Development of herbicide tolerant tomato

By

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Plant and Soil Sciences
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

December 2017

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Pages in Study: 113

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Tomato is a major horticulture crop grown across the globe. Unfortunately, its yield is reduced by 25% because of auxin herbicides and glyphosate drift. In this present study, wild germplasm of tomato was screened for herbicide tolerance. From the greenhouse study nine accessions for glyphosate and 2,4-D, eleven accessions for dicamba, five accessions for quinclorac, eight accessions for aminocyclopyrachlor, and two accessions for picloram and aminopyralid were identified to be tolerant. A few accessions were selected from each herbicide tolerant group for field trials at two locations in Mississippi in 2016 and 2017. Results indicated that TOM18 was most tolerant to dicamba herbicide, while TOM87 and TOM129 to glyphosate and quinclorac herbicide, respectively, on the basis of yield and injury. Molecular experiments were conducted to measure the genetic diversity among diverse germplasm. Genetic diversity analysis showed wild accessions to be highly diverse as compared to cultivated tomato.

DEDICATION

First and foremost, I would like to dedicate this thesis to my late grandmother Kaushalya Devi. This became possible because of your blessings. You taught me the importance of education, and getting a higher education was your dream which I am fulfilling now. I wish you could be here today. You have sacrificed a great deal for the education of my brother and me. I would like to dedicate this research to my father Raj Kumar Sharma, mother Ranjana Sharma, and brother Saurabh Sharma. Without their constant love, support, encouragement, blessings, and teaching, this would never have been possible. You three have sacrificed more than anyone to make this happen. I would also like to dedicate this to my grandmother Surindera Rani Joshi and late grandparents Kewal Krishan Sharma and Sham Lal Joshi. Your blessings and support helped me throughout this project. This work is also dedicated to my aunts, uncles, and cousins back in India. They have always encouraged, supported, and guided me in my good times and bad. I miss and love you all.

Last, but not the least, I would like to dedicate this research to my friends who are in India, U.S, and Canada. You all have always stood by my side and supported me whenever needed.

ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. Paul Tseng for trusting me and giving me the opportunity to earn an advanced degree in one of the world's best universities. His constant support and guidance encouraged me throughout my degree. I would like to thank my committee members, Dr. Dan Reynolds, Dr. Richard Snyder, Dr. David Nagel and Dr. Zhaohua Peng for their support and valuable suggestions whenever I needed it. I would also like to thank Dr. Janice DuBien for helping with my statistical analysis.

I would like to thank Zhiming Yue, Ericmar Santos, Marilia Ferreira, Shandera Stallworth, and most importantly Swati Shrestha, for their assistance throughout this project. I really enjoyed your friendship while working along with each other every day. I would like to thank Dr. Reynolds' crew for providing me herbicides, spray equipments and any other help whenever I needed it.

I would also like to thank the faculty and staff of the Plant and Soil Science department at Mississippi State University for their constant support. I never felt that I was away from home. Special thanks to Peter Hudson and the staff at Truck Crops Brach Experiment Station, Crystal Springs, and Dr. Casey Barickman, Thomas Horgan, and staff at North Mississippi Research and Extension Center, Verona, who helped us with our project immensely. I could not have done it without them.

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CHAPTER I

LITERATURE REVIEW

General Introduction

According to the definition by the International Union of Pure and Applied Chemistry (Duffus et al., 2007), herbicides are pesticides used for the control of unwanted plants or weeds. The first commercially used herbicide in the U.S. was 2,4-D, discovered during World War II when scientists were performing research on plant growth regulators (Rao 2000). Prior to the discovery of 2,4-D in 1900, Bolley in U.S., Schultz in Germany, and Bonnett in France, reported that inorganic compounds and solutions of copper salts could selectively control broadleaf weeds in cereals (Klingman et al., 1982). Dinitro compound (DNOC) was used in France during 1993 to act against annual weeds in cereals. DNOC and other dinitro compounds played a significant role in increasing food production during World War II (Cremlyn 1991). Today, herbicides are classified based on translocation time, method of application, chemical families, specificity, and site of action. Among all these classifications, site of action is widely used because it is helpful in effectively managing herbicide resistance (Vats 2015). According to the Environmental protection Agency (EPA 2017), worldwide pesticide market sales report from 2008 to 2012 indicate herbicides account for 45% of the total expenditure on pesticides; U.S. accounts for 21% of world expenditures on herbicides. In terms of global usage of pesticides, herbicides share the largest portion (approximately

50%) followed by insecticides, fumigants, and fungicides. In 2012, herbicides accounted for nearly 60% of total U.S. pesticide usage (EPA 2017). The usage of herbicides in developing countries in Asia increased dramatically because of the general rise in farm wages and growth in the non-farm employment (Pingali & Gerpacio., 1997). Today 96-98% of Pilipino rice farmers use herbicides (Marsh et al., 2009), Pakistan wheat farmers increase the grain yield by 19-21% with the help of herbicides (Khan et al., 2005), and the net income of rice farmers in Bangladesh adopting herbicides to manage weeds was 116% higher than without herbicides (Rashid et al., 2012). With the adoption of herbicides, agricultural greenhouse gas emissions can be reduced by decreasing fuel used by tillage equipment (EPA 2011). A moldboard plow consumes 17 times more diesel fuel per unit area than a herbicide sprayer; similarly, a row-crop cultivar requires 4 times more fuel per trip across a field compared to a herbicide sprayer (Hanna 2001). Thus, herbicides help to increase crop yield and enhances the profitability of farmers, while at the same time it reduces greenhouse gas emissions. Unfortunately, there are also some drawbacks of herbicides, such as the evolution of herbicide resistant weeds due to the overuse of herbicides with same mode of action, and herbicide drift to off-target crops.

Herbicide Drift

According to EPA, drift is the physical movement of pesticide droplets or particles through the air from the target site to any non-target site. Where target site is the area intended to be treated with pesticide, and non-target site as any area which is not designed to be treated. Drift can occur either by the movement of spray droplets or solid particles at the time of the application, or vapors soon after application/deposition (Carlsen et al., 2006). Herbicide drift can damage neighboring sensitive plants and crops,

reduce the efficacy of the applied herbicide on the target area, and affect human health (Nordby & Skuterud., 1974). Modern herbicide tolerant crop technology brings more risk of herbicide drift thus causing more loss of time and money. There is also the risk of expensive lawsuits due to herbicide drift. One such case occurred in Clay County, Arkansas where it took three years to resolve to result in the loss of millions of dollars (Schierholz 2010). The likelihood of crop damage increases when different crops requiring different herbicides are grown in proximity. A number of factors can influence the amount of drift but the most important ones are, spray droplet size (affected by nozzle type, herbicide formulation, operating pressure, and adjuvants), environmental conditions under which application occurs, and application height. The Spray Drift Task Force (SDTF) in the U.S. reviewed over 2500 studies related to drift and confirmed droplet size to be a key factor affecting drift. Smaller the droplet size higher the chances of off-target movement; since smaller droplets are lighter and therefore move slowly from the nozzle to target site as compared to larger droplets. Large droplets are less prone to drift because they have more momentum (Beckie et al., 1999). Al- Khatib et al. (1994) reported that small and concentrated droplets of thifensulfuron posed more damage to peas when compared to large and diluted droplets. Smaller droplets have the potential to adhere to plant stem and leaves whereas large droplets bounce off because of their greater velocity. The droplet diameter at which drift is more likely ranges from 100 - 200 microns (Dorr et al., 2013). According to American Society of Agricultural & Biological Engineers (ASABE 2009), droplets are classified into eight groups according to their approximate volume median diameter (VMD) range (microns) (Table 1.1); a value where 50% of the total volume or mass of liquid sprayed is made up of droplets larger than and 50%

smaller than this value. A larger VMD indicates a greater population of larger droplets. Droplet size also heavily depends on the nozzle choice, particularly the design and orifice size which in turn influences the amount of driftable particles in a spray (Nuyttens et al., 2009). TeeJet (TeeJet Technologies, Wheaton, IL, 60187) manufactures different spray nozzles which are commonly used in agriculture. Table 1.2 enlists some of the drift reducing nozzles and percentage (%) of spray volume in droplets < 200-micron diameter (TeeJet Technologies, 2017). The nozzle classification is as follows: XR stands for extended range; a flat fan nozzle type that holds a consistent spray pattern over a wide range of application pressures; TT stands for Turbo TeeJet, which impacts the liquid flow on a wall in a pre-chamber then out the exit orifice to produce larger droplets; TF stands for Turbo Floodjet, which is another preorifice design used to produce larger droplets; AI stands for Air Induction, which produces large droplets that splatter on contact by using a venturi effect to entrain air into the spray flow; and DG stands for drift guard which uses a preorifice to lower line pressure before mixing the flow in a chamber and ejecting it out a larger orifice . The XR, AI, TT and DG nozzles can be used for foliar applied pesticides, whereas DG and AI nozzles can also be used for soil applications. TF nozzles produce fewer, larger droplets than the others and are good for soil applications and applications of systemic herbicides. Stainier et al., (2006) tested 15 herbicide formulation and adjuvant combinations with 3 nozzles, flat fan and air induction (AI) with 110˚ spray angle, and a hollow cone with 80˚ angle; all with a flow rate of 0.8 L min^{-1} at 3 bar. With each spray combination, the AI nozzle produced droplets with the largest VMD and smallest volume of spray in droplets less than 100 microns followed by the flat

fan, while the hollow cone nozzle produced the smallest VMD and largest volume in droplets under 100 microns.

The height of the boom above the target is an important factor responsible for drift as it impacts the amount of time the herbicide will be exposed to the environment. Raising spray boom height above the target can, increase drift. Height can be increased or decreased depending on the spray angle. An 80˚spray angle requires a greater boom height to provide uniform coverage as compared to a 110˚angle. Nordby & Skuterud., (1975) reported that increasing boom height from 40 to 80 cm above the ground increased average drift from 1 to 3.2% of the spray volume. They performed this experiment with a mix of aminotriazole and fluorescent dye in water and used flat fan nozzles. Similarly, raising the boom height from 0.5 to 0.75 m consistently increased drift potential whereas lowering the height from 0.5 to 0.3 m decreased drift potential (Nuyttens et al., 2007). Boom height is a greater concern in aerial applications where the sprayer is higher than eight feet (Fishel et al., 2010). Herbicide drift potential was highest when an aerial application was made 9 m or higher, whereas at less than 9 m, no potential of drift was noticed (Hewitt et al., 2002).

Environmental conditions such as wind speed, temperature, and humidity during the time of application are other major factors responsible for herbicide drift. Wolf et al., (1993) reported that the higher wind speed, increased herbicide drift. Generally, the maximum acceptable wind speed for herbicide applications is 16 km/hr. According to EPA (2001), there should not be any herbicide applied, whether ground, aerially or chemigationally, if wind speeds exceed 16 km/hr. With the increase in wind speed from 7.2 to 14.4 km/hr, there was an increase in the average downwind drift as a percentage of spray volume

from 1.4 to 2.9% (Nordby & Skuterud,, 1975). Additionally, the volume of herbicide likely to drift during conventional ground application varies from 1.8 to 16.5% of the total spray volume (Wall 1994). The SDTF (1997) reported that wind speed affects herbicide drift of fine sprays in the range of distances less than 15 m from the sprayer. When wind speed was increased from 11 to 18 km/hr drift was increased 3.5 times at 8 m downwind from the sprayer with a nozzle that produces 26% of its volume in droplets less than 141 microns diameter (SDTF anonymous, 1997). Whereas, for a nozzle that produces 2% of its volume in droplets less than 141 microns diameter there was no difference in the amount of drift 8 m downwind from the sprayer. On the other hand, a gentle wind is needed during herbicide application otherwise inversion conditions are more likely to exist. In temperature inversions, cooler air is trapped close to the ground as we see early in the morning. Under inversions, turbulence is suppressed since adjacent air layers cannot mix with each other. Thus layers tend to remain distinct. These conditions cause small droplets to suspend in the air until inversion subsides, resulting in long distance transport of the drift cloud and severe damage to sensitive plants at considerable distances (Fishel et al., 2010).

Higher temperatures and low humidity should be avoided during application as these two conditions favor evaporation of spray droplets. Smaller droplets, as discussed earlier, increases the likelihood of drift, especially during stronger winds (Thistle, 2004). Storrie (2004) reported 28˚C and 60% to be the ideal temperature and relative humidity (RH) for herbicide application. At this RH, difference between wet and dry-bulb temperatures were less than 10˚C.

Other factors such as the herbicide formulations, adjuvants, application pressure and vehicle speed can be managed in a way to minimize herbicide drift. To predict herbicide drift, the USDA Agriculture Research Service (USDA ARS) in collaboration with Ohio State University developed software known as DRIFTSIM. It is a downloadable software package freely available at USDA ARS website (https://www.ars.usda.gov/news-events/news/research-news/2005/unique-software-for-preventing-pesticide-drift). The software can be used to calculate mean drift distances of water droplets up to 200 m under simplified field conditions. The user inputs information such as droplet size, wind velocity, temperature, relative humidity, droplet velocity, and discharge height, and the software generates either a single distance, if one diameter is entered, or a table of distances if an array of droplet sizes are entered. The applicator can then adjust each input to see how each choice condition affects the drift distance.

Herbicide Drift in Tomato and other Horticultural Crops

With the rapid adoption of new herbicide tolerant crop technologies, users can apply herbicides over large acreages. In some cases, these applications can occur at times and locations where sensitive crops are being grown in close proximity. There have been numerous studies showing how simulated drift rates of herbicides can affect crop growth and yield. Some horticultural crops such as tomato, potato, grape, pepper, and broccoli, are highly sensitive to auxin and glyphosate herbicides. Al-Khatib et al., (1993) observed injury to new and established vines of 'Lemberger' grape from 2,4-D simulated drift rate of 11.2 g ae ha^{-1} (1/100th labeled rate) and from 2,4-D plus glyphosate at 11.2 g ae ha^{-1} plus 4.3 g ae ha^{-1}, respectively. The 2,4-D damage observed lasted the entire season, and with the 11.2 g ae ha^{-1} rate of 2,4-D, cane dry weight was reduced by 48% versus

untreated. Wall (1994) reported 18% reduction in potato yield because of dicamba drift at 56 g ae ha^{-1}, whereas when sprayed at a rate of 1.0 g ae ha^{-1} it caused phenoxy-type symptoms, but potato yield was unaffected. Mohseni-Moghadam et al., (2015) reported a reduction in the yield of broccoli by 50% when 2,4-D was applied at 16.8 g ae ha^{-1} (1/50 of the labeled rate), with the greatest injury of 19% observed 28 DAT. Dicamba applied at 11.2 g ae ha^{-1} (1/50 of the labeled rate) caused slight injury but did not affect the overall yield as compared to untreated checks. On the other hand, when same rates for both herbicides were sprayed on bell peppers, yield was reduced by 50% with dicamba, whereas with 2,4-D the yield was similar to control treatments. Hemphill et al., (1981) observed a significant reduction in total marketable yield for carrot, cucumber, onion, pepper, radish, rutabaga, and turnip when applied with 2,4-D rates as low as 10.4 g ae ha^{-1}. Flessner at al., (2012) simulated aminocyclopyrachlor (AMCP) drift at a rate of 10 g ae ha^{-1} on cantaloupe and eggplant. A negligible amount of reduction in the marketable yield was observed in both crops (<0.005 kg). Therefore, drift rate of less than 10 g ae ha^{-1} is not a major concern in terms of fruit yield but great loss for marketable yield. In other vegetable crops, drift can also affect fruit or storage root quality. Sugarbeet when applied with a simulated drift rate of 2,4-D at 70 g ae ha^{-1}, caused a reduction in extractable sugar by 49% when compared with untreated (Dexter, 1993). Tomatoes are extremely sensitive to auxin herbicides and glyphosate. Kruger et al. (2012) reported a 25% tomato yield loss when a glyphosate drift rate of 8.5 g ae ha^{-1} was applied in the early bloom stage. At early vegetative stage, glyphosate dose of 43.9 g ae ha^{-1} was required to reduce the yield by 25%. The identification of glyphosate drift event on tomatoes is important and difficult to notice. Visible injury symptoms often take 4 to 7 days to manifest. It may also cause

discoloration or abortion of tomato flowers (Romanowski 1980). Fagliari et al., (2005) reported 92% reduction in a number of fruits per plant and 93% in total yield when tomato plants were sprayed with 2,4-D at a rate of 13.44 g ae ha^{-1}; all plants had just started anthesis of the first truss. When the same rate of 2,4-D was applied at sixth or fifth trusses, no significant yield reduction was recorded. Mature plants have thicker cuticle resulting in lesser penetration of 2,4-D into the leaves. Thus, mature plants have greater tolerance as compared to young plants. Dicamba when sprayed at early bloom stage of tomato at 7.5 g ae ha^{-1} caused 25% yield reduction (Kruger et al., 2012). A 2.4 g ae ha^{-1} rate can induce 5% flower loss at the early vegetative stage, and a 1.5 g ae ha^{-1} rate can induce some damage at early bloom stage. Quinclorac, another major drifted auxin herbicide in tomatoes can cause significant yield reduction and injury. Drifted rate of quinclorac above 0.42 g ae ha^{-1} has the potential to reduce tomato yield, and cause significant injury to the plants. The yield of tomatoes at the 0.42 g ae ha^{-1} rate of quinclorac was recorded as 17.3 MT/ha, whereas the yield was reduced further to 11.6 MT/ha when quinclorac rate was increased ten times (Lovelace et al., 2007). Although lower drift rate of quinclorac at 0.42 g ae ha^{-1} resulted in significant injury, as compared to untreated plots, no significant reduction in yield was reported. On the other hand, plants subjected to multiple application of quinclorac rates at 0.42, 2.1, and 42 g ae ha^{-1} resulted in yield reduction which was harder for the plant to recover and more likely to cause greater yield loss.

Tomato Production and Uses

Solanum lycopersicum, cultivated tomato is the world's second most commonly consumed vegetable crop after potato and also the most popular garden crop in the world

(Fooland & Panthee 2012). In the United States, it is one of the most economically important vegetable, with a production value of $2.55 B, in 2016 (USDA NAAS 2017). United States is the third largest producer of tomatoes after India and China (FAOSTAT 2017) and tomato is one of the third largest vegetable crop in terms of utilization and total production (USDA NAAS 2017). Although tomato is a tropical plant, it can be grown in almost every corner of the world. There are more varieties of tomatoes available today than any other vegetable crop (Robertson & Labate 2007). The versatility of tomato usage strongly contributes to its popularity. Tomato can be consumed raw, cooked or processed, where processed forms include juice, sauce, puree, paste, and dehydrated. Green, unripened tomato can be used in making pickles and candies. Tomato is about 90% water and is a good source of pro-vitamin A and vitamin C; and content of these two vitamins increases as fruit matures and develop color (Passam et al., 2007). Tomato ranks high in its nutritional contribution to the U.S. diet due to the large volume of processed tomato products and fresh tomato consumption (USDA, 2002). Fresh tomato contains dietary antioxidant lycopene, which has been demonstrated to inhibit some forms of cancer and plasma lipid peroxidation. Lycopene is the major carotenoid present in tomato and shows antioxidant activity both in vitro and in vivo (Peng et al., 2008). Tomato is a source of other compounds with antioxidant activities, including α- tocopherol, chlorogenic acid, plastoquinones, and xanthophylls (Charanjeet et al., 2004). Additionally, the fruit is a major source of fiber that helps prevent colon cancer and fluctuations of blood sugar levels. They are also an excellent source of chromium, folate, niacin, potassium, and vitamins B6 and K Niacin, which has the potential to lower high cholesterol levels (Leonardi et al., 2000).

Tomato origin and its domestication

Tomato is a C-3 perennial plant, cultivated as an annual crop belongs to the nightshade family Solanaceae, which falls in the division Magnoliophyta, class Magnoliopsida, subclass Asteridae, and order Solanales. The Solanaceae family comprises of 96 genera and over 2800 species which is divided into three sub-families, Solanoideae (in which tomato belongs), Cestroideae, and Solanineae (Knapp et al., 2004, Nee 1991). Solanaceae family consists of many economically important vegetable crops such as tomato, potato, pepper, and eggplant. Medicinal plants like deadly nightshade, henbane and Datura are also included in this family (Knapp 2016). In 1694, Tournefort named tomato as "wolf peach" in Greek, mainly because in old German folklore witches used plants of the nightshade family to evoke werewolves, practice is known as lycanthropy (Knapp et al., 2004). Linnaeus (1753) first started the system of giving plants a genus and species, known as binomial nomenclature as mentioned in the first edition of *Species Plantarum*. He classified tomato in the genus *Solanum* and species as lycopersicum but later in 1754 Miller use the generic name *Lycopersicon* based on certain fruit characteristics. A Number of twentieth-century authors recognized tomato as *Lycopersicum esculentum* based on the anther morphology (Rick & Holle., 1990, Correll 1958), but genetic sequence and morphology data indicated to change its botanical name to *Solanum lycopersicum* (Peralta et al., 2008).

Even in this era of advanced molecular tools and techniques, the unambiguity of the origin of tomato remains unsolved. Two hypotheses have been proposed for the original place of domestication; one is Mexico and the other Peru. Mexico is presumed to be the most likely region for domestication whereas Peru is the center of diversity for

wild tomatoes (Larry et al., 2007), but how and when tomatoes were first introduced into Europe has been debated since the nineteenth century (Jenkins 1948). Watercolor painting in Europe around mid-sixteenth century by Leonard Fuchs depicts the different shapes and colors of tomato, including some green fruits with stripes described as wild species (Peralta et al., 2008). Paintings clearly depicted that tomato in Europe was brought as a domesticated large fruited plant having yellow or red color fruits.

The plant group *Solanum,* section *Lycopersicon,* composed of 13 closely related taxa, of which 12 were classified as wild and one as cultivated tomato, *Solanum lycopersicum* (Peralta et al. 2008). The twelve-wild species are *S. pimpinellifolium, S. pennellii, S. corneliomulleri, S. habrochaites, S. neorickii, S. chmielewskii, S. arcanum, S. huaylasense, S. cheesmaniae, S. chilense, S. galapagense, and S. peruvianum* (Spooner et al. 2005 and Peralta et al. 2008). All of the wild species of section *Lycopersicon* occur on the western slopes of the Andes in the dry desert or pre-desert environments. Tomato and all its wild relatives are diploid with 2n=2x=24, and similar in chromosome structure and number (Rick 1956). Wild tomatoes are genetically diverse, especially self-incompatible species like *S. peruvianum* and *S. chilense* (Rick 1998). Cultivated tomato genome has genetic diversity <5% as compared to its wild relatives. This lack of diversity is due to the genetic bottleneck during domestication as the crop was migrated from the Andes to Central America and Europe (Peralta et al. 2008). The initial domestication process was conducted by selecting from existing germplasm and further selection on a single plant basis, thus leading to the narrow genetic variation (Tam et al., 2005). Due to this lack of diversity in cultivated tomato, wild germplasm of tomato is being exploited for various abiotic and biotic stress tolerance. Most genes and QTLs responsible for stress tolerance

have been transferred from wild species to cultivated tomato (Fooland 2007). Martin et al., (1991) reported mapping population NIL F_2 carrying a gene Pto which is resistant *to Pseudomonas syringae pv. (Tomato)* and is located on chromosome 5 of S. *pimpinellifolium*. Ganal et al., (1994) identified nematode (potato cyst) resistance gene against *Globodera rostochinesis* in S. *pimpinellifolium*. Fooland et al., (2002) identified QTLs responsible for early blight caused by *Alternaria solani* in a backcross population of *S. hirsutum* and *S. lycopersicum*. Recently, Lounsbery et al., (2016) reported QTLs controlling shoot turgor maintenance under root chilling in *S. lycopersicum X S. habrochaites* acc. Some wild species such as *S. habrochaites f. typicum,* and *S. habrochaites f. glabratum,* show resistance to at least sixteen insect pest species. Additionally, *S. pennellii* showed resistance to at least nine insect species including greenhouse whitefly, carmine mite, potato aphid, and spider mites (Dhall 2015). Fooland et al., (1997) reported five QTLs responsible for the salt stress tolerance mapped in the F_2 generation of *S. pennellii*. QTLs for cold tolerance was reported in *S. pimpinellifolium* (Fooland et al., 1998), for drought tolerance in *S. pimpinellifolium* (Martin et al., 1989), and for ion accumulation in *S. pennellii* (Zamir et al., 1987). Moreover, wild tomato germplasm has been used as a genetic source for improvement of flower and fruit related characteristics such as anther tube length, fruit size, diameter, elasticity, firmness, and total soluble solid content (Fooland 2007).

Molecular Markers

Kesawat & Kumar (2009) defined molecular markers as heritable differences in nucleotide sequences of DNA at the corresponding position on a homologous chromosome of two different individuals, which follow a simple Mendelian pattern of

inheritance. Today molecular markers have revolutionized all fields of biological sciences with its use in taxonomy, embryology, genetic engineering and physiology (Schlotterer 2004). In agriculture, they are used as a quick and cheap method to assess genetic diversity, gene mapping, phylogenetic analysis, map-based cloning of agronomically important genes, and marker-assisted selection (MAS) of desirable genotypes. With the help of MAS, the time span for developing better varieties can be reduced. There are a vast number of molecular markers used in plant science, and one should select these markers according to particular application and methodology. An ideal molecular marker must have some desirable characteristics such as high polymorphism (useful in genetic diversity studies); co-dominant inheritance (differentiates homozygous and heterozygous states of diploid organisms); easy, fast and cheap to detect; selective neutral behaviors (DNA sequences of any organism are neutral to environmental conditions or management practices), and highly reproducible (Weising et al., 1995).

Types of Molecular Markers and their application in diversity studies

There are a wide range of molecular markers that can be divided into different groups based on their mode of transmission (bi-parental nuclear inheritance, maternal nuclear inheritance, maternal organelle inheritance, or paternal organelle inheritance); mode of gene action (codominant or dominant markers); and, method of analysis (hybridization-based or PCR based markers). Hybridization based markers use restriction enzyme which digests the subject DNA followed by labeling the digested DNA using probes. Knowing the sequence of the DNA probe helps identify DNA polymorphism, as we already know the DNA probe sequence. On the other hand, polymerase chain reaction

based markers involve in vitro amplification of DNA sequences or loci with the help of primers (forward and reverse), and a thermos stable DNA polymerase enzyme. The amplified fragments are separated electrophoretically, and banding patterns are detected by staining and autoradiography.

The genetic variation in cultivated and wild tomato germplasm have been determined using various molecular marker techniques such as Amplified Fragment Length Polymorphism (AFLP), restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR), random amplified polymorphic DNA (RAPD) and single nucleotide polymorphism (SNP) (Bredemeijer et al., 1998, Park et al., 2004 and Garcia-Martinez et al., 2005). According to Dongre & Parkhi., (2005), RAPD was the first PCR-based molecular marker technique for the detection of pedigree breeding record of inbred parents and to determine genetic relationships amongst genotypes. It was an effective method to determine genetic diversity, polymorphism, gene mapping, genetic map construction and phylogenetic relationship in tomato varieties (Sharma and Sharma, 1999).

The genomes of higher organisms contain multiple copies of microsatellites, satellite DNAs, and minisatellites. These three are simple repetitive DNA sequences arranged in arrays of vastly differing size (Litt &Lutty., 1989). Microsatellites represent short tandem repeat motifs (1-6 bp) also known as simple sequence repeats (SSR). These tandem repeats can be mono- di- tri-, tetra-, or pent-nucleotides units. If nucleotide sequences in the flanking regions of the microsatellite are known, specific forward and reverse primers (generally 20-25 bp) can be synthesized to amplify the microsatellite region by PCR. Microsatellites and their flanking sequences can be identified by

constructing a small insert genomic library, screening the library with a labeled oligonucleotide repeat, followed by sequencing the positive libraries. Repeats can also be identified by screening sequence databases for microsatellite sequence motifs from which adjacent primers may then be designed. Primers may be used that have already been designed for closely related species. Likewise, primers for pepper can be used in tomato. The reason for the variation in a number of repeats could be slipped strand mispairing, DNA polymerase slippage during DNA replication, or unequal crossing over (Matsuoka et al. 2002). SSR polymorphisms can be visualized by agarose or polyacrylamide gel electrophoresis (PAGE). The strengths of microsatellites over other molecular marker techniques include the co-dominance of alleles, high genomic abundance, and random distribution throughout the genome (Morgante et al., 2002). Due to a high level of polymorphism consistently circulated throughout the genome, and having good analytical determination, SSR markers are a preferred choice of the marker (Matsuoka et al., 2002). Moreover, these markers significantly decrease the analytical costs. However, one drawback of microsatellites is its application in unstudied groups where no information is available related to the primers. In these cases, it may become expensive to synthesize primer sequences. Various researchers have used SSR markers to determine the genetic diversity in tomato. Benor et al., (2008) reported average genetic diversity by measuring polymorphism information content (PIC) of 35 SSR markers to determine the genetic diversity of 39 determinate and indeterminate tomato inbred lines. The average PIC was 0.31 and ranged 0.30 to 0.58. Korir et al., (2014) studied genetic diversity of 42 tomato varieties from different geographic regions using EST-SSR markers and reported genetic diversity between 0.18-0.77, with a mean of 0.49; the polymorphic information content

ranged from 0.17 to 0.74, with a mean of 0.45. Zhou et al., (2015) measured the genetic diversity in 29 cultivated, and 14 wild tomatoes and indicated low similarity coefficient of 0.627 in wild tomato whereas cultivated lines have high similarity coefficient of 0.845.

Table 1.1 Droplet size classification chart (ASABE)

Classification	Symbol	Approximate VMD (microns)
Extremely Fine	XF	<60
Very Fine	VF	60-145
Fine	F	145-225
Medium	M	226-325
Coarse	C	326-400
Very Coarse	VC	400-500
Extremely Coarse	XC	501-650
Ultra Coarse	UC	>650

Note: Adopted from ANSI/ASAE S572.1 (ASAE, 2009).

Table 1.2 Percentage of driftable droplets in several TeeJet nozzles using water at room temperature

Nozzle type with spray angle (1.16 L min^{-1} flow rate)	Approximate % of spray volume in droplets <200-micron diameter	
	Application Pressure	
	1.5 Bar	3 Bar
XR TeeJet 110˚	14	34
XR TeeJet 80˚	2	23
DG TeeJet 110˚	<1	20
DG TeeJet 80˚	<1	16
TT- Turbo TeeJet	<1	12
TE- Turbo FloodJet	<1	<1
AI TeeJeet 110˚	N/A	<1

Adapted from TeeJet Technologies, 2017

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CHAPTER II

SCREENING AND SELECTION FOR HERBICIDE TOLERANCE FROM A DIVERSE TOMATO GERMPLASM

Abstract

Injury on tomatoes from auxin herbicides and glyphosate were shown at rates as low as 0.01X. At present 2,4-D, dicamba, glyphosate, and quinclorac are herbicides with the greatest potential of being drifted to tomato plants from adjacent fields. This results in significant reduction in yield, and plant growth; at high drift rates plants may not recover. With the new crop technology, which includes 2,4-D and dicamba resistant crops, there will be increased usage of these herbicides causing more severe drift problems. There is a diverse germplasm of tomato that includes wild relatives known to be tolerant to numerous biotic and abiotic stresses. Chemical stress is an abiotic stress, and wild tomato accessions have a natural tolerance to herbicides in addition to other abiotic stresses. One hundred and ten tomato lines were used for screening of herbicide tolerance, representing numerous species; *Solanum habrochaites, S. cheesmaniae, S. pimpinellifolium, S. chilense, S. lycopersicum, S. pimpinellifolium, S. galapagense, S. chimelewskii, S. corneliomulleri, S. neorickii and S. lycopersicoides*. Plants from these accessions were sprayed with simulated drift rates of 2,4-D, dicamba, glyphosate, quinclorac, aminopyralid, aminocycloparachlor and picloram. The visual injury rating of each accession for each herbicide treatment was taken 7, 14, 21 and 28 DAT on the scale of 0-

100%. Numerous accessions were found to be tolerant to each herbicide tested; 9 accessions for both 2,4-D and glyphosate, 11 for dicamba and 5 for quinclorac,8 for aminocyclopyrachlor and 2 for both aminopyralid and picloram. From this study potential herbicide tolerant lines for different herbicides were identified. Thus, lines can be used to develop herbicide tolerant tomatoes that will help minimize or eliminate the negative impact of drift from non-labeled herbicides tested in this project.

Nomenclature: 2,4-D (2,4-dichlorophenoxy acetic acid); aminocyclopyrachlor (6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid); dicamba (3,6-dichloro-2-methoxybenzoic acid); glyphosate, N-(phosphonomethyl) glycine; tomato (*Solanum lycopersicum*); quinclorac (3,7-dichloro-8-quinolinecarboxylic acid)

Keywords: auxin herbicides, drift, glyphosate, herbicide tolerant tomatoes, wild/abiotic/biotic tolerant tomatoes

Introduction

Annual U.S. tomato production (fresh-market) is 2,703 million pounds, whereas processed tomatoes account for 29,509 million pounds (USDA 2016). Fresh and processed tomatoes account for more than $2 billion in annual farm cash receipts (USDA 2012). Tomatoes are widely known for their outstanding antioxidant content (Bramley, 2000; La Vecchia, 1999; Khachik et al., 1999) including, their oftentimes-rich concentration of lycopene. In Mississippi tomato is grown on over 444 acres across 627 farms (USDA, 2012). Even though the crop is primarily grown in a plasticulture system (Pan et al., 1999), weeds are still a major problem in tomato production. Major weeds in tomato are yellow nutsedge, purple nutsedge, large crabgrass, and Palmer amaranth. Among these weeds, yellow and purple nutsedge are the most problematic, causing significant yield losses and decreased fruit quality (Webster 2002). Herbicide options in tomato are limited, and only a few are highly effective on nutsedge. Herbicides registered in tomato for nutsedge control include halosulfuron, S-metolachlor, and trifloxysulfuron. Numerous studies (Haar et al., 2002; Bangarwa et al., 2009; Scott et al., 2012) have established that, although significant control of nutsedge and other weeds is achieved (60-90%) by these labeled herbicides, significant injury (15-54%) is also observed in tomato plants because of herbicide sensitivity. Moreover, injury from herbicides drifted to greenhouse tomatoes leads to deformed fruits and yield reduction.

Off-site herbicide drift is devastating to vegetable producers (Gilreath et al. 2000; Santos et al. 2007). For instance, in 2013 a small organic tomato grower in Tupelo lost $22,550 due to 2, 4-D drift in his field. Due to crop technologies such as glyphosate resistant corn, soybean and cotton (Green et al. 2009), growers have primarily depended

on glyphosate for weed control (Foresman 2008; Gustafson 2008). And with the recent commercialization of 2,4-D resistant corn, soybean and cotton by Dow AgroSciences, and dicamba resistance crops by Monsanto, the use of auxin herbicides will increase significantly, thus allowing a greater risk of drift of these herbicides to tomato fields. In 2014, USDA approved the commercialization of 2,4-D tolerant corn from DowAgrosciences (The Canadian Biotechnology Action Network); and then in 2015, the genetically engineered dicamba tolerant soybean and cotton from Monsanto was approved for seed sale. With this technology, the use of 2,4-D in corn is estimated to have increased 30 times (Benbrook 2012). There are 17 weed species in the US which are resistant to glyphosate (Heap 2017), and the best option to control these weeds will be using 2,4-D and/or dicamba, which are commonly used as POST treatments for glyphosate-resistant broadleaf weeds Thus, with these technologies, growers can apply on labeled crops to get better weed control but on the other hand, it could be a problem for sensitive non-target vegetables, crops, organic growers and rural home gardens. According to Caseley and Coupland (1985), glyphosate can alter the amount of endogenous plant growth regulators and enzymes produced, which could result in injury symptoms more typically associated with 2,4-D. Drifted rates of glyphosate can cause shortening of pollen tubes, change in the shape of generative cells from spindle-like to elongated cylinder-like, absence of microtubule, malformations in reproductive organs and delay in fruit ripening (Ovidi et al. 2001). Previous studies conducted by Romanowski (1980) showed 10% yield loss at the early vegetative stage with 28.5 g ae ha^{-1} of glyphosate, whereas the same yield loss at the early bloom stage with just 5.3 g ae ha^{-1} rate of glyphosate. A similar study looked at the effects of glyphosate applied at

different stages on flowering loss (Kruger at al. 2012). It was found that 32 g ae ha^{-1} ($1/20^{th}$ of 640 g ae ha^{-1}) of glyphosate is enough to induce a 5% flower loss at early vegetative stage, however in early bloom stage only 2.8 g ae ha^{-1} ($1/228^{th}$ of 640 g ae ha^{-1}) of glyphosate was enough to reduce flowering by 5%. Glyphosate also affected fruit ripening, where the number of ripe fruits harvested were more when glyphosate was applied at the early vegetative stage, than at early bloom stage (Kruger et al., 2012). Gilreath et al. (2001) found that tomato plants could withstand less than 60 g ae ha^{-1} rate of a glyphosate without reducing yield. In 1974, Jordan and Romanowski reported that tomatoes plants sprayed with dicamba at the early bloom stage had significantly higher yield losses than those sprayed at fruit set. Kruger et al in 2012 reported 2.4 g ae ha^{-1} rate is needed to induce a 5% flower loss when applied at early vegetative stage. On the other hand only 1.5 g ae ha^{-1} applied at the early bloom stage was sufficient to cause 5% flower loss. Tomatoes are therefore more susceptible to dicamba than to glyphosate, especially in the vegetative stages. The most commonly used synthetic auxin as a herbicide is 2,4-D. Synthetic auxin herbicides are volatile, resulting in vapor drift that may injure non-target plants (Behrens and Lueschen 1979; van Rensburg and Breeze 1990). In addition, the amount required to injure these non-target plants is minimal. A 0.001% of the label rate of 2,4-D can cause phytotoxicity on tomato (van Rensburg and Breeze 1990). 2,4-D drift to tomato fields at the beginning of the flowering stage is extremely harmful as it decreases the number of fruits per plant and reduces fruit yield (Fagliari et al. 2005). A 0.01X simulated drift rate of 2,4-D applied soon after transplanting resulted in up to 25% loss of ripe fruit and 43% increase in green fruit (Doohan et al., 2010). Quinclorac is another synthetic auxin herbicide commonly used in rice to control barnyardgrass, is the

only auxin herbicide with grass activity (Ronald E et al., 2007). However, tomato plants are very sensitive to quinclorac (De Barreda et al. 1993; Grossmann 1998). In Arkansas and delta region of Mississippi, it is sprayed aerially (Barrentine 1993), causing high drift to tomato fields. The most common symptoms are severe leaf curling and cupping, small plant size, lack of vigor, bloom abscission, and low fruit numbers (Lovelace et al., 2009). Drifted rate of quinclorac above 0.42 g ae ha^{-1} has the potential to reduce tomato yield, and cause significant injury to the plants. The yield of tomatoes at the 0.42 g ae ha^{-1} rate of quinclorac was recorded as 17.3 MT/ha, whereas the yield was reduced further to 11.6 MT/ha when quinclorac rate was increased ten times (Lovelace et al., 2007). Although lower drift rate of quinclorac at 0.42 g ae ha^{-1} resulted in significant injury, as compared to untreated plots, no significant reduction in yield was reported. On the other hand, plants subjected to multiple application of quinclorac rates at 0.42, 2.1, and 42 g ae ha^{-1}, resulted in yield reduction which was harder for the plant to recover from and more likely to cause greater yield loss.

Aminopyralid is a pyridine carboxylic acid herbicide that has negligible volatility (Senseman 2007; Strachan et al., 2010). It is a new synthetic auxin herbicide and is used only in permanent grass pastures and grass hay fields. There are no drift studies on tomato, but studies by Flessner et al., in (2012) reported that aminopyralid causes a higher reduction in dry biomass and height in cotton, as compared to 2,4-D. Aminocyclopyrachlor (AMCP) is a pyrimidine carboxylic acid type herbicide; with very low volatility (Turner et al., 2009; Stracban et al., 2010). It is used to control broadleaf weed in pastures, rangeland, and industrial rights of way. AMCP is the first pyrimidine carboxylic acid herbicide with a chemical structure similar to the pyridine herbicides

aminopyralid, clopyralid and picloram (Strachan et al., 2010). Lewis et al. (2011), simulated spray drift of aminocyclopyrachlor to flue-cured tobacco at five different rates from 0.31 g ae ha^{-1} to 31.4 g ae ha^{-1}; plant injury increased from 11% to 77 % (8 WAT) as the rate increased. Additionally, plant height and fresh weight reduced as rate increased; at 0.31 g ae ha^{-1}, plant height was 67 cm whereas at 31.4 g ae ha^{-1}, height was reduced by more than three times (21 cm); similarly, fresh weight at 0.31 g ae ha^{-1}, was 1285 gm (12 WAT), while at 31.4 g ae ha^{-1}, fresh weight was only 285 gm (12 WAT) including 22 to 32% injury. Flessner et al., 2012 reported that spray drift of amincyclopyrachlor less than or equal to 10 g ae ha^{-1} is not a major concern for cantaloupe and eggplant because there was a negligible change in the marketable yield. Picloram (4-amino-3, 5, 6-trichloro-2-pyridinecarboxylic acid) is an acidic herbicide in the pyridine carboxylic acid family, used to control annual and perennial dicot weeds, shrubs, and woody vegetation. Smith and Geronimo (1984) stated that picloram caused a significant yield loss at 11.2 g ae ha^{-1} in the field grown tomatoes. Cotton shows 32% yield reduction when sprayed with picloram at the rate 561 g ae ha^{-1} while the injury drastically increased to 95% when the rate was rose to 2244 g ae ha^{-1} (Molly et al 2007).

Breeding for herbicide tolerance in the tomatoes would be the most economical, environmentally friendly and feasible method to protect tomatoes from drift injury. Fortunately, scientist have conserved a huge germplasm of its wild species of tomatoes such as *Solanum pennellii, Solanum pimpinellifolium, Solanum peruvianum, and Solanum habrochaites.* These have valuable genes for various abiotic and biotic stresses. Breeding can be performed only if information for the tolerant line is available, and the

superior germplasm can serve as an excellent resource for the screening of herbicide tolerant lines.

Therefore, the objective of the study was to screen tomato germplasm for tolerance to herbicides that can be potentially drifted. Results from this study can be used by tomato breeders for breeding herbicide tolerance trait into the agronomically important tomato varieties, thus ultimately allowing the growers access to herbicide tolerant tomato varieties in the future.

Materials and Methods

A collection of 107 wild/abiotic/biotic stress tolerant tomatoes accessions was provided by the Tomato Genetic Resource Center at the University of California at Davis. Additionally, two accessions (Money Maker and Bonnie Best) were obtained from USDA at Geneva, New York, and eight cultivars (six heat and two drought stress tolerant) were purchased from a commercial seed company (Seedman.com ®, Mississippi) (Table 2.1). To improve the germination of the seeds, they were treated for 10 minutes with 10% bleach solution, rinsed 5-6 times with sterile distilled water at room temperature, and then kept in sterile distilled water overnight at 4˚C to allow the seeds to imbibe water. Imbibed seeds were then planted into cone-tainers (Greenhouse Megastore, Danville, IL) having diameter of 1.5 inches and a depth of 8.25 inch, filled with Sungro professional growing mix, (Sungro Horticulture ®, Agawam, MA) and maintained in greenhouse set at 23˚C for both day and night, light duration was set for 14 hours. Cone-tainers were placed in 7 by 14 cone-tainer trays measuring 24 x 12 x 6.75 inch. Tomato seeds were sown in a completely randomized design for all the three replications. At 4-leaf stage, plants were treated with simulated drift rates of 2,4-D, glyphosate, dicamba,

quinclorac, aminopyralid, aminocyclopyrachlor, and picloram in a spray chamber equipped with the TP8002VS Even Flat Spray Tip (TeeJet®, Spraying Systems Co. World Headquarters, and P.O. Box 7900, Wheaton, IL 60187), calibrated to a deliver 186 L ha^{-1} at 275.79 KPa, while maintaining the constant speed of 4.8 KPH. Drift rates were selected based on previous studies and vary from 0.01X to 0.05X. 2,4-D (Fagliari et al. 2005), dicamba and glyphosate (Kruger et al., 2012) was applied at 0.01X rate (11.2 g ae ha^{-1},2.8 g ae ha^{-1}, and 8.4 g ae ha^{-1}, respectively); similarly quinclorac (Lovelace et al., 2007) was applied at 0.01X rate (39.2 g ae ha^{-1}); and aminopyralid, aminocyclopyrachlor, and picloram (Trevor et al., 2013) was used at 0.05X rate (6.15, 15.65, and 28.0 g ae ha^{-1}, respectively). Table 2.2 lists all the herbicides used in the study along with their simulated drift rates used.

Visual injury was recorded 7, 14, 21, and 28 days after treatment (DAT) on the scale of 0-100 %, where 0 % indicates no injury, and 100% shows the death of the plant (Table 2.3). Accessions showing injury less than or equal to 20% were classified as tolerant accessions. After 28 DAT, the survivors (tolerant accessions) were transplanted into 7 ¼" high, 8" diameter and volume 4.44 qt pots (Greenhouse Megastore, Danville, IL) and maintained until harvest. Mature fruits were collected from each tolerant accession, seeds were extracted from the pulp, washed with 10% bleach solution for 30 min, rinsed with distilled water, air dried, and stored at room temperature for future studies.

In this experiment, data were pooled across experimental replications because experimental replication was considered a random effect whereas tomato accessions and herbicide dose were considered as fixed effect. The experimental design was a complete

randomized design and was setup to evaluate the response of herbicides on different accessions. Data were subjected to analysis of variance (ANOVA), and means were separated using Fischer's protected LSD test at P = 0.05 in the statistical program JMP® (Statistical Discovery™, from SAS). The ANOVA model used in this experiment is defined as $Y_i = \mu + \alpha_i + e_i$, where Y_i is the response variable which includes injury of tomato accessions, μ is mean of response variable alpha is treatment effect on the accessions and e_i is the error $e_i \sim N(0,\sigma^2)$ are independently identical distributed.

Result and Discussion

Accessions from germplasm were classified as tolerant when plants showed injury less than or equal to 20% at 28 DAT. Nine accessions were found tolerant to 2,4-D; injury for tolerant accessions ranged from 5 to 20% (Table 2.4). The effect of 2,4-D was significantly different on all accessions with p-value <0.0001 for injury. TOM17 showed the least injury of 5%. It belongs to species *S. pennellii* with unusual morphology and tolerance to extreme stress such as salinity makes it one of the most abiotic stress tolerant taxa of tomato (Robertson et al., 2007). The leaves of *S. pennellii* are very thick as compared to the cultivated tomato. Leaf analysis of a 5 week old *pennellii* plant has 0.94% of its dry weight in epicuticular lipids, whereas *Solanum lycopersicum* only has 0.16 % of the leaf dry weight in these lipids (Fobes 1985). This thick cuticle of TOM17 may have reduced the penetration of 2,4-D through leaves, thus leading to minor injury (Fagliari et al., 2005). The other two accessions showing injury of 5% or less were TOM83 and TOM56. TOM83 was reported to show moderate resistance to *Pepino mosaic virus* (PepMV), a highly contagious disease in greenhouse tomatoes (Ling et al., 2007); whereas, TOM56 has resistance to Black Mold, a disease of ripe tomato fruit

caused by *Alternaria alternata*, and this disease resistance trait from TOM56 has been bred into cultivated tomatoes (Cassol et la., 1994). According to Atkinson et al., (2012) there is a significant overlap in signaling and response pathways to different abiotic and biotic stresses which consists of cellular redox status, hormones, reactive oxygen species, protein kinase cascades, and calcium gradients as common elements. This overlap in signaling pathways is associated with cross-tolerance phenomena in which plants also develop resistance to other biotic or abiotic stresses (Pastori et al., 2002). Thus, the tolerance of these accessions to abiotic and biotic stresses may lead them to tolerance to herbicides.

For dicamba herbicide, there were eleven accessions with less than 20% injury, ranging from 7 to 20%. Accessions with least injury were TOM17, TOM13, and TOM1 with 7%, 7%, and 9%, respectively (Table 2.4). TOM17 is also 2,4-D tolernat and for the same reasons, it is tolerant to dicamba as well. Francis et al., (2001) showed that TOM1 is partially resistant to genetically distinct strains of *Clavibacter michiganensis subsp. Michiganensis* which causes bacterial canker, a serious pathogen causing significant yield losses in tomato grown in the humid conditions. Resistance from TOM1 was recovered in lines from a BC_2S_4 inbred backcross (IBC) population in both greenhouse and field trials. TOM13 belongs to species *S. pimpinellifolium* which is more specifically used to combat biotic stress such as disease resistance to tomato yellow leaf curl virus, *Botrytis cinerea* (Ignatova et al. 2000) and *Fusarium oxysporum f.sp. lycopersici* (Bournival et al. 1989). Additionally, TOM13 is found to have some drought tolerant traits (Labate et al., 2007).

Glyphosate tolerant accessions showed injury ranging from 3 to 20% with a total of nine accessions being tolerant (Table 2.4). Among the nine accessions, TOM60,

TOM61, and TOM46 showed the lowest injury. TOM60 is reported to be resistant to two insects: two-spotted spider mite [*Tetranychus urticae* (Koch.)] and sliverleaf whitefly (*Bemisia tabaci),* based on egg numbers using leaf disc and Tangle foot no-choice bioassays, and damage scores in choice bioassays (Rakha et al., 2017). TOM61 belongs to *Solanum chilense,* a drought tolerant species, and it is five times more tolerant to wilting as compared to cultivated tomato. This wild taxon of tomato has a longer primary root, and more extensive secondary root system which make it a drought tolerant species (O'Connell et al., 2006). TOM46 has tolerance to high temperature (Robertson et al., 2007).

Tomatoes are susceptible to quinclorac drift, and in this study, we found five tolerant accessions, where the lowest injury was 3% for TOM129 (Table 2.4). Two other accessions with the least injury were TOM66 and TOM63. TOM129 belongs to *S. lycopersicum var. cerasiforme* which is a cherry tomato biotype. Ciccarese et al., (1998) found that accessions from these species show high tolerance to powdery mildew caused by *Oidium lycopersici* and a single recessive gene was responsible for tolerance. Moreover, Cilo et al., (2007) showed that *S. lycopersicum var. cerasiformeis* were tolerant to cucumber mosaic virus stain Fny. TOM63 is a *S. pennellii* accession same as TOM17, while TOM66 belongs to *S. chmielewskii,* which has been found to be moderately resistant to the fungal pathogen *Oidium neolycopersici*. The production of reactive oxygen species (ROS) and peroxidase activity during infection of *O. neolycopersici* is associated with activation of defense responses in genotypes (Lebeda et al., 2014), thus indicating the presence of this defense system in TOM66.

Eight accessions were identified to be tolerant to aminocyclopyrachlor, of which TOM44 and TOM129 showed the least injury of 5%. Both these accessions belong to *S. lycopersicum var. cerasiforme*, as discussed previously, and TOM129 is also tolerant to quinclorac herbicide.

For the remaining herbicides, aminopyralid and picloram, only two tolerant accessions in each were identified; TOM17 (also tolerant to 2,4-D) and TOM47 (same species as TOM129, *S. lycopersicum var. cerasiforme* to picloram, and TOM76 and TOM84 to aminopyralid. Recently, a major QTL (known as *stm9*) on chromosome 9 was identified in TOM76, which is associated with maintenance of shoot turgor under root chilling. Root chilling (6 °C) induces rapid-onset of water stress by impeding water movement from roots to shoots. TOM76 responds to such changes by closing stomata and maintaining shoot turgor, while *S. lycopersicum* fails to close stomata and wilts (Arms et al., 2015). TOM84 is similarly found to be tolerant to low temperature from 2-4˚C (Robertson et al., 2007).

The majority of the tolerant accessions belong to the same species are commonly grown tomato cultivars belong to, *Lycopersicon* species (Fig. 2.1). Thus, indicating the ease of crossing between commercial cultivars and tolerant lines in breeding programs. The other two large groups used in this study were *Solanum habrochaites and S. chilense*. *Solanum habrochaites* is a source for various biotic stress tolerance and has recently been reported to be a potential source of resistance against *Bactericera cockerelli* (Hemiptera: Triozidae) and *Candidatus Liberibacter solanacearum* (Lewy et al., 2014). Similarly, *S. chilense* is found to be tolerant to low temperature (abiotic stress) where none of the plants showed any wilting or visible injury when exposed to 4 and 2 °C, which is atypical

for tomato species (Tetyana, et al., 2016). The other two groups widely studied and frequently used in abiotic stress breeding programs, are *S. pimpinellifolium* and *S. pennellii*. Bolger et al (2014) successfully sequenced the genome of the *S. pennellii*, and numerous QTL's have been identified for salt tolerance in this species (Frary et al., 2010). Linkage map of crosses between *Solanum lycopersicum* and *Solanum pimpinellifolium* display genomic locations of resistance gene analogs, candidate resistance/defense –response ESTs (Sharma et al., 2009)

Analysis of variance indicated that injury was significantly different among accessions for 2,4-D, picloram, dicamba, quinclorac and glyphosate (Table 2.5). However, aminocycloparachlor and aminopyralid did not show any significant difference in terms of injury for each accession; the p-value was 0.2912 and 0.1155, respectively. The order of the severity of the herbicide on different accessions was calculated in the one-way analysis of herbicide and injury (Fig. 2.2) which indicated picloram was most injurious, whereas was dicamba being least injurious on tomatoes. The order of the herbicide injury in ascending order is as follows picloram>aminopyralid>quinclorac>aminocycloparachlor> 2,4-D>glyphosate>dicamba. Wax et al. (1969) studied drift of picloram, 2,4-D, and dicamba on soybean and concluded that picloram was more injurious than other two herbicides. Flessner et al. (2012) reported that aminocyclopyrachlor is more injurious than 2,4-D in a study comparing drift of aminocyclopyrachlor and 2,4-D on cantaloupes, eggplant, and cotton, which is similar to our results. Drift studies of dicamba and glyphosate on tomato show that plants are equally sensitive to both herbicides, but dicamba causes more flower loss at the same rate in comparison to glyphosate at vegetative stages (Kruger et al., 2012).

Jordan and Romanowski (1974) reported injury symptoms for 2,4-D and dicamba in tomato were similar, but 2,4-D drift at early boom stage has the potential to cause higher yield loss than dicamba drift (Fagliari et al., 2005).

Conclusion and Implications

The study reveals tomato accessions tolerant to commonly drifted herbicides in tomato production. Majority of the herbicide tolerant accessions are also tolerant to other biotic/abiotic stresses. Tomato breeders can use lines identified in this study to breed new tomato varieties with herbicide tolerance. These lines can be used as an important genetic source in tomato breeding programs. Additionally, with the help of molecular biology techniques and information available on the tomato genome, breeders can find QTLs responsible for herbicide tolerance thus aiding them in marker-assisted breeding. Once successful tomato varieties are developed having herbicide tolerance and good yield and quality potential, they can be made available to tomato growers to help combat herbicide drift related issues; this includes field and greenhouse growers. Information regarding the tolerant lines and QTLs responsible for herbicide tolerance can be submitted to a tomato genetic database such as Tomato Genetic Resource Center at UC Davis and made available to researchers and breeders worldwide. Researchers will be able to study the mechanism behind herbicide tolerance and use it in other relative vegetable crops.

Table 2.1 List of all accessions used in this study along with their species and place of origin. These accessions are tolerant to various abiotic/biotic stress, it includes both wild and cultivated tomatoes.

S.No.	Accession Name	Taxon*	Place of Origin *
1.	TOM1	*S. habrochaites*	Ecuador
2.	TOM2	*S. peruvianum*	Chile
3.	TOM3	*S. pennellii*	Peru
4.	TOM4	*S. cheesmaniae*	Ecuador
5.	TOM5	*S. peruvianum*	Peru
6.	TOM6	*S. lycopersicum*	Peru
7.	TOM35	*S. habrochaites*	Peru
8.	TOM8	*S. galapagense*	Ecuador
9.	TOM9	*S. lycopersicum*	Ecuador
10	TOM10	*S. galapagense*	Ecuador
11.	TOM11	*S. pimpinellifolium*	Peru
12.	TOM12	*S. pimpinellifolium*	Peru
13.	TOM13	*S. pimpinellifolium*	Peru
14.	TOM14	*S. pimpinellifolium*	Peru
15.	TOM15	*S. pimpinellifolium*	Peru
16.	TOM16	*S. habrochaites*	Peru
17.	TOM17	*S. pennellii*	Peru
18.	TOM18	*S. pennellii*	Peru
19.	TOM19	*S. chilense*	Peru
20.	TOM20	*S. chilense*	Peru
21.	TOM12	*S. pennellii*	Peru
22.	TOM22	*S. chilense*	Peru
23.	TOM23	*S. chilense*	Peru
24.	TOM24	*S. lycopersicoides*	Peru
25.	TOM25	*S. chilense*	Peru
26.	TOM26	*S. chilense*	Peru
7.	TOM27	*S. chilense*	Peru
28.	TOM28	*S. sitiens*	Chile
29.	TOM29	*S. lycopersicum var. cerasiforme*	USA
30.	TOM30	*S. lycopersicum*	USA
31.	TOM31	*S. juglandifolium*	Ecuador
32.	TOM32	*S. lycopersicoides*	Chile
33.	TOM33	*S. pimpinellifolium*	Peru
34.	TOM34	*S. lycopersicum*	Nagcarlang
35.	TOM262	*S. lycopersicum*	La Huarpia
36.	TOM36	*S. orchranthum*	Peru
37.	TOM37	*S. lycopersicum*	Brazil
38.	TOM38	*S. lycopersicum*	Edkawi
39.	TOM39	*S. pimpinellifolium*	Peru

Table 2.1 (continued)

43.	TOM43	*S. sitiens*	Chile
44.	TOM44	*S. lycopersicum var. cerasiforme*	U.S.A
45.	TOM45	*S. cheesmaniae*	Ecuador
46.	TOM46	*S. lycopersicum*	Hotset
47.	TOM47	*S. lycopersicum var. cerasiforme*	USA
48.	TOM48	*S. peruvianum*	Peru
49.	TOM49	*S. pimpinellifolium*	Peru
50.	TOM50	*S. peruvianum*	Peru
51.	TOM51	*S. pimpinellifolium*	Peru
52.	TOM52	*S. pimpinellifolium*	Peru
53.	TOM53	*S. peruvianum*	Peru
54.	TOM54	*S. lycopersicum*	U.S.A
55.	TOM410	*S. habrochites*	Peru
56.	TOM56	*S. cheesmaniae*	Ecuador
57.	TOM57	*S. galapagense*	Ecuador
58.	TOM58	*S. cheesmaniae*	Ecuador
59.	TOM59	*S. cheesmaniae*	Ecuador
60.	TOM60	*S. galapagense*	Ecuador
61.	TOM61	*S. chilense*	Peru
62.	TOM62	*S. pennellii*	Peru
63.	TOM63	*S. pennellii*	Peru
64.	TOM64	*S. chmielewskii*	Peru
65.	TOM65	*S. chmielewskii*	Peru
66.	TOM66	*S. chmielewskii*	Peru
67.	TOM67	*S. neorickii*	Peru
68.	TOM68	*S. huaylasense*	Peru
69.	TOM69	*S. huaylasense*	Peru
70.	TOM70	*S. huaylasense*	Peru
71.	TOM129	*S. lycopersicum var. cerasiforme*	USA
72.	TOM72	*S. lycopersicum*	Mexico
73.	TOM18	*S. lycopersicum*	Philippines
74.	TOM74	*S. lycopersicum*	Brazil
75.	TOM75	*S. lycopersicum*	El Salvador
76.	TOM76	*S. habrochites*	Peru
77.	TOM77	*S. lycopericodies*	Peru
78.	TOM78	*S. lycopericodies*	Peru
79.	TOM79	*S. ochranthum*	Peru
80.	TOM80	*S. lycopersicum*	Sri Lanka
81.	TOM81	*S. lycopersicum*	India
82.	TOM82	*S. lycopersicum*	India
83.	TOM83	*S. chilense*	Chile
84.	TOM84	*S. chilense*	Chile

Table 2.1 (continued)

85.	TOM85	*S. juglandifolium*	Colombia
86.	TOM86	*S. lycopersicum*	Colombia
87.	TOM87	*S. sitiens*	Chile
88.	TOM88	*S. chilense*	Chile
89.	TOM89	*S. chilense*	Chile
90.	TOM90	*S. chilense*	Chile
91.	TOM91	*S. chilense*	Chile
92.	TOM92	*S. lycopersicum*	Hawaii
93.	TOM93	*S. juglandifolium*	Ecuador
94.	TOM94	*S. lycopersicum*	Venezuela
95.	TOM95	*S. lycopersicum*	Hawaii
96.	TOM96	*S. lycopersicum*	Hawaii
97.	TOM45	*S. habrochites*	Peru
98.	TOM98	*S. corneliomulleri*	Peru
99.	TOM108	*S. corneliomulleri*	Peru
100.	TOM100	*S. chilense*	Peru
101.	TOM101	*S. lycopersicum*	Fr. Oceania
102.	TOM102	*S. neorickii*	Peru
103.	TOM103	*S. lycopersicum*	Ecuador
104.	TOM104	*S. chilense*	Peru
105.	TOM105	*S. galapagense*	Ecuador
106.	TOM106	*S. habrochites*	Ecuador
107.	TOM107	*S. corneliomulleri*	Peru
108.	TOM108	*S. lycopersicum*	U.S.A
109.	TOM109	*S. lycopersicum*	U.S.A
110.	TOM110	*S. lycopersicum*	U.S.A
111.	TOM111	*S. lycopersicum*	U.S.A
112.	TOM112	*S. lycopersicum*	U.S.A
113.	TOM113	*S. lycopersicum*	U.S.A
114.	TOM114	*S. lycopersicum*	U.S.A
115.	TOM115	*S. lycopersicum*	U.S.A

*Source: Tomato Genetic Resource Center

Table 2.2 Common name, trade name, percentage of recommended rate and drifted rates (ae) for the seven herbicides used in the study

Herbicide	Trade Name	Rate used	Drift rates (g ae ha^{-1})
2,4-D	Weedar -64®	0.01X	11.2
Dicamba	Clarity®	0.01X	2.8
Glyphosate	Roundup Powermax ®	0.01X	8.4
Quinclorac	Facet L®	0.01X	39.2
Aminopyralid	Milestone®	0.05X	6.15
Aminocyclopyrachlor	Streamline ®	0.05X	15.65
Picloram	Tordon ®	0.05X	28.0

Table 2.3 Tomato plant visual injury (%) and its associated symptomology on to different plant parts (leaves, stem, petioles)

Tomato Injury (%)	Tomato symptomology
0-10	No symptoms of injury
10-30	Slight to moderate injury.
30-50	Epinastic and twisting of leaves in auxin herbicides, white/yellow discoloration at the base
50-70	Moderate to severe injury, callusing on the stems in auxins and growth reduction
70-95	Severe injury and no growth
95-100	Near to death or completely dead

Table 2.4 Tolerant accessions with their corresponding herbicide and mean injury (%) at 28 DAT, where tolerant accessions have injury less than or equal to 20%.

Herbicide	Accession	Mean Injury (%) (28 DAT)
2,4-D	TOM45	8 J*
2,4-D	TOM1	8 J
2,4-D	TOM56	5 J
2,4-D	TOM11	17 HIJ
2,4-D	TOM13	15 HIJ
2,4-D	TOM14	13 IJ
2,4-D	TOM22	12 IJ
2,4-D	TOM83	5 J
2,4-D	TOM17	5 J
Aminocyclopyrachlor	TOM27	13 FG
Aminocyclopyrachlor	TOM74	8 FG
Aminocyclopyrachlor	TOM54	20 EFG
Aminocyclopyrachlor	TOM78	10 FG
Aminocyclopyrachlor	TOM29	7 FG
Aminocyclopyrachlor	TOM44	5 FG
Aminocyclopyrachlor	TOM129	5 G
Aminocyclopyrachlor	TOM103	18 EFG
Aminopyralid	TOM76	10 F
Aminopyralid	TOM84	18 EF
Dicamba	TOM1	5 J
Dicamba	TOM3	10 J
Dicamba	TOM35	20 EFG
Dicamba	TOM18	18 EFG
Dicamba	TOM74	20 EFG
Dicamba	TOM13	7 FG
Dicamba	TOM14	15 FG
Dicamba	TOM17	7 G
Dicamba	TOM12	14 FG
Dicamba	TOM262	13 FG
Dicamba	TOM44	12 FG
Glyphosate	TOM46	10 IJ
Glyphosate	TOM60	3 J
Glyphosate	TOM61	10 IJ
Glyphosate	TOM64	12 IJ
Glyphosate	TOM108	17 HIJ
Glyphosate	TOM66	18 GHIJ
Glyphosate	TOM18	17 HIJ
Glyphosate	TOM102	20 GHIJ
Glyphosate	TOM87	15 HIJ

Picloram	TOM17	20 HIJ
Picloram	TOM47	13 J
Quinclorac	TOM66	7 H
Quinclorac	TOM129	3 H
Quinclorac	TOM77	10 EGH
Quinclorac	TOM410	15 GH
Quinclorac	TOM63	8 EGH

*Means followed by same letter are not different from each other at 0.05 significance level.

Table 2.5 Effect of the herbicide on all the tested accessions, each herbicide F ratio, sum of squares and mean square along with their significance.

Herbicide	Sum of Squares	Mean Square	F Ratio	Prob > F
2,4-D	97293.27	1201.15	7.5987	<.0001
Aminocycloparachlor	93162.80	970.446	1.1362	0.2912
Aminopyralid	66377.215	677.319	1.3343	0.1155
Dicamba	56952.692	720.920	1.5689	0.0445
Glyphosate	39430.523	788.610	2.4212	0.0036
Picloram	93820.05	1054.16	2.6679	<.0001
Quinclorac	43175.610	881.135	2.8772	0.0011

Probability of F greater, determined using student t test procedure for each treatment, at 0.05 significance level

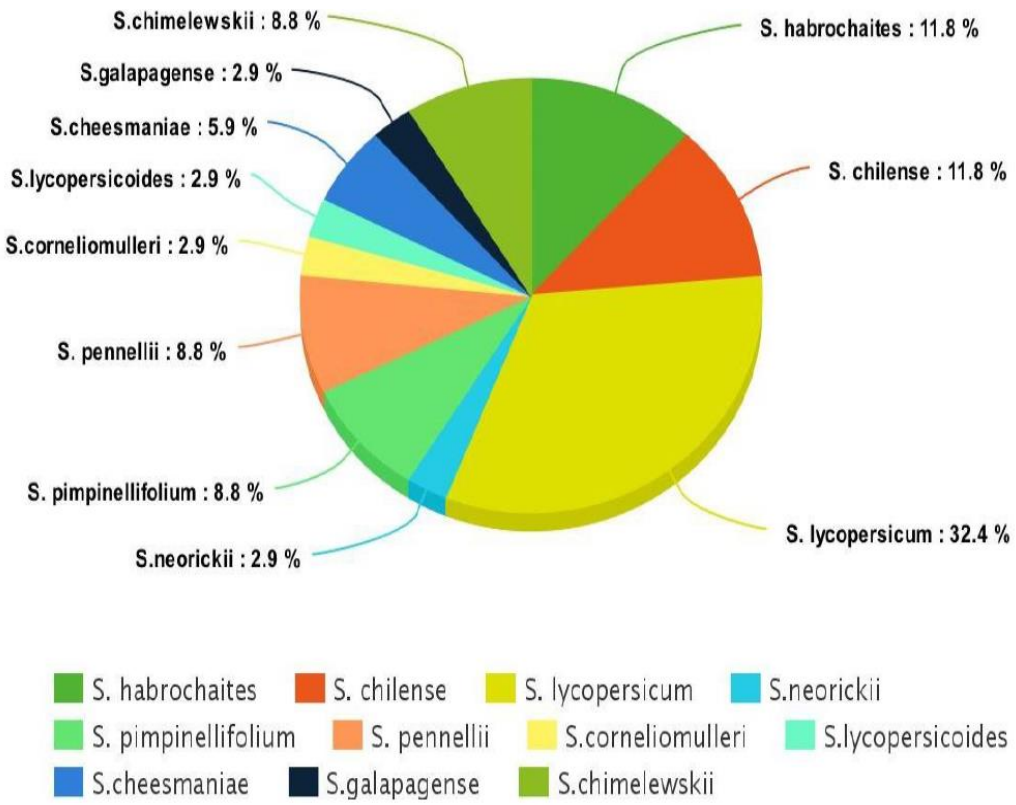

Figure 2.1 Distribution of tolerant accessions in different species (Cultivated and Wild relatives) of tomato.

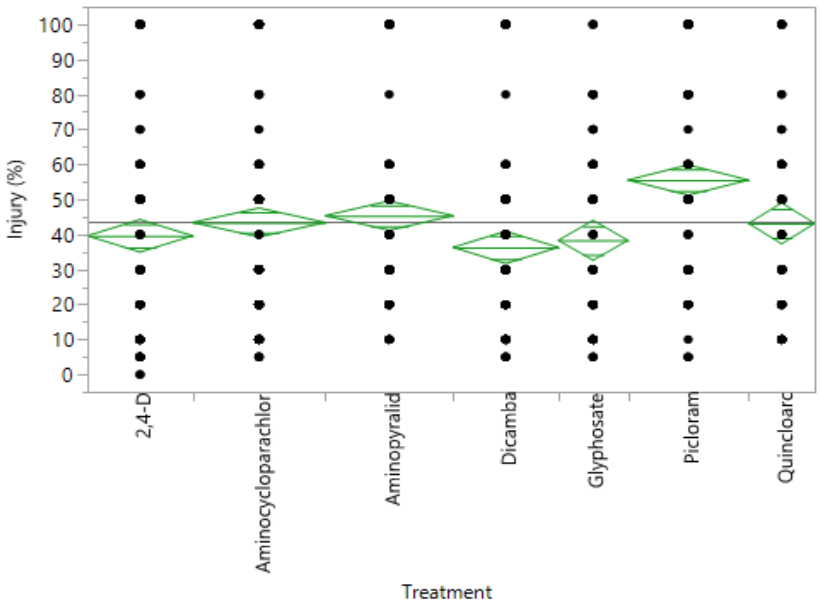

Figure 2.2 The Comparison of injury rating for all the herbicides among all the tested accessions.

Where black dot represents all the different values of injury for its respective herbicide. The top point on the green diamond is upper confidence interval whereas lower point is lower confidence interval.

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CHAPTER III

HERBICIDE TOLERANCE AND YIELD POTENTIAL OF TOMATO IN FIELD

Abstract

Solanum lycopersicum, the domesticated species of tomato, is one of most economically important horticulture crops is grown worldwide. Tomato is highly sensitive to auxin herbicides and glyphosate. Auxin herbicides and glyphosate results in injury and significant yield reduction in tomato, at rates as low as 0.01X. In this study, we conducted a field experiment at two different locations to characterize herbicide tolerant tomato lines, selected from our previous greenhouse study. Plants were treated with simulated drift rates of five herbicides namely, 2,4-D, dicamba, quinclorac, aminocycloparachlor, and glyphosate, after one week of transplantation. Visual injury on the scale of 0-100% and plant height was recorded every week following treatment, until 49 days after treatment (DAT). Fruits were harvested and yield was recorded. TOM18, TOM129, and TOM87 showed the least injury to Dicamba, quinclorac, and glyphosate respectively. TOM18 and TOM129 accessions belong to the *S. lycopersicum* species and are of the cherry tomato biotype. While TOM87 belongs to *S. sitines.* Plant heights of the tolerant tomato lines did not differ among themselves, or when compared to Better Boy cultivar. Based on the injury and fruit yield TOM129 , TOM18 and TOM 87 are accessions most tolerant to quinclorac, Dicamba and glyphosate respectively. Providing tomato growers access to tomato lines/varieties with improved herbicide tolerance

compared to the current varieties used in Mississippi can, therefore, protect these crops from herbicide injury, thus increasing the marketable yield and fruit quality.

Nomenclature: 2,4-D (2,4-dichlorophenoxy acetic acid); aminocyclopyrachlor (6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid); dicamba (3,6-dichloro-2-methoxybenzoic acid); glyphosate, N-(phosphonomethyl) glycine; tomato (*Solanum lycopersicum*); quinclorac (3,7-dichloro-8-quinolinecarboxylic acid)

Keywords: auxin herbicides, drift, glyphosate, herbicide tolerant tomatoes, wild/abiotic/biotic tolerant tomatoes

Introduction

Tomato is one of the most important vegetable crops grown all across the globe. The fruit provides a significant number of total antioxidants required in our diet (Martinez-Valvercle et al., 2002). One lipid soluble antioxidants found in tomato is lycopene and has been linked with decreased risk of cancer and cardiovascular diseases (Rao et al., 2000). Moreover, tomato by-products such as skin contain 2.5 times higher amount of lycopene as compared to the pulp (Ray et al., 2016). They also represent a good source of vitamins C and A, flavonoids, phenolics and are low in calories (Elbadrawy et al., 2011).

The U.S. is the third largest producer of the tomato, followed by China and India (FAOSTAT 2014), producing about 14,516,060 tons of tomato with a total harvested area of 163,380 ha (FAOSTAT 2014). In the US, tomatoes is produced for the fresh and processing industry. At commercial scale, the fresh market tomatoes is grown in almost

20 states of the US, among which California and Florida produce almost two-thirds of the total production. In Mississippi, the tomato are grown on about 444 acres across 627 farms (MDAC 2013). Nonetheless, significant yield reductions are caused due to pest attack, diseases, and herbicide drift, thus leading to an economic loss for the farmer. Drift studies by Kruger et al. (2012) showed that drift of glyphosate and dicamba on processing tomatoes at the early vegetative stage and early bloom could cause a yield reduction of 25%. Also, other related plants such as pepper are susceptible to drift rates of 2,4-D. A study by Mohseni-Moghadam (2015) reported that yield was reduced by 77 and 36% at 39 and 56 DAT, respectively, when drifted rates of 2,4-D (16.8 g ae ha^{-1}) were applied. Other auxin herbicides such as dicamba and quinclorac can cause significant yield reduction, and multiple applications of the same herbicide can lead to severe injury and yield loss. Lovelace et al. (2007) reported that drift rate above 0.42 g ae ha^{-1} of quinclorac could cause injury up to 68 %, and tomato fruit yield was reduced by half when drift rate was increased ten times.

Since the commercialization of Roundup Ready corn and soybean, the area under herbicide tolerant crops has increased. In 2009, Duke and Powles reported that glyphosate tolerant GMO crops represented more than 80 % of the 120 million ha of crops grown annually, worldwide. Growing these crops are economical for the farmers as it reduces cost and effort, and also promotes no-till practices. A study by Gardner et al. (2009) showed that a farmer switching from conventional soybean to GMO seeds would reduce labor for pesticide application and tillage by 374 hours. Similarly, in corn, labor was reduced by 184 hours by switching from conventional to GMO seeds. However, because of the ease of use and broad spectrum weed control provided by glyphosate,

farmers over relying too much on this chemical, thus reducing the use of other herbicides with a different mode of action. Integrated weed management practices are ignored, and tank mixing two or more herbicides with a different mode of action is less practiced (Johnson et al., 2009). The overuse of glyphosate increases the selection pressure on weeds and promotes the evolution of glyphosate resistant weeds. Thirty-seven glyphosate resistant (GR) weed species are found across 17 countries which include Argentina, Brazil, Chile, Italy and United States (Heap, 2017). Among the glyphosate resistant weed biotypes, *Amaranthus, Conyza,* and *Lolium* species are most common (Heap, 2017) with GR *Amaranthus* species being most problematic as they have evolved resistance to other mode of action herbicides. Both *Amaranthus* and *Conyza GR* species have potential to spread very rapidly (Bell et al., 2013, Norsworthy et al., 2014). The presence of GR weeds not only reduces crop yield but also increased management costs for the growers. Mueller et al (2005) reported that GR horseweed could increase the cost of production by \$28.42 ha^{-1} in soybean. Similarly, it costs an additional \$48 ha^{-1} to manage GR Palmer amaranth in cotton fields in Arkansas and Georgia (Norsworthy et al., 2011). To address the threat posed by GR resistant weeds, the agricultural industry came up with new herbicide technologies such as for as Enlist® (2,4-D-, glufosinate-and glyphosate-tolerant) and Xtend® (dicamba and glyphosate tolerant). These technologies allow farmers to use 2,4-D and dicamba herbicides, in addition to glyphosate, to control weeds. With the advent of these new herbicide technologies there will be an increase in the use of 2,4-D and dicamba herbicides, thus increasing potential off-site movement to sensitive horticulture crops such as tomato and grape-vines due to drift and volatility, ultimately causing economic loss to farmers (Johnson et al., 2012). In 1974, Jordan and

Romanowski reported that tomato plants sprayed with dicamba at the early bloom stage had greater yield losses than those sprayed at fruit set. Kruger et al in 2012 reported 2.4 g ae ha^{-1} rate is needed to induce a 5% flower loss when applied at early vegetative stage. On the other hand, only 1.7 g ae ha^{-1} applied at the early bloom stage was sufficient to cause 5% flower loss. Severe injury in tomato plants was caused by exposure to vapors of 2,4-D butyl ester (Baskin and Walker, 1953). Tomato plants are therefore more susceptible to dicamba and 2,4-D than to glyphosate, especially in the vegetative stages.

According to the Weed Science Society of America, herbicide tolerance is the inherent ability of a species to survive and reproduce after herbicide treatment, which implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant. Because genes associated with herbicide tolerance have pleiotropic effects or linkage with one or more other loci, herbicide tolerance may be related to fitness penalty (Mithila et al., 2011). There is no literature on herbicide tolerance and fitness cost for crops, although some information is available on fitness cost associated with herbicide resistance for weed species. Bourdot et al., (1996) reported that MCPA resistant *Ranunculus acris L* plants were ecologically less fit and less competitive as compared to their sensitive counterparts. Plant yield was also lower than sensitive plants when grown at higher densities. Similar results were reported by Hall et al., (1995) in phenoxy herbicide (2,4-D, dicamba, picloram and MCPA) resistant population of *Sinapsis arvensis*; resistant plants were stunted, with reduced leaf area, less developed root system, higher chlorophyll, and higher cytokinin levels. Baucom et al., (2004) reported that most glyphosate tolerant *Ipomoea purpurea* have a negative correlation with cost of fitness, and high levels of genetic variation among tolerant plants. A similar study

by Pedersen et al., (2007) with glyphosate resistant *Lolium rigidum* reported no reduction in vegetative growth when grown under competition with wheat. A F_2 hybrid, from a cross between weedy rice and glyphosate tolerant rice carrying EPSP synthase transgene, produced 48-125% more seeds than non-transgenic controls per plant without glyphosate application (Wang et al., 2014). Moreover, the hybrid had greater tryptophan concentrations, photosynthetic rates, and percent seed germination, than the non-transgenic control plants. Thus, morphological characteristics of herbicide tolerant crop plants such as injury, plant height, the number of seeds, and fruit yield are vital for crop improvement programs, as the success of the crop is limited by these factors (Koornneef et al., 2001).

Due to the potential increase in dicamba and 2,4-D herbicide usage and a corresponding increase in off target movements to sensitive crops such as tomatoes, significant changes in growth, morphology and fruit yield are expected in tomatoes with sub-lethal concentrations of the herbicide. Unfortunately, to date, no herbicide tolerant tomato cultivars have been reported or commercialized. Thus, the objective of this study was to identify tomato accessions having tolerance to drifted rates of auxin and glyphosate herbicide and characterize these tolerant accessions morphologically to determine if tolerance to herbicide cause any fitness penalty.

Materials and Methods

Experiment location

The field trials were conducted during the 2016 and, 2017 growing seasons in order to evaluate the tomatoes when exposed to drifted rates of herbicide. Each year, experiments were performed at Truck Crops Branch Experiment Station, Crystal Springs (31° 59' N, 90° 22' W) and North MS Research and Extension Center, Verona (34°11'N, 88° 42' W). The field in 2016 at both the locations consisted of 5 rows, each row 43 m long, and 18.5 m wide; in 2017, fields consisted of 10 rows, each 43 m long and 37 m wide. Fields were chisel-plowed twice, followed by disc plowing, and twice using a Triple K. Calcium was added at three different intervals throughout the growing season, with the first time before plastic mulch establishment using granular ammonium sulfate at 79 kg ha^{-1}. The other two applications were side dressed with granular calcium nitrate at 34.01 kg ha^{-1}, one at first fruit and the other two weeks after the first fruit. Phosphorus and Potassium were applied as 0-20-20 per row, equivalent to 7.25 kg. Weeds near tomato plants were regularly hand-weeded twice per week. Weekly spray schedule consisted of an alternative application of fungicide Bravo® (1.7 L/ha) and Quadris® (1.035 L/ha). Neem oil (2% by volume) and Veg Plus containing permethrin (10% by volume) was applied as an insecticide as needed.

Plant Materials

Tomato seeds were obtained from the Tomato Genetic Resource Center at the University of California, Davis, CA. Tolerant accessions were selected from our previous greenhouse screening (Sharma et al., 2017), and were chosen based on seed availability and level of tolerance to each herbicide. Seeds were first treated with 10% bleach

solution to improve germination, followed by sowing in the greenhouse at Dorman Hall, Mississippi State University. Dates for sowing and transplanting are in Table 2.1. Seeds were then grown in 48-cell tray (1.55" x 1.55" x 2.33" deep) filled with Metro Mix Professional Growing Mix (Sungro Horticulture ®, Agawam, MA) and maintained in a greenhouse set at 23˚C for both day and night, and at 14 hr of light per day. Table 2.1 contains a list of tomato accessions used in this study. In 2016, tomato was transplanted in the month of May as seed stocks were not available earlier than this date; while in 2017, tomato was transplanted in the month of April, the optimum planting date in Mississippi (Table 3.1) A commonly grown tomato cultivar, Better Boy (BB), was also included for comparison, and transplanted at the same stage as the tolerant accessions. Three replications of each treatment for both the locations.

Herbicide Treatment

Herbicide treatment was applied on a 2.44 x 0.61 m plot containing five plants. Herbicides treatments were applied 10 days after transplanting with the help of CO_2-pressurized backpack sprayer equipped with a two nozzle boom with TP8002VS Flat spray tip (TeeJet®, Spraying Systems Co. World Headquarters, and P.O. Box 7900, Wheaton, IL 60187). The spray boom was calibrated to deliver 186 L ha^{-1} at 275.79 kPa while maintaining the constant speed of 4.8 KPH. Wooden boards were used as blockers while spraying the plots to avoid off target movement of the herbicide to adjacent plots. Drift rates were selected based on previous herbicide drift studies on tomato and were similar from the greenhouse study (Table 2.2).

Data and statistical analysis

Crop visual injury and plant height were recorded at 7, 14, 21, 28, 35, 42 and 49 days after treatment (DAT) using a scale of 0 to 100%, where 0 = no injury, and 100% is complete death of the plant (Frans et al., 1986). Typical visual symptoms of auxin herbicide injury are leaf curling, epinasty (bending/elongation of stems and leaf petioles), and of glyphosate are yellow discolorations at the base of the youngest leaflets. Chlorophyll content in leaves from 5 plants in each plot (3 leaves from each plant) was recorded with the help of a spad meter (CCM-300 by Opti-Sciences) at 0, 7and 14 DAT. Fruits were harvested at the end of the season from each plant in the plots and fruit yield was recorded.

Experimental design was randomized complete block according to the model equation below

$$Y_{ijk} = \mu + \beta_i + \alpha_j + (\beta\alpha)_{ij} + e_{ijk}, \text{ Where } i=1,\ 2\ j=\ 1,\ 2,3,4,5,\ k=1,2,3,4,5 \quad \text{(Eq. 3.1)}$$

Where Y_{ijk} is the response variable, μ is the mean of the response variable, β_i is the location effect on the accessions, α_j is the treatment effect on the accessions, $(\beta\alpha)_{ij}$ is the interaction between the location and treatment and e_{ijk} is the error. Where $\beta_i \sim N(0,\sigma_\beta^2)$, $(\beta\alpha)_{ij} \sim N(0,\sigma_{\alpha\beta}^2)$, $e_{ijk} \sim N(0,\sigma^2)$ are independently identical distributed. Data from both years were analyzed separately because of an uneven number of treatments. Due to limited seed stock, untreated control plots were not included in the first year. For both years, data for injury, height, chlorophyll and fruit yield, were averaged across the locations and subjected to ANOVA using the PROC MIXED procedure of the SAS software (SAS 9.4 SAS Institute Inc., Cary, NC) in JMP®. Location and location x

treatment were considered as random effects whereas treatment was considered as fixed effect (Dodds et al., 2010, Yang 2010, Blouin et al., 2010). Treatment means were separated by Fisher's protected LSD at an alpha level of ≤ 0.05.

Results and Discussion

Visual Injury

Symptoms of 2,4-D injury such as petiole twisting and leaflet cupping were visible two days after treatment. Other symptoms that followed were parallel venation in new leaves, upper stem bending, and swollen stem with bumps; similar symptoms were reported by Marple et al., (2007) and Lewis et al., (2011). In 2016, BB and TOM45 showed injury of 10-30% and 4-18%, respectively (Table 3.4). Plants from both the accessions showed signs of recovery at 35 DAT. Although the mean injury was not significantly different for the two accessions, mean injury of TOM45 (15%) was significantly lower than BB (22%), the commercially used tomato cultivar (Table 3.5). In 2017, injury ranged from 5- 23% for TOM45 and 15-30% for BB (Table 3.6). It should be noted that TOM45 belongs to the *habrochaites* species that is well adapted to low temperature conditions where night temperature falls to 10°C (Venema et al., 1999). However, in Mississippi, average night temperature ranges 20-25°C, which is 10-15°C above their optimum growing temperatures. Thus, temperature conditions may be the reason why TOM45 did not perform better than BB as seen in the greenhouse study (Sharma et al., 2017). *S. Habrochaites* species has been reported to be resistant to *Bactericera cockerelli*, also known as tomato psyllid, which is one of the most destructive potato pests (Levy et al., 2014). Liu et al., (2015) reported that overexpression of DHN gene from *S. habrochaites* into cultivated tomato showed improved tolerance to

cold, drought, and salinity stresses. This suggests that TOM45 has a gene pool that makes it tolerant to abiotic and biotic stresses.

Visual symptoms observed with aminocycloparachlor treatment were drooping petioles, stunted leaflets on stringy petioles, epinasty of leaves, yellowing, and necrosis. Similar injury symptoms were reported by Marple et al., (2007), Lewis et al., (2011) and Strachan et al., (2013). In 2016, the injury of BB ranged from 19 to 43%, and that of TOM54 ranged from 21 to 43% (Table 3.4). Mean injury for BB and TOM54 were 35% and 32%, respectively, and were not significantly different from each other (Table 3.5). In 2017, the injury was similar ranging from 22 to 39% for TOM54 and 18 to 50% for BB (Table 3.6), with mean injury for TOM54 (35%) and BB (43%) not significantly different from each other (Table 3.7). Among all herbicides used in this study, aminocyclopyrachlor was most injurious to tomato. Patton et al., (2013) observed epinasty in tomato when aminocyclopyrachlor was applied as rates as low as <0.1 ppb, but the concentration of aminocyclopyrachlor in leaves was found to be 0.5 ppb. TOM54 is a commercial variety marketed as a drought tolerant cultivar and belongs to the same species as cultivated tomato *(Solanum lycopersicum).*

The most recognizable visual symptom of glyphosate injury was bleaching (white/yellow discoloration) at the base of young leaflets, which would turn brown at later stages. In 2016, visual injury for BB was 3% at 7 DAT and continued to increase until 35 DAT where an injury of 8% was recorded (Table 3.4). Similar results in potato were reported by Felix et al. (2011) where plants treated with a rate of 8.5 g ha^{-1} showed an injury of 2 % at 7 DAT. Mean injury of TOM108 and TOM87 was significantly higher than BB, and

were 20% and 11%, respectively (Table 3.5). Maximum injury of TOM108 and TOM87 was 27% (at 35 DAT), and 15% (at 28 DAT) respectively (Table 3.4). Surprisingly, the least mean injury of BB was 6% which was significantly lesser than TOM87 and TOM108 that had similar mean injuries. In 2016, BB was slightly advanced in its growth stage as compared to other accessions at the time of transplanting. This advanced growth stage of BB might be the reason why it was able to tolerate the herbicide better than other accessions. In 2017, BB injury ranged from 8 - 10% with a mean injury of 8% (Table 3.6, 3.7), the injury for TOM108 varied from 5 -24% with the mean injury of 15% which did not differ from BB. However, injury for TOM87 ranged from 8 – 13% with the mean injury of 10% which was lower than TOM108 and BB, indicating their higher tolerance to glyphosate (Table 3.6, 3.7). TOM87 and TOM108 belong to *S. sitiens* and *S. corneliomulleri*, and not many studies have been conducted with these wild species of tomatoes.

The visual symptoms for dicamba were parallel venation in leaves, petiole twisting and epinasty on the main stem. In 2016, injury for BB ranged from 8% to 11%, for TOM35 it ranged from 3% to 22% whereas for accessions TOM18, TOM12 and TOM262 it ranged from 6 to 3%, 5 to 12% and 16 to 19%, respectively (Table 3.4). TOM18 showed the lowest mean injury of 3%, while TOM35 and TOM262 showed the maximum mean injury of 14% and 15%, respectively (Table 3.5). TOM18 and TOM12 had significantly lower mean injury than TOM262 and TOM35. Mean injury of BB was 9% with a maximum injury of 10% at 21 DAT (Table 3.4, 3.5). Mohseni-Moghadam et al., (2015) reported maximum injury of 8% at 1.9 g ae ha^{-1} of dicamba in the white grape variety,

Riesling, at 42 DAT. Kruger et al. (2012) indicated a visual flower loss of 5% at the early vegetative stage for four tomato cultivars, when sprayed with dicamba at a rate of 2.4 g ae ha^{-1}, whereas, a rate of 1.5 g ae ha^{-1} caused flower loss of 5% at early boom stage. In our study, TOM18 showed the lowest mean injury of 8% at 14 DAT, and in the subsequent weeks, these plants completely recovered from the injury. Among the accessions tested, TOM35 showed the highest injury with a maximum of 22% at 35 DAT. In 2017, results were similar with TOM18 showing the least mean injury of 10%, ranging 8 to 11% (Table 3.6, 3.7). TOM12 and TOM262 showed mean injury of 9% and 12%, respectively. BB showed the highest mean injury of 16% with a range of 13 to 22% (Table 3.7). Similarly, tomato cultivar showed injury of 24% 14 DAT when dicamba was sprayed on the center of the tillage strip at a rate of 1,120 g ae ha^{-1} (Bauerle et al., 2015). TOM35 belongs to *habrochaites* species which is adapted to lower night temperature up to 10°C (Venema et al., 1999) whereas in Mississippi average night temperature ranges from 20 to 25˚C. Temperature difference may be the reason why TOM35 got severely injured as compared to the others. TOM18 belongs to same species as cultivated tomato, *Solanum lycopersicum*, but falls under cerasiforme variety, a cherry tomato biotype. Ciccarese et al. (1998) reported that *S. lycopersicum* var. cerasiforme has resistance to powdery mildew caused by *Oidium lycopersici.* This variety displays higher genetic diversity than other cultivated tomato varieties and higher phenotypic diversity than other wild tomato species (Ranc et al., 2012). The presence of these unique characteristics may be the reason why TOM18 exhibited lesser injury as compared to others.

The visual injury symptoms of quinclorac includes epinasty from meristematic regions to entire plant, stem twisting and leaf curling. Similar symptoms were reported by Lovelace et al., (2007). In 2016, the highest mean injury of 22% was recorded for BB with a range of 15 to 32% (Table 3.5). Lowest mean injury (13%) was recorded in TOM129, with a range of 13 to 16% (Table 3.4, 3.5). TOM410 showed mean injury of 19% and was not significantly different from BB and TOM129 (Table 3.6). Similar results were obtained in 2017, where TOM129 showed least mean injury of 6% which was significantly lower than TOM410 and BB (Table 3.7). Mean injury for TOM410 was 18%, ranging from 10-22%, and mean injury for BB was 22% with a range of 12 - 33% (Table 3.6, 3.7). Lovelace et al. (2007) reported maximum injury of 38% at 28 DAT on Mountain Supreme (commonly grown tomato cultivar). In both years, the tolerant accession, TOM129, showed a maximum injury of 16% at 28 DAT, but in consecutive weeks it recovered, and injury decreased to 10%. TOM129, similar to TOM18, belongs to the cerasiforme species, a cherry tomato biotype with resistance against leaf mold caused by fungi *Cladosporium fulvum (Passalora fulva)* (Gallo et al., 2011). This biotype is also resistant to the bacterial wilt caused by *Ralstonia solanacearum* (Mohamed et al., 1997). Cerasiforme is an admixture of wild and cultivated tomatoes, making it highly diverse, genetically (Gallo et al., 2011), and because of this diverse genetic makeup, TOM129 may have shown lower injury as compared to cultivated tomato.

Fruit Yield

Fruit yield across all herbicides was higher in 2017 as compared to 2016. This may be due to earlier transplanting in 2017, which also coincides with the optimum

planting date of tomato in Mississippi. Among all the herbicides, AMCP drift injury resulted in the lowest fruit yield in both years, and highest yield reduction. In 2016, the yield of TOM54 (105.65 kg ha^{-1}) was significantly less than BB (154.82 kg ha^{-1}) (Table 3.5). In 2017, the yield of TOM54 was 130.83 kg ha^{-1} but significantly lower than NTC. In 2017, yield of TOM54 with drifted rate of AMCP was 130.8 kg ha^{-1} however yield of NTC was 317 kg ha^{-1}. Thus, the yield reduction of TOM54 due to simulated drift rate was 59%. The yield of BB due to AMCP drift rate was reduced by 59%, which was not significantly different from the yield of TOM54 (Table 3.7). Highest injury was also observed with AMCP, which in turn may cause a reduction in fruit yield. Flessener et al., (2012) reported negligible weight loss, both total and marketable, in eggplant when AMCP was applied at 10 g ae ha^{-1}, and observed minor visible injury. Contrary to the finding of our study, tomato seems to be much more sensitive to AMCP than eggplant. Therefore, TOM54, although identified as tolerant to AMCP in greenhouse screening, is not tolerant in the current field screening, based on the high injury and yield loss recorded in both years.

Although in 2016, injury from 2,4-D on BB and TOM45 was similar, the average yield of BB (149.7 kg ha^{-1}) was significantly higher than TOM45 (114.16 kg ha^{-1}) (Table 3.5). TOM45 is a wild accession of tomato belonging to *S. habrochaites* species. Wild relatives of tomato generally do not have high yield but are beneficial in combating abiotic and biotic stresses and have improved fruit quality parameters (Hajjar et al., 2007). In 2017, although the yield of TOM45 (194.90 kg ha^{-1}) and BB (884.012 kg ha^{-1}) were similar, they were significantly lower than their respective NTC (Table 3.7). Yield

reduction of 25% and 22% was recorded in TOM45 and BB, respectively. Doohan et al. (2010) reported that drift of 2,4-D at the rate of 0.01X in tomato soon after transplanting can cause a 25% loss of ripe fruit, thus having an impact on overall yield of the plants. The nearly equal amount of yield reduction was recorded in TOM45 because of 2,4-D drift as in the commonly grown cultivar; thus, indicating that TOM45 may not be a suitable candidate for 2,4-D tolerance breeding.

In 2016, the average yield of TOM18, TOM262, and BB with drift rate of dicamba were 145, 255.9, and 277.1 kg ha^{-1}, respectively (Table 3.5). Average yields from these three accessions were not significantly different from each other. In 2017, for dicamba herbicide, TOM18 showed least yield reduction of 5%, and average yield from both treated (185.5 kg ha^{-1}) and non-treated (190.1 kg ha^{-1}) plots were not significantly different from each other (Table 3.7). Moreover, TOM18 showed the least injury among all accessions. Simulated drift rate of dicamba caused 11% yield reduction in TOM262, its mean yield of 366.19 kg ha^{-1} was significantly lower from NTC (413.215 kg ha^{-1}). Similarly, due to drifted rate of dicamba, yield reduction in BB was 12% (998.61 kg ha^{-1}), its yield of which was significantly lower than its NTC (1131.22 kg ha^{-1}). Kruger et al (2012) showed that dicamba drift rate of 2.4 g ae ha^{-1} at the early vegetative stage can cause 10% yield loss and 5% flower loss. TOM18 had the least injury and no significant yield reduction due to drift of dicamba thus indicating the natural tolerance of dicamba and potential use of TOM18 in dicamba tolerant tomato breeding programs. BB, the commonly grown tomato cultivar was highly effected in terms of yield from the

simulated drift rate of dicamba. Thus, causing economic loss to commercial tomato producers.

For 2017, among the two glyphosate tolerant accessions, TOM87 had a yield reduction of 2% and also showed least injury of 8% (Table 3.7). TOM108 failed to produce any fruit as it is a facultative allogamous species, thus requiring hand-pollination hence, chances of producing fruits were very low (Greenleaf et al. 2006). The yield of BB was reduced by 9% and the average yield was significantly lower than NTC (1131.2 kg ha^{-1}). In 2016, yields from TOM87 (136.7 kg ha^{-1}) and BB (147.1 kg ha^{-1}) were not significantly different (Table 3.5). As indicated earlier, a drift rate of 32.5 g ae ha^{-1} is enough to cause a 5% flower loss at early vegetative stage (Kruger et al., 2012), and the drift of 5.8 g ae ha^{-1} of glyphosate can cause 10% yield loss at early bloom stage (Romanowski 1980). Moreover, drifted rates of glyphosate can cause shortening of pollen tubes, malformations in reproductive organs, and delay fruit ripening (Ovidi et al. 2001), which in turn may affect fruit yield. TOM87 can, therefore, serve as a potential source of glyphosate tolerant trait since only 2% yield reduction, with the least injury recorded.

Among the three accessions screened with quinclorac, in 2016, TOM129 produced the highest yield (204 kg ha^{-1}) that was significantly higher than BB (130 kg ha^{-1}) (Table 3.5). In 2017, TOM129 had a yield of 316.6 kg ha^{-1} which was similar to the non-treated (348.3 kg ha^{-1}) (Table 3.7). Also, TOM129 showed the least injury in both years; thus, indicating a potential source of quinclorac tolerant genes. The other tolerant accession, TOM410, failed to produce fruits in both years, for the same reason stated for TOM108. Yield reduction of 24% was recorded for BB; yield of NTC (1131.2 kg ae ha-1) was

significantly higher than treated (864.3 kg ha^{-1}). Lovelace et al. (2007) reported that a 6% injury must occur in tomato during the season before yields are significantly reduced, and a drift rate above 42 g ae ha^{-1} causes significant injury and yield reduction in tomato up to 50%. Additionally, the study reported that fruit yield was reduced by half when drift rate was increased ten times.

Height

In 2017, all BB accessions treated with auxin herbicides showed a significant decrease in height as compared to NTC, with the highest decrease recorded with AMCP herbicide. Among all the tolerant accessions only TOM54 (60 cm), when treated with AMCP, showed a significant decrease in height as compared to the control (69 cm) (Table 3.7). No correlation of height with injury or fruit yield was found. Gilreath et al. (2001) reported that the height of pepper plants was decreased from 27.4 to 21.2 cm when sprayed with 2,4-D at the rate 11.2 g ae ha^{-1}. To our knowledge, there has not been any other research examining the effect of drift on tomato height reduction.

Chlorophyll

No significant difference was recorded for chlorophyll content for any of the accessions, as compared to NTC (data not shown). Neil et al. (2004) sprayed Arabidopsis seedlings with the recommended rate of 2,4-D and did not observe any differences in chlorophyll content between treated and untreated plants.

Conclusion

Results from injury and fruit yield indicate TOM18 to be tolerant to simulated drift rate of dicamba, while TOM129 and TOM87 were tolerant to quinclorac and glyphosate drift rates, respectively. These three accessions show the least injury in addition to higher yield as compared to their non-treated checks, and Better Boy. Fruit yield in these three tolerant accession was not affected by simulated rates of their respective herbicides. TOM18 and TOM129, being cherry tomato biotype, performed the best among all other accessions used in this study, across all herbicides. They both belong to the same species *S.lycopersicum* var. cerasiforme which also consists of accessions reported to be resistant to leaf mold caused by *Cladosporium fulvum (Passalora fulva)* (Gallo et al., 2011). On the other hand, TOM87 belongs to *S.sitines*, a wild taxon of tomato which is least studied. To overcome herbicide limitations, protect them from herbicide drift and preserve or improve tomato quality and yield for growers, there is a distinct need to select tomato lines or varieties having a higher tolerance to label, as well as non-labeled herbicides with high efficacy on problematic weeds, thus expanding the herbicide label for tomato. The lines identified in this study can serve as a genetic resource for breeding herbicide tolerant tomato varieties to protect the crop from accidental injury caused by herbidie drift, thus increasing the marketable yield and fruit quality. Ultimately, growers will be able to grow tomatoes without worrying about herbicide drift from nearby fields.

Table 3.1 Year, seeding date and transplanting dates for Verona and Crystal springs research stations

Year	Seeding Date (Greenhouse)	Transplanting Date
2016	May, 10	Verona: June, 6 Crystal Springs: June, 8
2017	March 3	Verona: April, 12 Crystal Springs: April, 13

Table 3.2 Location, year and harvesting schedule for both the locations

Location (Year)	1st Harvesting	2nd Harvesting	3rd Harvesting
Verona (2016)	9/9	9/16	------**
Verona (2017)	6/30	7/6	7/14
Crystal Springs (2016)	9/5	9/12	-----
Crystal Springs (2017)	6/28	7/5	7/12

**In 2016 only two harvest were sufficient to pick up all the fruits

Table 3.3 Herbicide tolerant accessions used for the field trails along their place of origin

Accession	Herbicide tolerant to	Place of origin*
TOM45	2,4-D	Yangas to Canta, Lima, Peru
TOM12	Dicamba	Rio Atico, Km 26, Arequipa, Peru
TOM18	Dicamba	Los Banos,Philippines
TOM262	Dicamba	La Huarpia
TOM35	Dicamba	Huaraz- Caraz, Ancash, Paeru
TOM54	Aminocyclopyrachlor	USA
TOM129	Quinclorac	Kauai: Poipu, Hawaii, USA
TOM410	Quinclorac	Cajamarca, Peru
TOM108	Glyphosate	Rio Canete, Lima, Peru
TOM87	Glyphosate	USA

*Source: Tomato Genetic Resource Center

Table 3.4 Herbicide,mean injury (%) for accessions (tolerant and commonly grown), from 0 to 35 days after treatment (DAT) for the year 2016

DAT/Injury (%)						
Herbicide= 2,4-D						
Accession	35DAT (%)	28 DAT (%)	21 DAT (%)	14 DAT (%)	07 DAT (%)	0 DAT
TOM45	18 AB *	14 AB	10 B	8 B	4 B	0 B
Better Boy	29 ABC	27 ABC	26 ABC	15.0 BC	11 C	0D
Herbicide= AMCP						
Better Boy	43 AB	43 AB	41 AB	29 AB	19 BC	0 C
TOM54	43 A	44 A	38 AB	24 AB	22 AB	0 B
Herbicide= Dicamba						
TOM12	12 A	13 A	9 AB	9 AB	6 AB	0 B
TOM18	3 AB	3 AB	4 AB	8 A	7 A	0 B
TOM262	18 A	18 A	19 A	18 A	17 A	0 B
TOM35	22 AB	16 AB	13 AB	6 AB	3 B	0 B
Better Boy	10 A	10 A	11 A	10 A	8 A	0.B
Herbicide= Glyphosate						
TOM87	15 A	15 A	10 B	10 AB	9 AB	0 C
TOM108	27 BC	21 BCD	13 CDE	10 DE	7 DE	0 E
Better Boy	8 AB	8 AB	5 BC	6 BC	3 CD	0 D
Herbicide= Quinclorac						
TOM129	16 A	16 AB	15 A	16 A	13 A	0 B
TOM410	20 AB	18 AB	11 B	11 B	8 B	0 B
Better Boy	27 AB	27 AB	25 AB	18 AB	15 BC	0 C

*Means followed by same letter are not different from each other at 0.05 significance level.

Table 3.5 Herbicide, accession (tolerant and commonly grown), mean injury (%) and mean yield (kg ha^{-1}) for the year 2016

Herbicide	Accession	Mean injury (%) **	Mean Yield (kg ha^{-1})
2,4-D	TOM45	15 A*	114 B*
2,4-D	BB	22 A	150 A
Aminocyclopyrachlor	BB	35 A	155 A
Aminocyclopyrachlor	TOM54	32 A	106 B
Dicamba	TOM262	15 A	256 A
Dicamba	TOM18	3 C	146 AB
Dicamba	TOM12	9 B	------***
Dicamba	TOM35	14 A	------
Dicamba	BB	9 BC	147 A
Glyphosate	TOM87	11 A	137 A
Glyphosate	TOM108	20 A	---------
Glyphosate	BB	6 C	270 A
Quinclorac	TOM129	13 B	204 A
Quinclorac	TOM410	19 AB	----------
Quinclorac	BB	22 A	131 B

*Means followed by same letter are not different from each other at 0.05 significance level.

**Injury averaged across all the days for each accession

***No fruits produced by these accessions

Table 3.6 Herbicide,mean injury (%) for accessions (tolerant and commonly grown), from 0 to 35 days after treatment (DAT) for the year 2017

DAT/Injury (%)						
Herbicide= 2,4-D						
Accession	35 DAT (%)	28 DAT (%)	21 DAT (%)	14 DAT (%)	07 DAT (%)	0 DAT
TOM45	23 A*	21 AB	13 AB	12 AB	5 A	0 B
BB	34 A	33 AB	26 BC	22 CD	16 D	0 E
Herbicide= AMCP						
TOM54	38 AB	39 A	38 AB	32 AB	23 BC	0 C
BB	49 AB	42 BC	33 CD	27 D	18 DE	0 E
Herbicide= Dicamba						
TOM12	10 AB	13 AB	15 A	12 AB	8 ABC	0 C
TOM18	8 AB	11 A	10 AB	9 AB	11 A	0 C
TOM262	12 AB	13 A	14 A	17 A	17 A	0 C
TOM35	21 A	23 A	12 AB	10 B	9 B	0 C
BB	22 ABC	28 A	24 AB	22 ABC	13 CDE	0 E
Herbicide= Glyphosate						
TOM87	8 A	9 AB	10 BC	13 BC	11 C	0 D
TOM108	24 A	20 AB	17 B	10 C	5 C	0 D
BB	8 BC	10 B	18 A	14 A	10 B	0 E
Herbicide= Quinclorac						
BB	33 A	30 BC	23 BC	17 CD	13 D	0 E
TOM129	16 A	16 A	17 A	16 A	11 AB	0 C
TOM410	22 AB	23 A	16 AB	13 AB	10 AB	0 B

*Means followed by same letter are not different from each other at 0.05 significance level.

Table 3.7 Accession name (tolerant and commonly grown), herbicide, mean height (cm), mean yield (kg ha^{-1}), yield reduction (%) and mean injury (%) for the year 2017

Accession	Herbicide	Mean Height (cm)[1]	Mean Yield (kg ha^{-1})	Yield Reduction (%)[2]	Mean Injury (%)[3]
TOM45	2,4-D	44 B*	195 D*	25 A *	15 B*
TOM45	NTC	42 B	260 C	0 B	0 C
BB	2,4-D	59 B	884 B	22 A	22 A
TOM54	AMCP	60 B	131 C	59 A	35 A
TOM54	NTC	69 A	317 B	0 B	0 C
BB	AMCP	49 C	461 B	59	43 A
TOM35	Dicamba	60 B	-----	------**	10 B
TOM35	NTC	52 B	------	------	0 D
TOM18	Dicamba	57 B	186 E	5 A	10 C
TOM 18	NTC	59 B	190 E	0 B	0 D
TOM12	Dicamba	44 C	-------	-------	9 BC
TOM12	NTC	48 CD	--------	------	0 D
TOM262	Dicamba	45 C	366 D	11 A	12 B
TOM262	NTC	45 C	413 C	0 B	0 D
BB	Dicamba	57 B	999 B	12 A	16 A
TOM87	Glyphosate	55 B	137 C	3 A	7 CB
TOM87	NTC	60 B	141 C	0 C	0 C
TOM108	Glyphosate	31 D	------	------	15 A
TOM108	NTC	36 D	-------	------	0 C
BB	Glyphosate	64 A	1024 B	9 B	8 A
TOM129	Quinclorac	67 A	317 B	9 B	6 CD
TOM129	NTC	71 A	348 B	0 C	0 D
TOM410	Quinclorac	43 D	-------	--------	18 B
TOM410	NTC	48 CD	-------	--------	0 D
BB	Quinclorac	52 C	864 B	24 A	22 A
BB	NTC	66 AB	1131 A	0 C	0 D

[1] Height of five plants form a plot were averaged across all the days

[2] yield reduction relative to non-treated control

[3] Injury averaged across all the days for each accession

*Means followed by same letter are not different from each other at 0.05 significance level

** No fruits produced by these accessions, NTC= Non-treated control

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CHAPTER IV

GENETIC DIVERSITY AMONG TOMATO GENOTYPES WITH DIFFERENT HERBICIDE TOLERANCE LEVEL BASED ON MICROSATELLITES (SSR) MARKERS

Abstract

The United States is one of the world's leading producers of tomatoes, third only to China and India. Fresh and processed tomatoes account for more than $2 billion in annual farm cash receipts. In terms of consumption, the tomato is the nation's fourth most popular fresh-market vegetable behind potato, lettuce, and onion. To improve tomato through breeding and germplasm characterization, assessment of genetic diversity plays an important role. Thirty-five accessions (different in their tolerance to herbicides) were selected from our previous greenhouse study, and 18 SSR markers were used to analyze their genetic diversity. In DNA profiling, a total number of 81 alleles with an average of 4.5 alleles per locus were detected. Polymorphism Information Content (PIC) value ranged from 0.3074 to 0.778 with an average of 0.6289; while the average gene diversity over all SSR loci for the 35 genotypes was 0.6785, with varied from 0.3750 to 0.7917. The Unweighted Pair Group Method of Arithmetic Means (UPGMA) dendrogram constructed from Nei's (1978) genetic distance produced 6 distinct clusters for the 35 tomato accessions. Cluster analysis based on SSR markers separated tomato accessions into groups based on genetic relatedness which did not correspond to herbicide tolerance

level. Clusters 1, 2, 4, and 5 consisted of wild accessions, while cluster 3 was comprised of mostly cultivated tomato. Cluster 6 represented an equal number of wild and cultivated tomato accessions. Wild accessions were significantly more diverse than the cultivated accessions. Thus, results indicate that wild accessions can be used to diversify gene pool of cultivated tomato. Additional markers covering the whole genome of tomato needs to be used to characterize accessions based on herbicide tolerance level.
Key words: Microsatellites (SSR), herbicide tolerance, genetic diversity, wild germplasm, tomatoes

Introduction

Tomato is one of the most important vegetable crop because of its diverse use, nutrition, and taste (Fooland 2007). China, India and United States of America are the top three producers of tomato, averaged across years from 2010 to 2014. Americas in total accounts for 15.6% total production throughout the world from the year 2010 to 2014 (FAOSTAT 2014). In U.S. the two different industries for tomato include fresh and processing tomato. Processing tomato is usually grown under a contract between the processing industry and farmer, whereas fresh tomato is produced according to the demand in the open market. Hence, fresh tomato prices vary as compared to the processing tomato. California and Florida produced almost two-thirds of total fresh market tomato, making them the top two states (USDA 2016). There are numerous health benefits associated with tomato. The fruit is a rich source of vitamin A and C, minerals, and antioxidant (Nguyen et al., 1999), and fresh tomato provides 22 % RDA vitamin A, 47 % RDA vitamin C, with only 23 calories (Vinson et al., 1998). Among fruits and

vegetables, tomato ranks first as a source of minerals and vitamins in the U.S. diet (Rick 1980).

Today various varieties, shapes, and sizes of tomato are grown all across the globe. Cultivated tomato belongs to *Solanum* genus whereas the other twelve related wild species falls under the *Lycopersicon* genus (Rick 1979). Tomato originated in the Andean region which includes Bolivia, Ecuador Chile, Colombia and Peru (Rick 1976). They were first introduced in the sixteenth century to Europe from Southern and Central America, where they were grown as ornamental plants (McCue 1952), as they were considered poisonous and not fit for human consumption. It was first cultivated as a plant in Italy referred by Saccardo. Two centuries later, the tomato was successfully grown in Italy, France, and Spain (Soressi 1969, Esquinas-Alcazar et al., 1995), and from Europe, it was introduced into North America during the 18th century (Rick 1976). However, a few morphological characteristics related to the shape of fruit became prominent in North America and Europe. In European countries, ribbed and flat angled, pear and heart shaped, elongated and plum forms of tomato were commonly consumed (Noble 1994); whereas, in North America, the solid, smooth and globular fruit was in higher demand. Some of these fruit types were prevalently used, even until now, for commercial production in their respective regions (Ruiz et al., 2005).

The habitat of *Lycopersicon* species are highly variable, ranging from very wet to very dry, from mountainous to coastal regions, thus making these species highly variable, genetically and morphologically (Warnock 1988). In order to improve the crop for tolerance to various abiotic and biotic stresses, germplasm diversity plays a crucial role. For example, QTLs conferring cold tolerance (abiotic stress) during seed germination

were mapped in a BC_1F_2 line developed from a cross between wild (*L*. pimpinellifolium) and cultivated tomato (Foolad et al., 1998). QTLs associated with early blight (biotic stress) was mapped in the wild tomato, *L. hirsutum* (Foolad et al., 2002). To improve the yield, color, and total soluble solids, genes were introgressed from a wild tomato, *S. habrochaites*, to cultivated tomato (Fooland 2007). The Tomato Genetics Resource Center (TGRC) at the University of California in Davis contain more than 2,750 tomato lines which include wild, abiotic, and biotic stress tolerant lines, in addition to mutant lines. These lines can serve as an important source of variation that can be used for the improvement of the crop.

Domesticated tomatoes are explicitly different from their wild relative because of natural selection and constant breeding to select for traits such as fruit shape and size. This domestication has resulted in the narrow genetic variation of cultivated tomato, a process also referred to as the 'domestication syndrome' (Bauchet et al., 2012). Rick, in 1976, mentioned that the domestication of tomato in different parts of the world rather than in its natural place of origin has caused a narrow genetic basis. Molecular analyses show that genetic diversity among cultivated tomato varieties is very low as compared to the other self-compatible, autogamous species (Broun et al., 1996). Miller et al. (1990) reported that cultivated tomato has less than 5% allelic diversity as compared to its wild relatives. With changes in the climate and environment, there is a need to develop crop varieties that can withstand these changes. In nature, interspecific hybridization between wild species, *S. pimpinellifolium, and S. lycopersicum*, was shown to enrich the gene pool in Ecuador and Peru (Campbell 1946). To enlarge the genetic basis in climate changing conditions first, we need to find out the genetic diversity in the tomato with the help of

wild germplasm and then introgression of the desirable traits through breeding in cultivated tomatoes (Singh 2007). The most preferred method for estimating genetic diversity is using molecular markers. As compared to morphological (root and shoot markers) and biochemical markers, molecular markers provide detailed information about the genetics of the plant (Sudre et al., 2007, Goncalves et al., 2009). Moreover, molecular marker techniques are easy to use, reproducible, and with the advent of whole genome sequencing, it has become easier to understand the genetic diversity at base pair level (Souza et al., 2008, Goncalves et al., 2008). With the help of polymerase chain reaction (PCR) and molecular markers, map-based cloning of agronomically important genes, genetic diversity studies, phylogenetic analysis, and marker-assisted breeding, has become possible (Saker et al., 2005). There are different types of molecular markers used in genetic diversity studies, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), and single nucleotide polymorphism (SNP). Among these markers, SSRs are widely used in different plant breeding and genetic studies as they have co-dominant inheritance, are highly reproducible, multiallelic in nature, cheap, time efficient and provides good genome coverage (Jiang 2013). SSR markers are segments of DNA consisting of tandemly repeating penta-, tetra-, tri-, di-, and mono-nucleotide units, and usually contain repeats with 1 to 10 base pairs (Powell et al., 1996). SSR markers are thus widely used in tomato genetic diversity studies.

Benor et al. (2008) evaluated the genetic diversity of 39 inbred tomato lines collected from USA, China, South Korea, and Japan, and classified them as determinate and indeterminate type. The study used 60 SSR markers of which 41 of them were

polymorphic. They concluded that there were 150 alleles with moderate levels of diversity, average polymorphism information content (PIC) was 0.31, and the average genetic similarity among inbred lines was 0.71 with values ranging from 0.45 to 0.98. Unweighted pair group method with arithmetic mean (UPGMA) classified these inbred lines into four clusters. Lines of the same origin were clustered together indicating their genetic similarity. The study, therefore, suggests the potential of wild/exotic lines as an important genetic resource for increasing genetic diversity of cultivated tomato. Zhou et al. (2015) performed a study with 13 EST-SSR and 15 SSR markers combined with morphological traits to access genetic diversity in 29 cultivated, 14 wild, and 7 introgression tomato lines. According to morphological traits analyses, all 50 tomato lines were categorized into 4 clusters. SSR markers detected a total of 64 alleles whereas EST-SSR markers detected 52 alleles. The dendrogram analysis clustered them into 8 different groups in which wild were in 7 clusters and the other consisted of cultivated and introgression lines in the same cluster. Wild lines showed lower similarity coefficient of 0.627 than cultivated lines (0.845), thus indicating a lower genetic diversity in cultivated as compared to wild lines.

Materials and Methods

Plant Materials

From our previous greenhouse study to screen for herbicide tolerance among a diverse germplasm of tomato, we classified tomato accessions into three groups based on their injury: tolerant (T) with <20% injury, intermediate tolerant (I) with 20-80% injury, and susceptible (S) with 80-100% injury. We selected a total of 35 different accessions for our genetic diversity analysis, which includes the 9 major taxa of the wild relative of

tomato (Table 4.1). These accessions have different tolerance/susceptible level for seven different herbicides (Table 4.2).

DNA Extraction

Leaf samples were collected from 4-5 leaf stage tomato plants and stored at-80° C until use. Total genomic DNA was extracted from the harvested leaf tissues, using a modified hexadecyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1990). Briefly, about 0.1 g of leaf tissue was placed in a 2 mL Precellys® tube containing 4-5 ceramic beads, and homogenized into a fine powder in Precellys® Evolution (Bertin Technologies, USA). Following homogenization, 500µL of CTAB buffer (containing 100Mm Tris-HCL, 2 M NaCl, 2 % CTAB, 20Mm EDTA, 2 % polyvinylpyrrolidone-40 and 0.003 beta-mercaptoethanol) was added into the Precellys® tube. The tube was then mixed thoroughly, vortexed for 60 seconds, and then incubated in water bath for 45 minutes at 55° C. To remove the protein contaminants from the cell lysis, an equal volume of chloroform isomyl-alcohol (500 µL) was added, tubes were mixed gently by inverting 5-6 times, and then centrifuged at 12,000 rpm for 10 min. After centrifugation, tubes were separated into three layers. The uppermost transparent layer containing the DNA was carefully transferred to new tube, and an equal volume of absolute isopropanol, stored at-20° C, was added and gently mixed by inverting 5-6 times. Tubes were incubated overnight at -80° C, followed by centrifugation at 12000 rpm for 10 min. Supernatant was then discarded, DNA pellet was washed with absolute ethanol (500 µL), air dried, resuspended in 50 µL of 1X TE buffer (10 mM Tris-HCl, 1mM EDTA), and stored at -20° C for further use. The DNA quantity and quality was measured using a Nanodrop 2000 (Wilmington, USA) spectrophotometer.

Polymerase chain reaction with SSR primers

Eighteen different primers were selected from genetic diversity studies in tomato (Solomon et al., 2008, Korir et al., 2014) (Table 4.3). DNA amplification was carried out in 0.2 mL tubes containing a total reaction volume of 25 µL. The PCR reaction contained 200 ng DNA, 0.4 mM dNTPs, 25U/mL Taq DNA polymerase (New England Biolabs), 3 mM $MgCl_2$, 1µM of each forward and reverse primers (Table 4.4). All reactions were prepared in a 96-well PCR plate, and subjected to thermal profile mentioned in Table 4.5, in a BioRad MyCycle Thermocycler (BioRad, CA, USA). The PCR products were electrophoresed in a 6% denaturing polyacrylamide gel at 180V for 70 min. Gels were stained with ethidium bromide, and bands were photographed.

Data Analysis

Cross Checker 2.91 (Buntjier, 1999) was used to score the individual bands from the gel as codominant markers. Data were entered into a binary matrix as discrete variables, 1 for presence and 0 for the absence of the band. The binary matrix was used to estimate the observed alleles (na), effective alleles (ne), number of alleles per locus (A), percentage of polymorphic loci (P), genetic distance (D), Shannon's index (I), and Nei's gene diversity (h)/heterozygosity using POPGENE software version 1.32.(Yeh at al., 1997). The heterozygosity (H) of a locus is defined as the probability that an individual is heterozygous for the locus in the population, which is calculated as:

$$H = 1-\sum_{i=1}^{l} P_i^2 \qquad \text{(Eq. 4.1)}$$

Where P_i is the frequency of the i^{th} allele among a total of l alleles. Another important parameter which provides an estimate of the discriminating power of the marker is polymorphism information content value (PIC). It is defined as the probability that the

marker genotype of a given offspring will allow the deduction, in the absence of crossing over of which of the two marker alleles of the affected parents it received. It can be calculated as:

$$\text{PIC}=\sum_{i=1}^{l} P_i^2 - \sum_{i=1}^{l-1}\sum_{j=i+1}^{l} 2P_i^2 P_j^2 \qquad \text{(Eq. 4.2)}$$

Where Pi and Pj are the population frequency of the i^{th} and j^{th} allele. PIC values above 0.5 indicate highly polymorphic loci whereas values between 0.25 and 0.5 are considered moderately informative and PIC values less than 0.25 are considered uninformative (Botstein et al. 1980). The genetic cluster analysis was conducted with UPGMA algorithm and dendrogram was constructed using Tree Viewer by NCBI (https://www.ncbi.nlm.nih.gov/tools/treeviewer/). Population structure for herbicide tolerance level was determined using STRUCTURE 2.3.3 software with the Bayesian clustering approach that divided the accessions into three populations. A total of 10,000 loci were randomly chosen for each analysis to accommodate capacity limitations in STRUCTURE. Original analyses were run from K = 1 to K = 10, with four runs per K value, 100,000 burn-in period, and 500,000 replications. The best-fit K value was determined using Structure Harvester (Earl, 2012) by assessing ΔK and maximum likelihood scores.

Results and Discussions

Marker analysis

All 18 SSR markers produced a total of 81 alleles with an average of 4.5 alleles per locus (Table 4.6). The length of the fragment generated by these markers ranges from 100 to 450 base pairs (bp). Locus SLR50, SLR19, and Tom236-237 produced the highest number of alleles (6 alleles) whereas locus SLR21 produced the least number of alleles (2

alleles). Similar to our findings, Bredemeijer et al., (2002) reported 2 to 8 alleles per locus with an average of 4.7 alleles per locus in 521 tomato varieties. Garcia-Martinez et al. (2006) reported that a number of SSR alleles detected with 19 markers in 48 tomato accessions ranged from 2 to 10, with all the 19 markers being polymorphic. He et al. (2003) reported 2 to 6 alleles for each locus, with 65 SSR loci in 19 tomato accessions. Kwon et al. (2009) evaluated 63 varieties of tomato with 33 SSR markers and identified a total of 132 alleles with an average of 4 alleles. In the current study, the SSR allele number ranged from 2 to 6 with an average of 4.5 alleles per locus, which was similar to studies indicated above. SSR markers with a higher number of alleles per locus showed the lowest frequency of the predominant allele. Thus, markers with a lower frequency of the predominant allele have more differentiation ability than other markers (Moghaddam et al., 2009). A Higher number of alleles per locus observed in this study is an indication of allelic variants per locus. Tomato accessions used in this study were thus genetically diverse. Muñoz et al. (2010) reported that lower number of alleles with microsatellite markers could be related to the origin of the plant material and its genetic diversity. Thus, a small number of alleles can be explained by a narrow geographical collecting area and low genetic diversity.

Genetic Diversity

Shannon's index (I) and Gene diversity (h) are most commonly used indices for measuring genetic variation (Nei, 1978). Shannon's index is a measure of the degree of uncertainty in determining which species an individual would belong to if randomly picked from a group of species, while gene diversity is a measure of expected heterozygosity, higher values of gene diversity and Shannon's index would indicate

higher genetic diversity. The mean Shannon information index was 1.2939 with values ranging from 0.5623 to 1.7087. Overall gene diversity for all the locus was 0.6785, and among all markers, Tom236-237 showed highest gene diversity with an H-value = 0.7917, whereas marker SLR21 showed lowest gene diversity with H-value of 0.3750. