Indian J. Plant Genet. Resour. 35(2): 257–263 (2022)
DOI 10.5958/0976-1926.2022.00029.8

RESEARCH ARTICLE

SSR Marker Based Genetic Diversity and Fusarium Wilt Resistance Screening of Tomato (*Solanum lycopersicum* L.) Genotypes

K Sushma[1*], P Saidaiah[1], Harikishan Sudini[2], A Geetha[3] and K Ravinder Reddy[1]
[1*]*Department of Vegetable Science, Sri Konda Laxman Telangana State Horticultural University (SKLTSHU), Rajendranagar, Hyderabad-500030, Telangana, India*
[1]*College of Horticulture, Sri Konda Laxman Telangana State Horticultural University (SKLTSHU), Rajendranagar, Hyderabad-500030, Telangana, India*
[2]*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad-502324, Telangana, India*
[3]*College of Agriculture, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Palem-509215, Nagarkurnool District, Telangana, India*

(Received: 06 April, 2022; Revised: 14 July, 2022; Accepted: 15 July, 2022)

Fusarium oxysporum is one of the devastating diseases of tomato (*Solanum lycopersicum* L.) causing high yield losses in fields and commercial greenhouses, inferring necessity for development of disease resistance. An research experiment was laid out with 23 diverse genotypes including susceptible check at research farm during wet season-2018 for screening of tomato genotypes for fusarium wilt resistance. Phenotypic screening of 23 genotypes revealed varied disease resistance as highly resistant (AVTO1219 and EC631), resistant (Pant bahar, EC620428, EC620378, EC631369 and EC620503), moderately resistant (EC615055, EC620389, EC620394, EC620422, EC620406 and AVTO9803) and moderately susceptible (PKM1, EC620382, EC620427 and EC620395) over the highly susceptible checks (Pusa Ruby, Arka Vikas). Employing 95 SSRs for molecular profiling resulted in 33 polymorphic markers, 58 monomorphic markers and remaining 4 markers as unamplified. A total of 74 alleles were detected using 33 polymorphic markers with an average allele number of 2.24 for each marker. Two markers viz., TES60 and TGS633 produced maximum (4) alleles. The polymorphic information content (PIC) value ranged from 0.28 to 0.76 with an average of 0.53 and marker TGS633 was found to be the most suitable marker with the highest PIC value. Cluster analysis through UPGMA method classified twenty three genotypes into five clusters and the coefficient among 23 genotypes was varied from 0.5 to 0.86. Over all, EC631379, EC631369, EC620428, EC620378 and EC620503 were identified as new resistant genetic resources against Fusarium wilt at both phenotypic and genotypic level with the presence of *I-2* gene loci. Based on the results, the identified genotypes can be further tested and be used in Marker Assisted Selection or gene pyramiding programs to develop disease resistant commercial cultivars.

Key Words: Cluster analysis, Fusarium wilt, Tomato: *Solanum lycopersicum*, SSRs

Introduction

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop in the world, belongs to the family *Solanaceae*, with its probable origin at Peru Equador region (Rick 1969) and it is second popular widely grown and consumed vegetable in the world, next to potato (Anonymous 2005; Reddy *et al.*, 2013). The fruits are rich source of vitamins (A and C), minerals (potassium, phosphorus, magnesium, calcium, and fair concentrations of protein and Niacin (Onyekachukwu and Adefoyeke 2017). In addition, it is also considered as 'Protective food' because of its special nutritive values and antioxidant properties due to the presence of lycopene and flavonoids (Sepat *et al.,* 2013). Lycopene is treasured for its anticancer attribute and have antiseptic and blood purifier properties. It acts as an antioxidant which is often colligated with anti-carcinogenic nature (Giovannucci 2002; Miller *et al.,* 2002; Bai and Lindhot 2007). Besides rich nutritional values, tomato also has good agronomical characteristics like wider adaptability, high yielding potential and multipurpose uses in the form of fresh and processed food Despite of the few competitors in the value addition chain of tomato, hindrances like biotic and abiotic stresses are playing detrimental role in yield formation. . Among all these hurdles, biotic stress solely effecting 10-50% of yield (de

**Author for Correspondence: Email: kothasushma20@gmail.com*

Carvalho *et al.,*, 2012). In tomato, over twenty diseases were reported from different parts of country and the diseases like wilt, damping-off, early blight, late blight, septoria leaf spot, leaf curl, tobacco mosaic, root-knot etc were noticed as major diseases.

Fusarium wilt (FW), caused by *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) is one of the most devastating diseases, which is a soil borne disease with mechanical mode of pathogen entry into the host and the crop is susceptible throughout all growth stages. Pathogen entry is succeeded by colonization in the xylem, resulting in inhibition of water flow and wilt like symptoms, yellowing and drooping of leaves on one side of the plant. Leaf wilting, plant stunting, browning of the vascular system, leaf death and lack of fruit production also occurs. *Fusarium oxysporum* f. sp. *lycopersici* (FOL) causes disease only in plants of the genus lycopersicon and inhabits most tomato growing regions worldwide, causing yield losses. The variation in genetic architecture of varieties will bring in them the disease resistance. Thus, the assessment of genetic diversity is essential to enhance the genetic yield potential, nutritional properties along with sustainability to different stress.

As the number of varieties continuously increase, the discrimination among cultivars based on morphological traits becomes complex as these traits are influenced by environmental factors. Molecular markers can be used as a complementary tool to overcome this and their frequent availability with the characteristics of high level of polymorphism, multi-allelic and can be assayed with minute DNA concentrations. Moreover molecular markers have also been successfully applied in registration activities, such as cultivar identification where the goal is to obtain specific pattern for each variety (Lombard *et al.,* 2001). Among different molecular markers, SSRs (microsatellites) have been most widely used over past 20 years due to its co-dominant, multi-allelic and reproducibility nature (Mason 2015). Many of the researches explored SSRs for different studies of genetic characterization and genetic assessment in tomato (Benor *et al.,* 2008; Dhaliwal *et al.,* 2011; El-Awady *et al.,* 2012; Sanghani *et al.,* 2013; Kaushal *et al.,* 2017). A combination of microsatellites can be useful in distinguishing cultivars of tomato, which are genetically strongly related to each other. The objective of this study was to evaluate tomato genotypes as new resistant sources for Fusarium wilt and assessment of genetic diversity among these tomato genotypes using molecular markers.

Materials and Methods

Plant material

A total of twenty three genotypes were collected from different sources of India. Among them 19 genotypes were procured from NBPGR, Regional Station, Hyderabad and 4 were released varieties. Details of seed material and their source were given under Table 1. The present study was carried out in wet season 2018, at the PG Research Block, Department of Vegetable Science, College of Horticulture, Sri Konda Laxman Telangana State Horticultural University, Rajendranagar, Hyderabad.

Table 1. List of genotypes and their sources used for diversity study

S. No.	Genotypes	Source
1.	EC615055	NBPGR, Hyderabad
2.	EC620463	NBPGR, Hyderabad
3.	EC620428	NBPGR, Hyderabad
4.	AVTO1219	WVC, Taiwan, China
5.	EC620378	NBPGR, Hyderabad
6.	EC620382	NBPGR, Hyderabad
7.	EC620389	NBPGR, Hyderabad
8.	EC620395	NBPGR, Hyderabad
9.	EC620406	NBPGR, Hyderabad
10.	EC620427	NBPGR, Hyderabad
11.	EC620394	NBPGR, Hyderabad
12.	EC620422	NBPGR, Hyderabad
13.	EC631369	NBPGR, Hyderabad
14.	EC631379	NBPGR, Hyderabad
15.	EC620503	NBPGR, Hyderabad
16.	AVTO9803	WVC, Taiwan, China
17.	AVTO9804	WVC, Taiwan, China
18.	AVTO1002	WVC, Taiwan, China
19.	AVTO0101	WVC, Taiwan, China
20.	Pusa Ruby	IARI, New Delhi
21.	PKM1	Periyakulam, TNAU
22.	Pant bahar	GBPUAT, Uttarakhand
23.	Arka vikas	IIHR, Bengaluru

Morphological identification and characterization of strains of FOL

For identification of Fusarium wilt causing organism strains, the morphological characteristics of micro conidia, macro conidia, phialides and chlamydospores, single-spored isolates were grown for 10-15 days on PDA medium as described by Booth (1971); Gerlach

and Nirenberg (1982); Nelson *et al.* (1983); Burgess *et al.* (1994); and Leslie and Summerell (2006). Fusarium wilt culture preparation was done followed procedure given by Nirmaladevi and Srinivas, 2012. The colour and pigmentation of the isolates on PDA medium varied between white, creamish white to cream, light pink to pink and light purple to violet.

Morphological screening

Twenty three genotypes were sown in the trays filled with coconut pith compost and raised them by following standard agronomical cultural practices. Morphological screening of 21 days old seedlings for fusarium wilt was conducted using root dip method. Conidia of all the isolates were recovered from one week old cultures. The race2 of FOL was purified and used for screening all the tomato genotypes under test. Seedlings were then removed from the trays, shaken to remove the adhering particles and washed carefully under tap water. The roots were trimmed with a sterile scissor and were submerged in the conidial suspension for 30 minutes. The inoculated seedlings were transplanted to polybags (15 cm diameter), after surface sterilized with 0.1% mercuric chloride containing soil and sand 1:1 ratio. The severity of the disease was assessed from 2 weeks of inoculation up to 45 days (Nirmaladevi and Srinivas 2012). Symptoms were recorded from 0 to 4 scale given by Bahar *et al.* (2012). 0 - No symptoms, 1- Slight chlorosis 2- Moderate chlorosis, wilting or stunting of the plant, 3- Severe chlorosis, wilting or stunting of the plant, 4- Death of the plant. In addition to this, percent incidence of Fusarium oxysporum was also calculated using a scale of 0-4 as adopted by Silme and Cagirgan (2010) which was based on infection percent as follows: 1-0.33-25%, 2-26-50%, 3-51-66.66%, 4-66.67-100%. Where: 0= Highly resistant (HR), 1= resistant (R), 2 = moderately resistant (MR), 3 = moderately susceptible (MS), 4= susceptible (S) and highly susceptible (HS).

Isolation of Genomic DNA and PCR amplification

Approximately 100 mg of fresh young leaves were collected and DNA isolation was performed by using modified CTAB method (Doyle and Doyle 1990). The quality of DNA was checked using 1% Agorase gel electrophoresis and quantity of DNA was determined with a nanodrop. These samples were diluted to the required concentration for PCR analysis and stored at -20^{o}C until use (Velpula *et al.*, 2017). PCR amplification was performed as follows: one cycle of 94^{o}C for 5 min; 35 cycles of 55^{o}C for 1 min, 72^{o}C for 2 min, and 94^{o}C for 1 min. After the final cycle, 1 cycle of 55^{o}C for 1 min and 72^{o}C for 7 min was added. The PCR amplification was verified by ethidium bromide (EtBr) were used for visualization using gel documentation unit.

SSR and Cluster analysis

A total of 95 SSRs were used to analyse the genetic diversity of 23 tomato genotypes, which were selected from the linkage map of tomato genome by covering all the 12 chromosomes. The amplified alleles for 23 genotypes with each primer were scored based on presence or absence of the allele at a given locuswith1 and 0 respectively. The polymorphism information content (PIC) for each SSR was calculated according to the formula PIC = 1 - $\sum pi^2$ .Where pi is the frequency of the i^{th} allele for each SSR marker locus in the set of 23 tomato genotypes investigated (Weir 1990). The binary matrix data retrieved from the molecular marker analysis was used for diversity assessment. The Jaccard's similarity coefficient values were calculated for 23 tomato germplasm accessions using NTSYS-pc version 2.02e (Rohlf 2000) and the dendrogram was constructed based on UPGMA (Unweighted pair group method with arithmetic mean) method.

Results

Morphological screening

In the present study, 23 genotypes were studied for Fusarium wilt resistance at morphological level. Scoring of Fusarium wilt infection severity based on morphological symptoms revealed five groups (Table 2) *viz.,* asymptomatic/no chlorosis in two cultivars (AVTO1219 and EC631) (Supplementary Fig. 1), slight chlorosis of leaves in five genotypes (Pant bahar, EC620428, EC620378, EC631369 and EC620503), Moderate chlorosis with wilting or stunting of the plant in six genotypes (EC615055, EC620389, EC620394, EC620422, EC620406 and AVTO9803), severe chlorosis with wilting and stunting of the plant in four genotypes (PKM1, EC620382, EC620427 and EC620395) and plant death was observed in six genotypes (EC620463, AVTO9804, AVTO1002 and AVTO0101) including susceptible checks (Pusa Ruby and Arka Vikas). The percent incidence of disease in genotypes was ranged from 0% to 100% (Supplementary Table 1).

Table 2. Response of genotypes for *Fusarium* wilt incidence

S. No.	Reaction	Score	Number of genotypes	Genotypes
1.	Highly Resistant (HR)	0	2	AVTO1219, EC631379
2.	Resistant (R)	1 (0-1)	5	Pant bahar, EC620428, EC620378, EC631369, EC620503
3.	Moderately Resistant (MR)	2 (1-2)	6	EC615055, EC620389, EC620394, EC620422, EC620406, AVTO9803
4.	Moderately Susceptible (MS)	3 (2-3)	4	PKM1, EC620382, EC620427, EC620395
5.	Susceptible (S) and Highly Susceptible (HS)	4 (3-4)	6	Pusa Ruby, Arka Vikas, EC620463, AVTO9804, AVTO1002, AVTO0101

Molecular screening

The PCR analysis using 95 SSRs resulted 33 markers as polymorphic (Supplementary Fig. 3), 58 markers as monomorphic and remaining 4 markers were not amplified. These 33 polymorphic markers were further used in analysis of molecular diversity with yielded allelic data. The binary matrix data was prepared by considering clear variation among alleles. The total number of alleles were 74 with an average of 2.24 and the the number of alleles were varied from 2 to 4. Highest number of alleles was observed with TES60 and TGS633. PIC value among SSRs was varied widely from 0.28 to 0.76 with an average of 0.53 (Supplementary Table 2). Highest PIC value of 0.76 was observed with TGS633 and SSR46, TGS1093 were followed with 0.73 PIC value. Whereas the lowest PIC value of 0.28 was observed with TEI0396 marker.

Genetic diversity pattern

Based on UPGMA cluster analysis using 33 polymorphic primers, the 23 genotypes were classified into 5 clusters *i.e.* cluster I, cluster II, cluster III, cluster IV and cluster V with the similarity coefficient value from 0.5 to 0.85 (Supplementary Fig. 2). List of genotypes in clusters I to V were given under Supplementary Table 3. The highest similarity was observed between EC620382 and EC620389, placed in sub cluster II with 86% similarity.

Discussion

A total of 23 tomato genotypes were screened for Fusarium wilt resistance after proper confirmation of fungal isolates (*Fusarium oxysporium)* symptoms. Interestingly, different levels of resistance was observed among the genotypes (Table 2), indicating high diversity among the genotypes. The two genotypes AVTO1219, EC631379 with 0% disease incidence and with score of "0" were showing their high resistance nature against Fusarium wilt, this resistance nature of AVTO1219 was also explained by World Vegetable Centre database, Loganathan Indian Institute of Spices Research annual report (2012-13). Genotypes, Pusa Ruby, EC620463, AVTO1002, Arka Vikas, AVTO0101 and AVTO9804 were susceptible genotypes with disease incidence of 100-83.33 (3-4 score). In the present study, genotypes with varied disease symptoms (scored as 0-4) and disease incidence (80% -100% incidence) were observed. These results are in agreement with the finding of Mahmoud *et al*. (2006); Ahmadvand *et al.* (2010) and Antonio *et al.* (2017).

In addition to this, genetic diversity among 23 genotypes was assessed using 95 SSR's markers, which were selected from the linkage map of tomato genome by covering all the 12 chromosomes. The PCR analysis using 95 SSRs revealed 33 polymorphic markers, 58 monomorphic markers and 4 unamplified markers. Thus, these markers showed low levels of polymorphism among genotypes (Alvarez *et al.,* 2001; Yang *et al.,* 2005), it may be due to the non specificity of markers and the lower levels of polymorphism was detected by interrupted and imperfect SSRs may be associated with the initial stages of mutational decay, so that replication slippage is less-likely to occur (Smulders *et al.,* 1997; Benor *et al.,* 2008) or probably due to its autogamous nature (El-Awady *et al.,* 2012). A total of 74 alleles were observed with 33 polymorphic markers with an average of 2.24 alleles for each marker. The highest number of alleles (3) was resulted with markers TES60 and TGS633 showing the importance of these markers in the diversity analysis. PIC value was calculated based on allelic information to study the discrimination power of each marker among the genotypes. As a result it was in the range of 0.28 to 0.76 with an average value of 0.53. This results was almost similar to Jones *et al.*, (2001) in the range of 0.2 and 0.8 with an average value

0.56 and it was higher than the previously reported PIC values of different diversity studies conducted in tomato like 0.3 (Kaur *et al.,* 2018), 0.31 (Benor *et al.,* 2008), 0.35 (Varshney *et al.,* 2009), 0.39 (Frary *et al.,* 2005), 0.37 (He *et al.,* 2003), 0.4 (Bredemeijer *et al.,* 2002), 0.45 (Glogovac *et al.,* 2013),. Interestingly, highest PIC value of 0.76 was observed with TGS633 and SSR46, TGS1093 were followed with 0.73 PIC value indicating these markers would be further useful to discriminate the genetic variability in tomato germplasm. Moreover the marker TGS633 was making remarkable role with highest allele value along with highest PIC value.

Based on cluster analysis using UPGMA was revealed all the genotypes into five major clusters (I, II, III, IV and V). Similarity coefficient among 23 genotypes was observed with the range of 0.5 to 0.86. The superior nature of Pusa Ruby was once again proved by forming into a separate cluster (I) among all genotypes (Yogendra and Gowda 2013). In second cluster the two genotypes EC620382 and EC620427 were shown highest similarity (86%), this highest similarity is may be due to the similarities in evolution pattern. Interestingly, these two genotypes showed negligible (0.81) similarity coefficient, though from different pedigree. The remaining two released varieties PKM1 and Pant Bahar were also located in the same clustered and paired with AVTO1219 with different levels of resistance against Fusarium wilt. Most of the remaining lines in cluster II were shown similar results at both genotypic and phenotypic level except EC631379 and Pant bahar, it may be due to the presence of other resistance gene loci of Fusarium wilt and the resistant of Pant bahar was also explained by Agarwal *et al.* (2000). In cluster III, all the genotypes were followed the same pattern in both the cases (phenotypic and genotypic). As expected, all the AVTO genotypes were placed in cluster IV along with EC620463 except AVTO1219. Even though EC620463 was located in sub cluster B2, it was showing the superiority over the other genotypes of cluster IV. In addition to this, AVTO1219 was placed very nearer to the Pant bahar and PKM1. Similarly in cluster IV all the genotypes were phenotypically susceptible to FW except AVTO9803 and it was resulted same with the use of FW gene specific primer. Whereas the genotype EC620406 classified as a cluster V was not identified with any allele with FW specific primer but it has shown moderately resistance at phonotypical screening. Interestingly all the AVTO genotypes were aligned as one group and all the EC lines were aligned in another group with three released varieties and AVTO1219. Whereas, the two genotypes including one released variety (Pusa Ruby) and one Exotic collection (EC620406) were found to be most diversifying among all the genotypes used in this study by truncated into separate clusters with lowest similarity. Over all, EC631379, EC631369, EC620428, EC620378 and EC620503 were identified as new resistant genetic resources against Fusarium wilt at both phenotypic and genotypic level with the presence of *I-2* gene loci. Thus, the identified highly diversified/ related tomato genotypes can be improved further using various breeding and crop improvement programmes.

Conclusion

In the present study a total of 23 genotypes were screened for Fusarium wilt resistance. The morphological screening was revealed EC631379 and AVTO1219 as highly resistant genotypes. Some more genotypes *i.e.,* Pant bahar, EC620428, EC620378, EC631369 and EC620503 were also resulted as resistance against Fusarium wilt at phenotypic level. Remarkably this resistance of some EC lines *viz*., EC615055, EC620428, EC620378, EC620389, EC620394, EC620422, EC631369 and EC620503 were also confirmed genetically using Fusarium wilt resistance I-2 gene specific primer along with AVTO1219 and AVTO9803. In addition to this the genetic assessment using SSRs revealed primer TGS633 as most useful marker in tomato genetic diversity and using the cluster analysis. Pusa Ruby and EC620406 were resulted as high diversified genotypes among 23 genotypes using SSRs. Over all, the new genetic resources were identified for resistance to Fusarium wilt race2 at phenotypic and genotypic level. Hence, the genotypes identified with the presence of I-2 gene can be further useful for marker assisted breeding program for the improvement of tomato crop.

Acknowledgement

Authors are thankful to SKLTSHU, Rajendranagar, Hyderabad for providing facilities to carry out the research trial.

*Supplementary Table or Figure mentioned in the article are available in the online version.

References

Agarwal MK, MS Fageria and RS Dhaka (2000) Breeeding for multiple disease resistance in vegetables. *Agricultural Review*. **21(2):** 125-128.

Ahmadvand RZ, SM Abdoljamil and M Kahbazi (2010) Screening of tomato genotypes to determine the resistance sources to fusarium wilt disease. *International Information System for the Agricultural Science and Technology.* **23**: 40-44.

Alvarez AE, CCM van de Wie and MJM Smulders (2001) Use of microsatellites to evaluate genetic diversity and species relationships in the genus Lycopersicon. *Theor Appl Genet*. **103**: 1283-1292.

Anonymous (2005) FAO statistical databases. Rome, Italy: Food and Agriculture Organization of the United Nations. *http://www.faostat.fao.org*.

Antonio ESC, SC Fabio, CH Alan and SC Alexandre (2017) Resistance to fusarium wilt in watermelon accessions inoculated by chlamydospores. *Sci. Hortic*. **228**: 181-186.

Bahar M, S Hajmansoor and N Kakvan (2012) Screening of resistance genes to fusarium root rot and fusarium wilt diseases in tomato (*Lycopersicon esculentum* Mill.) cultivars using RAPD and CAPs markers. *Pelagia Research Library*. **2(4)**: 931-939.

Bahattin T and A Cengiz (2010) Screening of resistance genes to fusarium root rot and fusarium wilt diseases in F_3 family lines of tomato (*Lycopersicon esculentum*) using RAPD and CAPs markers. *Afr J. Biotechnol*. **9**(19): 2727-2730.

Bai Y and P Lindhot (2007) Domestication and breeding of tomatoes: What have we gained and what can we gain in the future. *Ann Bot*. **100**(5): 1085-1094.

Benor S, M Zhang, Z Wang and H Zhang (2008) Assessment of genetic variation in tomato (*Solanum lycopersicum* L.) inbred lines using SSR molecular markers. *J. Genet. and genomics*. **35**: 373-379.

Booth C (1971) The Genus Fusarium. Commonwealth Mycological Institute. *CAB International*. Kew, Surrey, England.

Bredemeijer GMM, RJ Cooke, MW Ganal, R Peeters, P Isaac, Y Noordijk, S Rendell, J Jackson, MS Roder, K Wendehake, M Dijcks, M Amelaine, V Wickaert, L Bertrand and B Vosman (2002) Construction and testing of a microsatellite containing more than 500 tomato varieties. *Theor Appl Genet*. **105**: 1019-1026.

Burgess LW, BA Summerell, S Bullock, KP Gott and D Backhouse (1994) Laboratory Manual for Fusarium Research, 3rd ed. *University of Sydney and Botanic Garden, Sydney, Australia.*

de Carvalho LM, IR de Oliveira, NA Almeida and KR Andrade (2012) The effects of biotic interaction between tomato and companion plants on yield. *Acta Hortic*. **933**: 347-354.

Dhaliwal MS, M Singh, K Singh and DS Cheema (2011) Genetic diversity analysis and DNA fingerprinting of elite genetic stock of tomato using SSR marker. *Indian J Genet Pl Br*. **71**: 341-348

Doyle J J and JL Doyle (1990) Isolation of plant DNA from fresh tissue. Focus. **12**: 13-15.

El-Awady M, AAE El-Tarras and MM Hassan (2012) Genetic diversity and DNA fingerprint study in tomato (*Solanum lycopersicum* L.) cultivars grown in Egypt using simple sequence repeats (SSR) markers. *Afr J Biotechnol*. **1**(1): 16233-16240.

Frary A, Y Xu, J Liu, S Mitchell, E Tedeschi and S Tanksley (2005) Development of a set of PCR-based anchor markers encompassing the tomato genome and evaluation of their usefulness for genetics and breeding experiments. *Theor Appl Genet*. **111**: 291-312.

Gerlach W and H Nirenberg (1982) The genus Fusarium-A pictorial atlas. Mitteilungen aus der Biologischen Bundesanstalt Für Land- und Forstwirtschaft (Berlin-Dahlem) **209:** 1-405.

Giovannucci E, W Erdman, J Schwartz, K Clinton and Miller E (2002) Tomato products, lycopene and prostate cancer risk. *Urologic clinics North America*. **29**(1): 83-93.

Glogovac S, A Takac, L Brbaklic, D Trkulja, J Cervenski, JG Varga and V Popovic (2013) Molecular evaluation of genetic variability in tomato (*Lycopersicon esculentum* Mill.) genotypes by microsatellite markers. *Original Scientific Paper* **50**: 1-3.

Jones ES, MP Dupal, R Kolliker, MC Drayton and JW Forster (2001) Development and characterisation of simple sequence repeat (SSR) markers for perennial ryegrass (*Lolium perenne* L.). *Theor Appl Genet*. **102**: 405-415.

Kaur S, AK Singh and B Sreshti (2018) Assessment of Genetic Diversity in Cultivated Tomato (*Solanum lycopersicon* L.) Genotypes using Molecular Markers. *Int. J. curr. microbiol. appl. sci.* **7**(4): 2129-2143.

Kaushal A, A Singh and AS Jeena (2017) Genetic diversity in tomato (*Solanum lycopersicum* L.) genotypes revealed by simple sequence repeats (SSR) markers. *J. Appl. Nat. Sci.* **9**(2): 966-973.

Leslie JF and BA Summerell (2006) The Fusarium laboratory manual. 1st ed. Blackwell Publishing Ltd, Oxford, London.

Mahmoud A, Hifzi AB and Sameer M (2006) Determination of Resistance of Locally Grown Tomato Varieties to *Fusarium oxysporum* f. sp. *Lycopersici* in Jordan under Greenhouse Conditions. *Jordan J. Agric. Sci.*. **2**(3): 18-26.

McCouch SR, X Chen, O Panaud, SX Temnykh, YG Cho, N Huang, T Ishii and M Blair (1997) Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Mol Biol*. **35**: 89-99.

Miller EC, CW Hadley, JW Schwartz SJ, Erdman, TMW Boileau and SK Clinton (2002) Lycopene, tomato products, prostrate cancer prevention. Have we established causality. *Pure Appl Chem*. **74**(8): 1435-1441.

Nelson PE, TA Toussoun and WFO Marasas (1983) Fusarium species, An Illustrated Manual for Identification. Pennsylvania State University Press, University Park Pennsylvania, USA 193pp.

Nirmaladevi D and C Srinivas (2012) Cultural, Morphological, and Pathogenicity Variation in *Fusarium oxysporum* f. sp. *Lycopersici* Causing Wilt of Tomato. *Batman University Journal of Life Sciences*. **2**(1): 1-16.

NTSYS-pc, RF (2000) Numerical taxonomy and multivariate analysis system, version 2.2. Setauket, NY, USA: Exeter Publishing.

Onyekachukwu OA and Adefoyeke OA (2017) Fusarium wilt disease of tomato: screening for resistance and in-vitro

evaluation of botanicals for control; the Nigeria case. *J. Microbiol. Biotechnol. Food Sci.* **7**(1): 32-36.

Reddy BR, MP Reddy, Begum HD and N Sunil (2013) Genetic diversity studies in tomato (*Solanum lycopersicum* L.). *IOSR J. Agric. Vet. Sci.* **4**(4): 53-55 [19].

Rick CM (1969) Origin of cultivated tomato, current status of the problem. Abstract XI International Botanical Congress, p.180.

Rohlf F J (2000) NTSYSpc numerical taxonomy and multivariate analysis system (Version 2.1).

Sanghani AO and MK Mandavia (2013) Characterization of tomato (*Lycopersicum esculentum* Mill.) genotypes through RAPDs, ISSRs, and SSRs marker. *Ind J. Agric. Biochem*. **26**(2): 141-147.

Sepat NK, SR Sepat, S Sepat and A Kumar (2013) Energy use efficiency and cost analysis of tomato under greenhouse and open field production system at Nubra valley of Jammu and Kashmir. *Int. J. Environ Sci*. **3(4):** 1233-1241.

Silme RS and Cagirgan MI (2010) Screening for resistance to Fusarium wilt in induced mutants and world collection of Sesame under intensive management. *Turk J. Field Crops*. **15**(1): 89-93.

Smulders MJM, G Bredemeijer, W Rus-Kortekaas, P Arens and P Vosman (1997) Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other Lycopersicon species. *Theor. Appl. Genet.* **97**: 264-272.

Varshney R, S Pande, S Kannan, T Mahendar, M Sharma, P Gaur and D Hoisington (2009) Assessment and comparison of AFLP and SSR based molecular genetic diversity in Indian isolates of *Ascochyta rabiei*, a causal agent of Ascochyta blight in chickpea (*Cicer arietinum* L.). *Mycol. prog*. **8**(2): 87-97.

Velpula PK, DS Parihar and R Pinnamaneni (2017) Genetic diversity analysis of ripening specific genotypes using potential public domain ssr markers in tomato (*Solanum lycopersicum*). *J. Plant Breed. Genet*. **5**(2): 39-44.

Weir BS (1990) Genetic data analysis-methods for discrete population genetics data. (Sunderland: Sinauer Associates, Inc).

Yang WC, E Sacks, MLL Ivey, SA Miller and DM Francis (2005) Resistance in *Lycopersicon esculentum* intraspecific crosses to race T1 strains of *Xanthomonas campestris* pv. *vesicatoria* causing bacterial spot of tomato. *Phytopathology* **95:** 519-527.

Yogendra KN and PR Gowda (2013) Phenotypic and molecular characterization of a tomato (*Solanum lycopersicum* L.) F. *Genetics and Molecular Research*. **12**(1): 506-518.

Supplementary Table 1. Scores and percent incidence of fusarium wilt in 23 genotypes

S.No.	Genotype	Score	Percent incidence	S.No.	Genotype	Score	Percent incidence
1.	Pusa Ruby	4.00	100.00	14.	EC620427	2.33	58.33
2.	PKM1	2.33	58.33	15.	EC620394	2.00	50.00
3.	Pant bahar	0.67	16.66	16.	EC620422	1.67	41.66
4.	Arkavikas	3.33	83.33	17.	EC631369	1.00	25.00
5.	EC615055	1.33	33.33	18.	EC631379	0.00	0.00
6.	EC620463	4.00	100.00	19.	EC620503	0.67	16.66
7.	EC620428	0.33	8.33	20.	AVTO9803	2.00	50.00
8.	AVTO1219	0.00	0.00	21.	AVTO9804	3.33	83.33
9.	EC620378	0.33	8.33	22.	AVTO1002	3.67	91.66
10.	EC620382	2.33	58.33	23.	AVTO0101	3.33	83.33
11.	EC620389	1.67	41.66		C.V	8.32	14.89
12.	EC620395	2.67	66.66		C.D (P=0.05)	2.13	9.86
13.	EC620406	1.33	33.33				

Supplementary Table 2. List of polymorphic primers with sequence, Chromosome number, Annealing temperature, Number of alleles and PIC

S.No.	Primer	Sequence 5^1 to 3^1	Chromosome number	Alleles	**PIC**
1	TGS2458	F:GTGAATTTTTCAAACCCTGGC	1	2	0.58
		R: ATTTGGAAATGAACTCGGCA			
2	TES109	F:GTCAACAACTATTCCAGGCCC	1	2	0.53
		R: CTCCCGTGCAAAATCTAAGC			
3	SSR222	F: TCTCATCTGGTGCTGCTGTT	1	2	0.58
		R: TTCTTGGAGGACCCAGAAAC			
4	TES134	F: GTCATTTTTCCCCAGCTGTTC	1	2	0.53
		R: AAGGAAAAGACCCAGGTGTG			
5	SSR37	F: ATTGAAGACCGAAACGGTTG	1	2	0.53
		R: CTGATAAACCCGGCAAGACT			
6	SSR32	F: TGGAAAGAAGCAGTAGCATTG	2	2	0.53
		R: CAACGAACATCCTCCGTTCT			
7	SSR22	F:GATCGGCAGTAGGTGCTCTC	3	2	0.62
		R:CAAGAAACACCCATATCCGC			
8	SSR3	F:CTAATATAGTAGAGTAGGAGTAAG	3	2	0.53
		R:GCTCTAATGATAAGGAGAGAGTCTG			
9	TES60	F:GTTCCTCCTCCTCCTCCTTTC	4	4	0.45
		R: ACACAATTCCCCAAAATCCA			
10	SSR214	F:AAATTCCCAACACTTGCCAC	4	2	0.53
		R:CCCACCACTATCCAAACCC			
11	TES1652	F:AAAAAGTCAGCTTCAGTGGTAGTATAG	4	3	0.30
		R:GCAACCTCCTACTCTGCTGG			
12	TGS633	F: GTTTTTACCAATTCTTCCGGC	5	4	0.76
		R:AGGAAATAGAATAAAACTAACCCAAA			
13	TGS914	F: GCCAGGCATTCCAACAATACA	5	2	0.61
		R: TCACTTGTGCAATGAGGTTGA			
14	SSR162	F: GCTCTCTACAAGTGGAACTTTCTC	5	2	0.50
		R: CAACAGCCAGGAACAAGGAT			
15	TES1743	F: GAGTGTCTCGATCTCGCACCT	6	2	0.53
		R: CCATGTGTCCAACCTTTTCC			

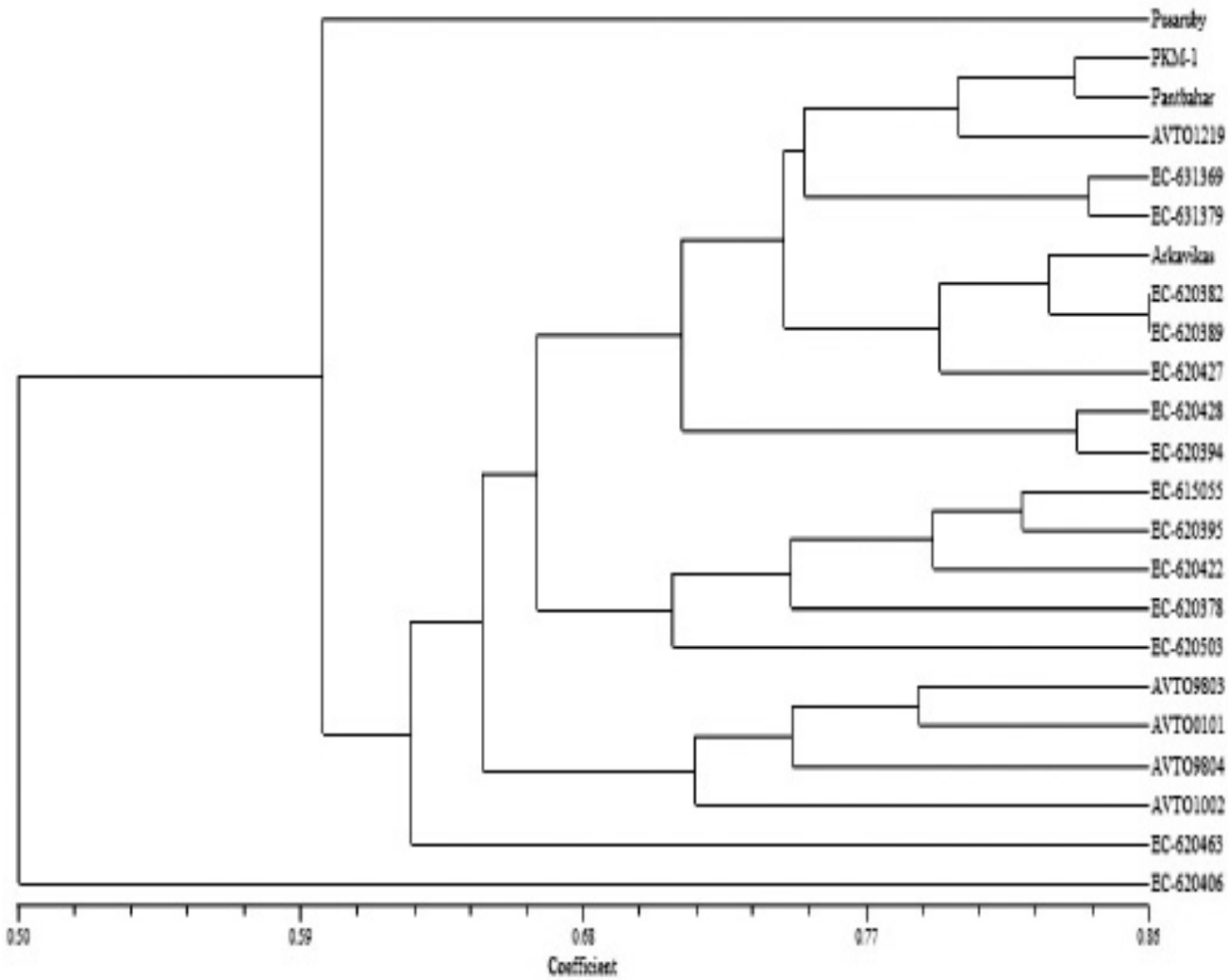
S.No.	Primer	Sequence 5[1] to 3[1]	Chromosome number	Alleles	**PIC**
16	TGS2005	F: GGGTGAAAGGATAAGGGAAA	7	2	0.58
		R: CGGATTTCTTGTTGTGTTGC			
17	TGS192	F: GTCAGTTGCTTTTTATCCAACAA	7	2	0.36
		R: CACTGATGGGAATGCCTTTT			
18	SSR8-0.5	F:GTAATCTTACTTTAGATGACATG	8	2	0.58
		R:CCATAAGAATACAATCCACTTG			
19	TGS610	F: GTTAGTGAAGTGAAGAGGAAGCAA	8	3	0.42
		R: CCGGCAAGCTGCATTTTT			
20	TES36	F: GGACCAAGCGAAGTTGGATA	9	2	0.61
		R: CGAGTGTTTCGCTTCTCCTC			
21	TES184	F: GCGTCATCAACCAGTCAGCAG	9	2	0.64
		R: TATTTCTGTGCCAATGGACG			
22	SSR112	F: GGAACACAACCAAGAAGTGGA	9	2	0.35
		R: TATCGGCTTAGGGTTGTTGG			
23	TES1592	F: GCCAATTTGGTGGCTACCCT	9	3	0.65
		R: CGGGATATCTGCCTCTACCA			
24	TES1154	F: GAGCGACCTCAACTTGTTTGG	10	2	0.49
		R: AACCAGATGACCCCATTTGA			
25	TGS643	F: GTTTCTCCCAAGGGGGATATT	10	3	0.53
		R: ACTTCCAAGCGGGGATAGAT			
26	TEI0396	F: GCTATGTATAGGAAGCAACACAAGA	10	2	0.28
		R:TAGCAGCTTCTTGGGCGATA			
27	SSR223	F: TGGCTGCCTCTTCTCTGTTT	10	2	0.53
		R: TTTCTTGAAGGGTCTTTCCC			
28	TES0426	F: TTTGAGGAGGGCTGAAGAGA	11	2	0.58
		R: GCAGGATAACAGCCTCTTGC			
29	SSR46	F: CCGAGGCGAATCTTGAATAC	11	2	0.73
		R: GCACCATCTCTTGTGCCTCT			
30	TES152	F: GTGTTTCTATTCGTGAACCATGA	11	2	0.65
		R: CCGTGAGTTAGCTAATGAGGTT			
31	TGS1476	F: GTCATGGGAATGACACTAACGAG	11	2	0.29
		R: AGTGTGTGTGTTTGTGTGCG			
32	TES1420	F: GCAGCTCGTCATTTCTTCAA	12	2	0.61
		R: AGTGGCTGAAGAAGAACGGAA			
33	TGS1093	F: GTTTCTTCTTTGTAAATCGGCG	12	2	0.73
		R: CGAGTCAACCCCTAGGCTAC			

PIC: Polymorphic information content

Supplementary Table 3. Clustering pattern of genotypes obtained by genetic diversity analysis

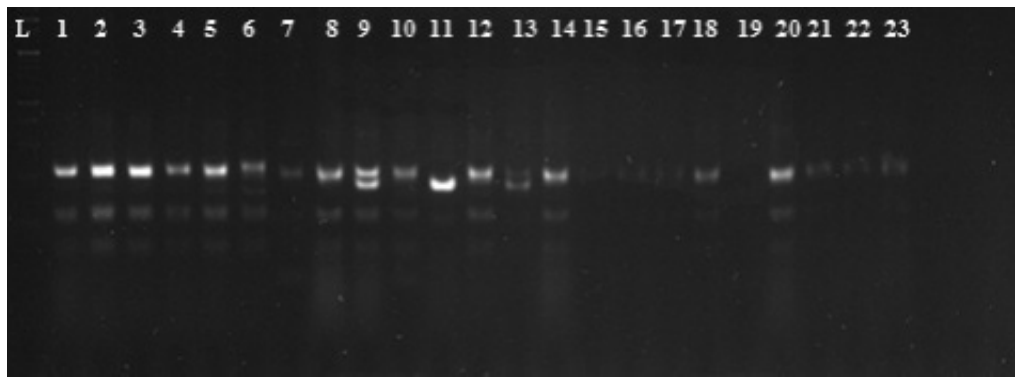
Cluster Number	Number of genotypes	**Name of genotypes**
I	1	Pusa Ruby
II	11	PKM1, Pant bahar, AVTO1219, EC631369, EC631379, Arka Vikas, EC620382, EC620389, EC620427, EC620428, EC620394
III	5	EC615055, EC620395, EC620422, EC620378, EC620503
IV	5	AVTO9803, AVTO0101, AVTO9804, AVTO1002, EC620463
V	1	EC620406

Supplementary Fig. 1. Disease reaction in genotypes AVTO1219, EC631379 (Resistant), Pusa Ruby (Susceptible check)



Supplementary Fig. 2. Dendrogram obtained from SSRs analysis using UPGMA analysis based on 33 SSR primers Banding pattern of SSR markers

TGS610



TES109

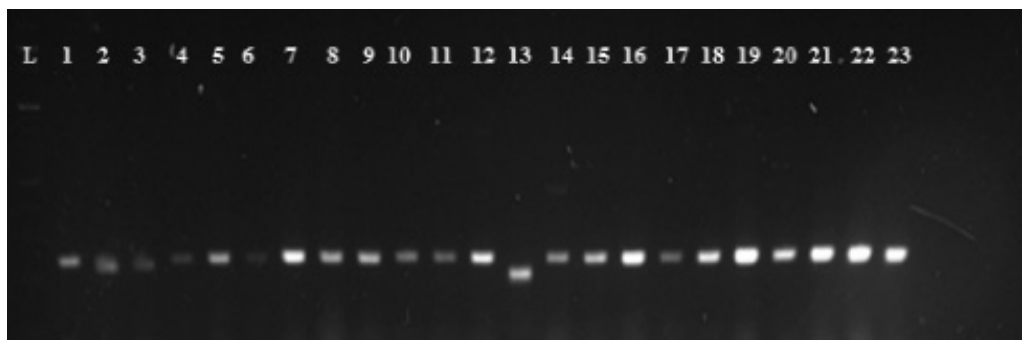


Fig. 3. Banding pattern of SSR markers