United States Department of Agriculture
Project Initiation

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Project Title

Identification of genetic resistances against economically important tomato diseases

Project Details

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Project Director

SHARMA, A.

Recipient Organization

SAES - UNIVERSITY OF FLORIDA

235 Tigert Hall

Gainesville, FLORIDA 68071

Performing Department

Gulf Coast Research and Education Center, Bradenton

Non Technical Summary

Tomato (Solanum lycopersicum) is the most popular and valuable vegetable crop in the world. In 2021, the United States (US) produced ~10.5 tonnes of tomatoes, and the industry is valued at ~1.8 billion dollars annually. Florida is the biggest producer of fresh market tomatoes in the US. The warm and humid climate in Florida is conducive for the emergence and rapid spread of many fungal and bacterial diseases of tomato. Economic losses from these diseases result from reduction of yield and marketability and from increased cost of production associated with pesticide application. Identification of genetic resistances against these pathogens and their incorporation into commercial tomato cultivars can help mitigate the economic damage associated with these diseases. Bacterial spot of tomato is caused by a complex of several Xanthomonas species. Historically, the disease was managed by application of metal-based bactericides and antibiotics, but loss of efficacy due to bacterial evolution has prompted urgency to develop disease resistant cultivars. While sources of resistance have been identified in wild tomato and landraces, they have not been deployed commercially either due to race shift or difficulty in introgression. Two dominant resistances

effective against race T4 have been identified in wild tomato, Ptr1 from S. lycopersicoides and Zar1/Jim2 from S. pennellii. Introgression of these genes into cultivated tomato will confer high level of resistance against T4 race that threatens Florida tomato production today. bs5 is a non-race-specific recessive resistance against bacterial spot of pepper. Our ongoing investigation shows that genome-edited tomato carrying mutation in Bs5 is resistant against bacterial spot. bs6 and bs8 are other recessive resistances identified in pepper; identification and mutation of bs6 and bs8 homologs in tomato may enable development of resistance tomato germplasm. The phytopathogenic necrotrophic fungus, Corynespora cassiicola, is responsible for the target spot disease of tomato and several other crops, such as soybean, cucumber, rubber tree, and tobacco. In the last decade, target spot has emerged as a serious foliar and fruit pathogen of tomato in Florida, partly because of the emergence of isolates resistant to traditionally effective fungicides, such as quinone outside inhibitors. The problem is exacerbated by the fact that multiple phylogenetically distinct populations of C. cassiicola are able to infect tomato. There are no target spot resistances available in tomato cultivars. Recently, major QTLs for resistance to target spot were identified three wild tomato species, S. pimpinellifolium, S. galapagense, and S. cheesmaniae, and mapped to a common locus in tomato chromosome 12. The resistances appeared to be controlled by a single recessive gene, which is attractive for durable resistance breeding. Fusarium wilt of tomato is caused by Fusarium oxysporum f. sp. lycopersici (Fol), a soil inhabiting fungus specialized to be pathogenic on tomato and threatens the tomato production system globally. This disease was traditionally well-managed through soil fumigation in Florida, but regulations prohibiting use of methyl bromide, an ozone-depleting substance, resurfaced a need to manage the disease using disease resistance genes. Three races of the pathogen have been reported and can be managed by resistance genes I, I-2, and I-3.A recent survey in Florida tomato fields identified multiple isolates that overcome all three resistances, highlighting an urgent need for new sources of resistance effective against this new race (Fol4). More Fusarium wilt resistances have been reported in the literature; however, their efficacy against this new race has yet to be determined. Since all currently commercialized resistances were identified in wild relatives of tomato, it may be necessary to screen more wild germplasm to identify resistance to Fol4. This work aims to identify sources of resistance against economically important diseases of tomato. The specific objectives are to (i) incorporate resistances against all species causing bacterial spot into cultivated tomato, (ii) identify genes in wild tomato conferring resistance to target spot disease, and (iii) screen tomato accessions and wild germplasm for resistance to Fusarium wilt race 4. The long-term goal of this research is to manage tomato diseases and promote food security by identifying and utilizing genetic resistance. At the end of the project, multiple sources of disease resistances will be evaluated for efficacy and made available for tomato breeding. Markers linked to the resistance locus will be publicized for developing commercial cultivars.

