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Original Research Paper

Genetic diversity and screening for bacterial wilt in tomato (*Lycopersicon esculentum*)

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ABSTRACT

Thirty-four tomato genotypes from different geographical locations were evaluated for genetic diversity and screened for bacterial wilt (BW) caused by *Ralstonia solanacearum*. Results revealed that plant height, fruits per cluster, fruit weight, fruit diameters, locules per fruit, fruit firmness, yield per plant, and quality parameters exhibited high heritability and genetic advance. Clustering based on D^2 analysis, classified genotypes into four clusters. Maximum intra-cluster distance was recorded within cluster I and maximum inter-cluster distance between cluster II and IV followed by cluster I and IV, indicating existence of wide genetic variability. Genotypes in cluster IV (AVTO 1711, AVTO 1717 and AVTO 1718) recorded high fruit weight coupled with high yield. These may be explored as promising donors for developing large sized bacterial wilt resistant tomatoes. The large fruited genotypes in cluster IV can also contribute to the genetic improvement of existing bacterial wilt resistant varieties placed in cluster I. Out of 34 genotypes screened for BW disease, 5 genotypes were classified as resistant and 7 as moderately resistant.

Keywords : Bacterial wilt, genetic advance, heritability, humid tropics

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), the second most important vegetable in the world after potato excels as a good source of vitamin A, C, E, contains large quantity of water, calcium and niacin. The crop largely attracts farmers due to its short duration, low input costs and feasibility for cultivation throughout the year. In India, tomato has registered a production of 20.30 million tonnes from 830.75 thousand ha area (NHB, 2022). Madhya Pradesh is the leading producer of tomato with 2970.0 thousand metric tonnes from an area of 1,03,000 hectares. Successful crop breeding depends on the variability and genetic diversity in the base population. Yield and its components, with their polygenic inheritance, are vulnerable to environmental sways. Variability present in the base population could be segmented into heritable, and non-heritable, segments with genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), heritability and genetic advance. GCV and PCV indicates the amount of variability present in the base population, while, heritability and genetic advance assist in determining environmental influences, and the degree to which improvement is achievable (Patel *et al*., 2013).

Diverse parents bring about hybrid vigor, consequently, examination of genetic diverseness is necessary to determine the breeding strategy (Harrington, 1940). According to D^2 statistics (Mahalanobis, 1936), genetic divergence helps in identifying diverse parents which on hybridization yield bumptious transgressive segregants (Naveen *et al*., 2018).

Bacterial wilt caused by *Ralstonia solanacearum* have caused havoc in the commercial cultivation of tomato leading to heavy yield losses. It causes 26% loss of fresh fruit production in hybrid tomatoes and yield losses reach up to 90.62% (Dharmatti *et al*. 2009). Development of resistant varieties can be employed as alternative to overcome bacterial wilt disease. Most of the bacterial wilt resistant sources have only small fruit size due to linkage drag of wilt resistant gene with small fruit size (Wang *et al*., 1998). Identifying a resistant genotype with better fruit size will help in easy transfer of resistance into different background. With this foreground, the present study was carried

out to analyse the diversity in tomato genotypes and screening for bacterial wilt disease.

MATERIALS AND METHODS

The present investigation was accomplished employing 34 tomato genotypes. Out of 34 genotypes, 23 were collected from the ICAR-NBPGR, New Delhi, 3 from World Vegetable Centre, Taiwan and remaining 8 genotypes (3 advanced lines and 4 varieties) from Kerala Agricultural University, Kerala. Two experiments were carried out. In first experiment, 34 genotypes were planted in pots in a completely randomized block design with 2 replications. Standards package of practices recommended by Kerala Agricultural University was followed. Data on growth, yield and quality traits were subjected to statistical analysis as per Comstock and Robinson (1952), Johnson *et al*., (1955), and Allard (1961). Mahalanobis D^2 analysis (Mahala nobis, 1936) and Euclidean clustering (Spark, 1973) was used to elucidate divergence and consequent selection of parents for hybridization.

