traits were governed by additive gene actions and controlled by polygenes. This trend was also reported by Akhter et al. (2021).

Table 4 : Analysis of variance for yield component and quality traits in Anagha × FBT-41 cross

Source of variation	Mean sum of squares													
	DF	PHT	PB	NOFPC	NOCPP	FLO	DFF	D50F	FL	FD	AFW	NOF	YPP	TSS	PH
Block	2	1	0.3	0.11	0.08	0.17	0.11	1.33	0.0033	0.03	7.48*	0.88	0.0017	0.02**	0.00053
Entries	153	196.09**	2.49**	1.54**	22.88**	0.63**	6.32**	10.33**	0.64**	0.72**	70.14**	769.35**	1.33**	0.06**	0.05**
Checks	6	817.6**	2.01**	1.37**	19.05**	0.89**	18.55**	15.08**	0.61**	2.94**	89.11**	691.83**	2.05**	0.49**	0.06**
Lines	146	171.59**	2.47**	1.4**	22.69**	0.61**	5.84**	8.09**	0.63**	0.56**	69.76**	771.36**	1.28**	0.04**	0.05**
Checks vs. Lines	1	45.1**	7.91**	22.87**	72.95**	2.41**	2.76	304.09**	3.49**	11.51**	11.58*	940.75**	4.33**	0.69**	0.27**
Error	12	0.89	0.28	0.08	0.49	0.05	0.87	1.56	0.02	0.03	1.46	1.9	0.00082	0.002	0.0048

*Significant at 5% probability level; **Significant at 1% probability level

PHT: plant height (cm), PB: number of branches per plant, NOFPC: number of fruits per cluster, NOCPP: number of cluster per plant, FLO: number of locules per fruit, DFF: days to first flowering, D50F: days to 50 per cent flowering, FL: fruit length (cm), FD: fruit diameter (cm), AFW: average fruit weight (g), NOF: number of fruits per plant, YPP: total yield per plant (kg), TSS: total soluble solid

The selection of disease resistance lines through utilizing molecular markers which linked to an allele of a gene conferring resistance has an advantage without phenotyping of the germplasm at the seedling stage. In bacterial wilt resistance breeding, the accurate selection of germplasms for disease resistance is very difficult due to several uncontrollable factors including change in environmental factors such as temperature, relative humidity and pH of the soil. These problems can be overcome by identifying closely linked molecular markers to disease resistance loci and using the same in marker-assisted selection, which helps in rapid identification of the trait of interest in plants and development of durable resistant cultivars. Furthermore, markers can be exploited if they exhibit a tight association with the trait of interest across the breeding lines and populations (Pandiyaraj et al., 2019). In the present study, out of 147 recombinant inbred lines developed and evaluated for yield and its attributing traits only 43 lines were yielded more than 3 kg of fruits per plant. Among the 43 lines, only eight lines were identified as superior inbred line based on yield and quality traits. In the present study four DNA markers were utilized which were previously reported along with green house sick plot screening. Among the four markers used, only one SCAR marker *i.e.* SCU176-534 showed the polymorphism between the parents at band size 400 bp for resistance and 370 bp for susceptible. So, this marker was further forwarded marker assisted selection for bacterial wilt resistance in the superior inbred lines identified. The results found that among eight high yielding superior inbred lines, seven lines were identified as superior inbred line coupled with bacterial wilt resistance *viz*., TRIP2-8, TRIP2-17, TRIP2-18, TRIP2-22, TRIP2-24, TRIP2-35 and TRIP2-110 (Table 6) (Fig. 1).

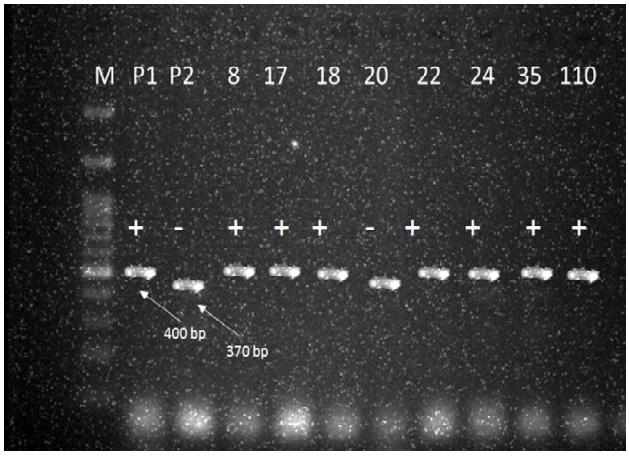
These molecular results was corroborative with phenotypic screening data. The similar findings were also reported by Sujeetkumar et al. (2018) by using SCU176-534 marker at significant level $P < 0.0001$ to validate for bacterial wilt resistance in 57 tomato genotypes and Truong et al. (2015) in F_9 lines derived from cross of *S. lycopersicum* Hawaii 7996 (resistant parent) and *S. pimpinellifolium* WVa 700 (susceptible parent) in tomato, who developed SCU176-534 marker through bulk segregant analysis. The failure of remained markers could be due to innumerable explanations including absence of genomic sequences

Table 5 : Genetic variability parameters for yield and component traits in Anagha × FBT-41 cross

Trait	Skewness	Kurtosis	PCV (%)	GCV (%)	Heritability (%)	GAM (%)
Plant height	0.19	2.21	15.93	15.89	99.48	32.7
Number of branches per plant	0.45	2.53	24.71	23.28	88.77	45.25
Number of cluster per plant	0.16	1.73	31.38	31.04	97.85	63.34
Number of fruits per cluster	0.3	2.25	27.67	26.9	94.46	53.93
Number of locules per fruit	0.16	1.73	27.06	25.99	92.25	51.5
Days to first flowering	0.3	2.25	9.12	8.41	85.06	16.00
Days to 50 percent flowering	0.19	2.37	8.99	8.08	80.77	14.97
Fruit length	0.57	3.46	17.99	17.7	96.8	35.93
Fruit diameter	0.9	3.88	13.72	13.36	94.8	26.84
Average fruit weight	0.8	3.3	20.57	20.35	97.91	41.54
Number of fruits per plant	0.19	2.21	42.86	42.81	99.75	88.2
Yield per plant	0.45	2.53	43.51	43.49	99.94	89.7
TSS	0.71	3.07	4.63	4.51	94.7	9.05
pH	1.16	4.10	5.33	5.05	89.63	9.86

Table 6 : Identification of superior inbred lines derived from Anagha × FBT-41 cross coupled with bacterial wilt resistance using both by greenhouse sick plot screening and DNA marker SCU176-534 genetically linked to an allele of a gene conferring bacterial wilt resistance

Parents/RILs	Pericarp thickness (mm)	Firmness (kg/cm^2)	Ascorbic acid (mg/100 g)	Lycopene (mg/kg)	Yield/ plant (kg)	PDI (%)	Reaction	Amplicon Status (±)
Anagha	3.50	3.85	12.50	11.60	3.93	0.00	HR	+
FBT- 41	3.40	3.74	10.20	12.50	3.33	84.00	HS	-
TRIP2-8	3.90	4.29	11.30	16.30	6.05	6.00	MR	+
TRIP2-17	4.20	5.25	15.30	13.20	5.08	8.00	HR	+
TRIP2-18	5.90	7.38	16.30	14.30	3.69	18.00	HR	+
TRIP2-20	4.10	4.51	14.00	15.30	3.80	48.00	MS	-
TRIP2-21	4.90	5.39	13.00	14.30	3.98	30.00	R	+
TRIP2-22	5.90	6.49	12.30	15.30	5.88	14.00	HR	+
TRIP2-24	5.30	5.57	15.30	16.10	4.52	30.00	R	+
TRIP2-35	3.80	3.99	12.60	13.20	5.89	14.00	HR	+
TRIP2-110	5.20	5.72	13.10	11.20	3.53	18.00	HR	+



[+: resistant, - : susceptible, P1: Anagha, P2: FBT-41, (8, 17, 18, 20, 22, 24, 35, 110) - RILS derived from Anagha × FBT-41 cross]

Fig. 1 : Identification of superior inbred lines derived from Anagha × FBT-41 cross coupled with bacterial wilt resistance using DNA marker SCU176-534 genetically linked to an allele of a gene conferring bacterial wilt resistance

corresponding to the primers used, the breakdown of linkages between markers and genes and differences in PCR conditions.

CONCLUSION

The study of the genetic evaluation of recombinant inbred line population for yield and its attributing traits is very helpful for ease of gainful selection in any breeding programme. So it can be concluded that these characters may be considered in selection criteria for the improvement of yield in tomato. High genetic parameters such as PCV, GCV, h^2_{bs} and GAM was observed in plant height, branches per plant, clusters per plant, fruits per cluster, fruit locules, fruit length, fruit diameter, average fruit weight, fruits and yield per plant indicated additive nature of gene action so there is scope for selection. One SCAR marker *i.e.* SCU176-534 showed polymorphism between parents so it can be used for marker assisted selection to identify superior inbred lines coupled with bacterial wilt resistance.

REFERENCES

Agong, S. G., Schittenhelm, S., & Friedt, W. (2000). Genotypic variation of Kenyan tomato (*Lycopersicon esculentum* L.) germplasm. *The Journal of Food Technology in Africa*, doi: 10.4314/JFTA.V6I1.19277

Akhter, M., Apon, F. N., Bhuiyan, M. M. R., Siddique, A. B., Husna, A., & Zeba, N. (2021). Genetic variability, correlation coefficient, path coefficient and principal component analysis in tomato (*Solanum lycopersicum* L.) genotypes. *Plant Cell Biotechnology and Molecular Biology*, 46-59. https://ikprress.org/index.php/PCBMB/article/view/6160.

Aslam, M. N., Mukhtar, T., Hussain, M. A., & Raheel, M. (2017). Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. *Journal of Plant Diseases and Protection*, *124*(6). 585-590. doi: 10.1007/s41348-017-0100-1

Chaudhary, L. B., & Prasad, B. (1968). Genetic variation and heritability of quantitative characters in Indian mustard (*Brassica juncea*). *Indian Journal of Agricultural Sciences*, *38*, 820-825.

Darshan, S. P., Reshma, J. K., & Mathew, A. (2013). Estimation of lycopene content in different tomato varieties and its commercial products. *Australasian Journal of Environmental Management*, *8*(2), 122-124.

Federer, W. T., & Raghavrao, D. (1975). On augmented design, *Biometrics*, *31*, 29-35.

Gawel, N. J., & Jarret, R. L. (1991). A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea. Plant Molecular Biology Reporter*, *9*(3), 262-266. doi:10.1007/BF02672076

Glaszmann, J. C., Kilian, B., Upadhyaya, H. D., & Varshney, R. K. (2010). Accessing genetic diversity for crop improvement. *Current Opinion in Plant Biology*, *13*(2), 167-173. doi: 10.1016/j.pbi.2010.01.004

Hanson, C. H., Robinson, H. R., & Comstock, R. S. (1956). Biometrical studies of yield in segregating population of Korean lespedeza. *Agronomy Journal*, *48*, 268-272.

Jayaramachandran, M., Kumaravadivel, N., Eapen, S., & Kandasamy, G. (2010). Gene action for yield attributing characters in segregating generation (M2) of sorghum (*Sorghum bicolour* L.). *Electronic Journal of Plant Breeding*, *1*(4), 802-805.

Jenkins, J. A. (1948). The origin of the cultivated tomato. *Economic Botany*, *2*(4), 379-392. https://doi.org/10.1007/BF02859492

Johnson, H. W., Robinson, H. F., & Comstock, R. E. (1955). Estimation of genetic and environmental variability in soybean. *Agronomy Journal*, *47*, 314-318.

http://dx.doi.org/10.2134/agronj1955.00021962004700070009x

Lush, J. L. (1940). Intra-sire correlation and regression of offspring on dams as method of estimating heritability of characters. *In: Proceedings of the American Society of Animal Production*, *33*, 293-301.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T. G., Yano, M., Bhatia, C. R., & Sasaki, T. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding*, *3*, 87-103. https://doi.org/10.1023/A:1009651919792

Pandiyaraj, P., Singh, T. H., Reddy, K. M., Sadashiva, A. T., Gopalakrishnan, C., Reddy, A. C., Pattanaik, A., & Reddy, D. L. (2019). Molecular markers linked to bacterial wilt (*Ralstonia solanacearum*) resistance gene loci in eggplant (*Solanum melongena* L.). *Crop Protection*, *124*, 104822. doi: 10.1016/j.cropro.2019.05.016

Pearson, K. (1894). III. Contributions to the mathematical theory of evolution. *Philosophical Transactions of the Royal Society*, *185*, 71-110. https://doi.org/10.1098/rsta.1894.0003

Rick, C. M. (1969). Controlled introgression of chromosomes of *Solanum pennellii* into *Lycopersicon esculentum*: segregation and recombination. *Journal of Genetics*, *62*(4), 753. doi: 10.1093/genetics/62.4.753

Sadasivam, S., & Manickam, A. (1992). Biochemical methods for agricultural sciences. *Wiley Eastern Limited*.

Sujeet, K. S., Ramanjini Gowda, P. H., Saikia, B., Debbarma, J., Velmurugan, N., & Chikkaputtaiah, C. (2018). Screening of tomato genotypes against bacterial wilt (*Ralstonia solanacearum*) and validation of resistance linked DNA markers. *Australasian Plant Pathology*, *47*(6), 365-374. doi: 10.1007/s13313-018-0567-7

Truong, H. T. H., Kim, S., Tran, H. N., Nguyen, T. T. T., Nguyen, L. T., & Hoang, T. K. (2015). Development of a SCAR marker linked to bacterial wilt (*Ralstonia solanacearum*) resistance in tomato line Hawaii 7996 using bulked-segregant analysis. *Horticulture, Environment, and Biotechnology*, *56*(4), 506-515. doi: 10.1007/s13580-015-1050-9

Winstead, N. N., & Kelman, A. (1952). Inoculation techniques for evaluating resistance to *Pseudomonas Solanacearum*. *Phytopathology*, *42*(11), 628-634.

Yang, W., & Francis, D. M. (2007). Genetics and breeding for resistance to bacterial diseases in tomato: prospects for marker-assisted selection. *Genetic Importance of Solanaceae Crops*, *2*, 379-419.

(***Received : 19.4.2023; Revised : 20.7.2024; Accepted : 25.7.2024)***