Research Effort Categories

Basic	50%
Applied	50%
Developmental	0%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
201	1460	1080	75%
202	1460	1080	25%

Knowledge Area

[201] Plant Genome, Genetics, and Genetic Mechanisms

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[202] Plant Genetic Resources

Subject Of Investigation

[1460] Tomato

Field Of Science

[1080] Genetics

Keywords

Goals / Objectives

Project Methods

Objective 1: Incorporate resistances against all species causing bacterial spot into cultivated tomato Introgression of dominant resistance genes: We have acquired introgression lines carrying Ptr1 resistance from S. lycopersicoides LA2951 in VF36 background (LA4245) and Zar1/Jim2 from S. pennellii LA0716 in M82 background (IL5-3 and IL6-2-2). We will advance the resistances in Fla 8059 background, which is suitable for Florida environment. We will carry out comparative genomics studies to design high resolution melting (HRM) markers as well as a high-density SNP genotyping panel. LA2951 and LA0716 genomes are publicly available and Fla 8059 sequence is available at the UF tomato breeding program. Developing transgenic Ptr1 tomato: Our preliminary studies have indicated a low recombination frequency between LA4245 and Fla 8059 in Ptr1 region. Extremely large F2 populations (5000-10000 progenies) may be necessary for reducing the introgression interval. Prior to a large-scale experiment, we will develop transgenic tomato carrying Ptr1 gene to evaluate its efficacy in tomato without S. lycopersicoides background. For this, a 35S constitutive promotor will be fused to LA2951 Ptr1 cDNA, inserted into pCambia plasmid, and transformed into Fla 8059 through agro-inoculation. Transformants will be evaluated for resistance to X. perforans carrying xopJ2 gene. Genome editing for recessive resistance: Tomato homologs of pepper bs6 and bs8 are expected to confer susceptibility to bacterial spot and we are currently in the process of mutating the homologs of the candidate genes. Once we get the transformants from an external agency, we will screen for mutants using molecular markers at those loci. Mutants with disruptive mutations in bs6 and bs8 homologs will be screened for resistance to X. perforans and X. gardneri, respectively, to identify the resistance genes. An additional source of resistance against X. gardneri, bs9, is being studied in pepper; similar mutational analysis will be performed when the candidate resistance genes are identified. Objective 2: Identify genes in wild tomato conferring resistance to target spot disease. Reduce candidate genes: Identify differential polymorphism: Target spot resistance QTLs from tomato accessions LA2093 and LA0483 have both been mapped to chromosome 12 and fine-mapping efforts are ongoing. Varitome data is available for both LA2093 and LA0483. We will use that information to identify and narrow down the number of candidate genes in the fine-mapped interval by identifying polymorphisms in amino acid sequence or insertions-deletions in the promoter region. In case of variation in promoter, we will run qRT-PCR to check if the resistance phenotype is associated with a difference in transcript level. Differential accessions from varitome data: The varitome data for more than 200 tomato cultivars, breeding lines, landraces, and wild species are available through Sol Genomics, and UF tomato breeding program. We will mine the data to identify lines that vary in the set of polymorphisms in different genes. Preliminary results indicate that S. lycopersicum var cerasiforme is differential for the polymorphisms in candidate genes. We will acquire the necessary germplasms from the Tomato Genetics Resource Center at UC Davis and screen them for resistance to reduce candidate genes. Genome editing and functional validation: Once we have a satisfactory number of candidate genes, we will knock out the candidate genes to determine and validate the resistance gene. A third party will perform the transformation and tissue culture steps. We will screen for the mutants among the transformants, develop homozygous mutants, and conduct necessary disease screens. Objective 3: Screen tomato accessions and wild germplasm for resistance to Fusarium wilt race 4. Screening for resistance: Evaluate previously identified

resistances: Previously identified Fusarium wilt resistances, such as I-5, I-6, and I-7 with unknown reaction to Fol4 will be screened for their efficacy against it. Further characterization for resistance against Fol4 will also be conducted for novel Fol resistances in UF tomato breeding lines, such as introgressions from S. pennellii accessions LA750 and LA1367. Screening tomato accessions: Novel resistance to Fol4 may be present in the wild tomato germplasm. Therefore, we will conduct an extensive screening to identify such resistances. For tomato accessions that have been screened before, we will focus on detecting multiple recessive resistances that may complement each other to provide a high level of resistance. Accessions collected by TGRC and currently present within UF tomato breeding programs as well as tomato PI lines in USDA Germplasm Resources Information Network will be screened under this initiative. Estimation of breeding values: Once one or more sources of resistance are identified, we will conduct genetic analyses to determine the breeding value for those resistances. Monogenic or oligogenic resistances with efficacy against all Fol races will be selected for resistance breeding. Linkage with undesirable traits will be identified and such traits will be selected against when advancing the backcross lines. Mapping and marker development: Once several desirable sets of resistances are shortlisted, we will map the resistances using SNP genotyping panel or whole genome bulked segregant analysis. The genes will be further fine-mapped and linked markers will be identified for marker-assisted selection.