Second experiment was laid out in completely randomized block design with 2 replications to screen the genotypes for bacterial wilt incidence under field conditions with one susceptible check variety Pusa Ruby. Prior to crop establishment, the soil was tested for pathogen load by serial dilution, which recorded an inoculum load of 61 x 10^6 cfu/ g soil. Plants were observed on daily basis during the entire crop period for bacterial wilt symptom which was confirmed by ooze test. Bacterial wilt incidence was recorded and per cent wilt incidence was calculated by the following formula.

$$\text{PDI} = \frac{\text{number of plants infected}}{\text{total number of plants observed}} \text{ X } 100$$

The genotypes were grouped into different categories based on the per cent disease incidence (PDI) and the reaction of the genotypes to bacterial wilt as described by Mew and Ho (1976).

Reaction	Per cent disease incidence
R (Resistant)	0-20
MR (Moderately resistant)	21-40
MS(Moderately susceptible)	41-60
S (Susceptible)	61-100

RESULTS AND DISCUSSION

Heritability, variance components and genetic advance

Significant variations were recorded for growth and yield traits in the base population (Table 1). The PCV was imperceptibly higher than GCV, indicating the environmental impact on the expression of these traits. Estimates of GCV and PCV were high for yield per plant, fruit weight, number of fruits per plant, secondary branches per plant, fruit firmness, ascorbic acid acidity, lycopene, and beta carotene. This designated greater magnitude of phenotypic and genotypic variability in the base population. GCV alone, cannot be depended upon to decide the magnitude of heritable variation, and hence, the knowledge on heritability also is entailed.

Heritability plays decisive role in breeding, expressing the reliability of phenotype as an indicator of its breeding values. Heritability was high (61.31% - 97.97%) for most of the traits, suggesting less influence of environment factors, and hence, effectiveness in selection. High genetic advance as percentage of mean was observed for all traits except for days to flowering, days to harvest, and total soluble solids (Ara *et al*., 2009), suggesting the predominance of additive gene action. TSS recorded high heritability with moderate genetic advance, while days to flowering and days to harvest recorded low heritability and low genetic advance implying the control by non-additive gene action.

On the basis of D^2 analysis, 34 genotypes were grouped into four highly divergent clusters (Table 2 and Fig. 1). High inter-cluster and low intra cluster values highlighted the cluster divergence. Numbers of genotypes in clusters were in the order: Cluster I >cluster III >cluster II > cluster IV. The clustering pattern showed that accessions from different geographical areas were clubbed in single cluster indicating that there existed no parallelism between genetic diversity and geographical origin (Meena and Bahadur, 2015). Similarly, accessions from same geographical origin were distributed into different clusters, indicating that these accessions must have under gone changes for characters under selection which could be attributed to selection or genetic drift, creating more diversity rather than genetic distance. This clearly explained that selection of parents for hybridization must be emphasized on genetic diversity rather than geographical diversity (Naveen *et al.,* 2018).

Table 1 : Estimates of variance for yield and yield contributing traits in tomato

Characters	Range	Mean	GV	PV	GCV (%)	PCV (%)	H^2	GA	GAM
Plant height (cm)	35.25-76.5	52.9	63.00	102.55	15.00	19.14	61.43	12.82	24.23
Days to flowering	47.5-59.5	55.38	4.82	19.08	3.96	7.89	25.27	2.27	4.11
Days to harvest	84-98.5	89.94	8.42	19.27	4.90	3.24	43.7	3.95	4.39
Primary branch	4.75-10.5	7.46	1.02	1.85	13.53	18.21	55.20	1.54	20.70
Secondary branch	7.5-25.75	12.00	11.70	13.58	28.48	30.69	86.11	6.54	54.45
Fruits per cluster	2.1-4.7	3.07	0.34	0.55	18.86	24.08	61.31	0.94	30.42
Fruits per plant	13.38-69	24.91	191.31	198.85	56.27	57.37	96.21	27.95	113.70
Fruit weight (g)	15.15-118.4	50.01	602.55	655.61	49.03	51.15	91.91	48.48	96.83
Polar diameter (cm)	11.1-21.1	14.40	4.02	4.95	13.92	15.45	81.22	3.72	25.85
Equatorial diameter (cm)	9.95-21.7	13.81	4.03	4.92	14.54	16.05	81.99	3.75	27.11
Locules per fruit	2-5	3.67	0.40	0.47	17.17	18.70	84.31	1.19	32.48
Fruit firmness	0.52-1.8	1.16	0.15	0.16	33.02	34.34	92.48	0.76	65.42
TSS (ºBrix)	4.4-7.15	6.12	0.35	0.48	9.73	11.33	73.62	1.05	17.19
Ascorbic acid (mg/100g)	8.16-26.53	13.28	26.78	27.34	38.98	39.38	97.97	10.55	79.48
Acidity (%)	0.25-1.21	0.52	0.05	0.06	43.30	47.00	84.87	0.43	82.17
Lycopene (mg/100g)	1.49-10.74	4.97	5.46	6.14	47.00	49.83	88.97	4.54	91.33
Beta carotene (mg/100g)	0.93-7.29	3.32	1.88	2.01	41.32	42.69	93.68	2.73	82.39
Total sugars (mg/100g)	1.96-3.26	2.52	0.11	0.12	13.35	13.58	96.58	0.68	27.03
Shelf life (days)	7.25-16.5	10.29	7.19	8.58	26.06	28.47	83.76	5.05	49.13
Yield (kg)	0.36-2.42	1.10	0.46	0.48	62.07	63.27	96.25	1.37	125.45

GV-genotypic variance, PV-phenotypic variance, GCV-genetic coefficient of variation, PCV-phenotypic coefficient of variation, H^2-heritability, GA-genetic advance, GAM- genetic advance as percentage of mean

Table 2 : Cluster wise distribution of tomato genotypes

Cluster No.	Total number of accessions	Name of Accessions
I	19	EC-914087, EC-914094, EC-914100, EC-914107, EC-914091, EC-914096, EC-914099, EC-914093, Sakthi, Mukthi, Anagha, Manuprabha, EC-914090, EC-914103, EC-914109, EC-914098, EC-914102, EC- 9140107, EC-914085
II	4	Sln-2 (Mukthi x IIHR 2195-F2-38-5-1),Sln-6 (Mukthi x IIHR 2195-F2-38-3-6), Sln-7 (Mukthi x IIHR 2196- F2-57-4-45),Sln-9 (LE-1-2 x H24-F2-59-3-20)
III	8	EC-914089, EC-914108, EC-914086, EC-914092, EC-914097, EC-914087, EC-914100, EC-914104
IV	3	AVTO-1718, AVTO-1711, AVTO-1717

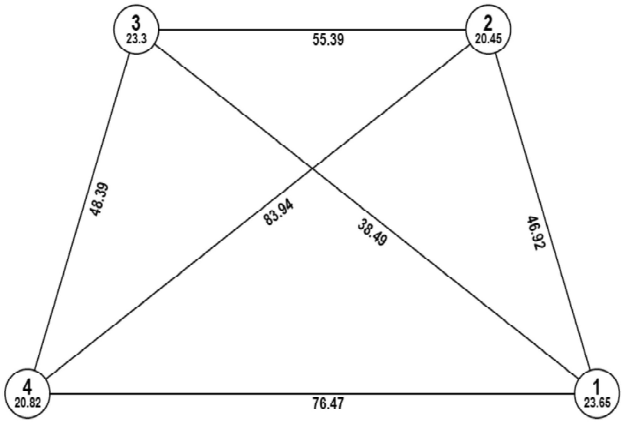

Fig. 1 : Dendrogram showing clustering of tomato genotypes

Average inter and intra cluster distance (Table 3 and Fig. 2) revealed that inter cluster distances were higher than that of intra cluster distances, suggesting homogeneous and heterogeneous nature of the germplasm within and between the clusters, respectively (Rai *et al*., 2017). Cluster I recorded the highest intra cluster distance suggesting the presence of maximum diversity among the genotypes in it. At inter cluster level, minimum distance was recorded between cluster I and cluster III, while, cluster II and cluster IV recorded the maximum inter cluster distance. Minimum inter cluster distance indicated that these genotypes are closely related, and a higher inter cluster distance indicated wider genetic diversity among the genotypes, hence, parents for hybridization must be selected from these clusters, to generate maximum heterotic progenies and for getting desirable transgressive segregants (Naveen *et al*., 2018).

Table 3 : Intra and inter cluster distance in tomato genotypes

Cluster No.	I	II	III	IV
I	23.65	46.93	38.49	76.47
II		20.45	55.39	83.94
III			23.30	48.39
IV				20.82

The cluster means of characters indicated the presence of appreciable amount of genetic variation among clusters (Table 4). Intercrossing among the genotypes with outstanding mean performance (cluster mean) gives heterotic crosses (Kumar *et al*., 2013). The genotypes in the cluster II recorded high mean values for days to harvest, fruits per cluster, fruits per plant, TSS, ascorbic acid, lycopene, and beta carotene. Cluster III showed maximum mean values for primary branches, secondary branches, locules per fruit, and fruit firmness. Genotypes from cluster III could give plants with more branches, and firm fruits when

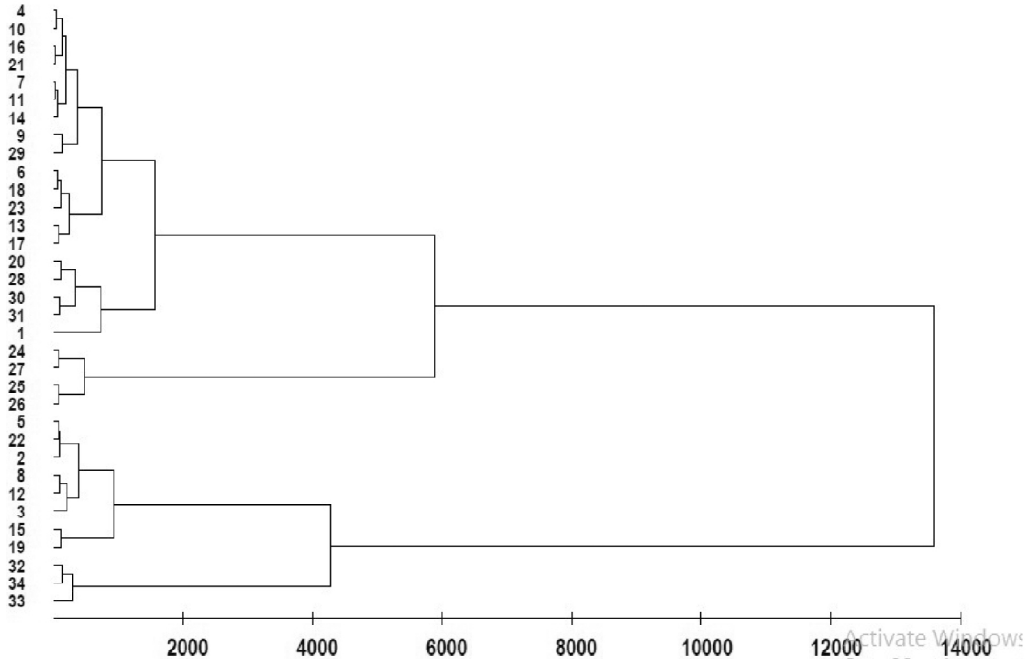

Fig. 2 : Mahalnobis Euclidean Distance (not to scale)

Table 4 : Cluster wise mean performance of tomato genotypes

Character	I	II	III	IV
Plant height (cm)	53.78	38.06	53.72	65.25
Days to flowering	55.87	54.97	55.95	51.33
Days to harvest	90.22	87.09	91.27	88.50
Primary branch	7.67	5.81	7.88	7.25
Secondary branch	11.51	8.88	15.09	11.08
Fruits per cluster	3.13	3.35	2.91	2.77
Fruits per plant	20.51	58.75	18.98	23.50
Fruit weight (g)	35.92	39.93	66.76	108.03
Polar diameter (cm)	13.67	12.19	15.86	18.14
Equatorial diameter (cm)	13.03	12.39	14.43	19.08
Locules per fruit	3.68	3.85	3.40	4.07
Yield per plant (kg)	0.64	2.11	1.23	2.28
Fruit firmness (kg/cm^2)	1.13	0.93	1.34	1.23
TSS (^{o}brix)	6.09	6.38	6.28	5.53
Ascorbic acid (mg/100g)	12.88	17.54	11.11	15.92
Acidity (%)	0.51	0.59	0.48	0.60
Lycopene content (mg/100 g)	4.66	7.16	5.21	3.40
Beta carotene content (mg/100 g)	3.24	3.97	3.50	2.46
Total sugars (%)	2.61	2.21	2.59	2.14
Shelf life (days)	10.11	9.88	10.56	11.25

used in hybridization. Plant height, fruit weight, polar diameter, equatorial diameter, yield per plant, acidity, shelf life recorded maximum cluster mean values in Cluster IV, and minimum value for days to first flowering. When breeding for earliness, high fruit weight, yield, acidity, and improved shelf life, genotypes from clusters IV, could be effectively utilized (Meena and Bahadur, 2013).

Screening for bacterial wilt resistance

Based on the PDI, the genotypes were classified into four groups (Table 5). Five genotypes *i.e.*, Sakthi, Mukthi, Anagha, Manuprabha and AVTO-1711 appeared as resistant, while, seven genotypes were categorized as moderately resistant to the bacterial wilt, however, five genotypes were rated as moderately susceptible and seventeen were susceptible.

Table 5 : Classification of tomato genotypes based on per cent disease incidence (PDI)

Disease reaction	Genotype
Susceptible (61-100 PDI)	EC-914085, EC-914087, EC-914088, EC-914089, EC-914092, EC-914093, EC-914095, EC-914096, EC-914097, EC-914098, EC-914099, EC-914101, EC-914102, EC-914103, EC-914105, EC-914107, EC-914109
Moderately susceptible (41-60 PDI)	EC-914086, EC-914100, EC-914104, EC-914108, Sln-9,
Moderately resistant (21-40 PDI)	EC-914090, EC-914091, AVTO-1718, AVTO-1717, Sln-2, Sln-6, Sln-7
Resistant (0-20 PDI)	Sakthi, Mukthi, Anagha, Manuprabha, AVTO-1711

CONCLUSION

Significant diversity among tomato genotypes could be effectively exploited in developing promising and high yielding bacterial wilt resistant hybrids. High heritability and genetic advance as percentage of mean were observed for plant height, fruits per cluster, fruit weight, polar and equatorial diameter, locules per fruit, fruit firmness, yield per plant and quality parameters, referring that these traits could be focused for developing promising high yielding tomato hybrids. Cluster analysis grouped the exotic large fruited genotypes in cluster IV, and the bacterial wilt resistant genotypes in cluster I, and small fruited bacterial wilt moderately resistant improved genotypes in cluster II. Maximum inter cluster distance was recorded between cluster II and cluster IV, followed by cluster I and cluster IV, indicated that exotic genotypes from World Vegetable Centre could be one of the promising parents and the small fruited bacterial wilt resistant improved genotypes as the counter parent for getting maximum heterotic hybrids as they are genetically diverse. The large fruited exotic lines in cluster IV can be used for improving the fruit size of bacterial wilt resistant varieties.

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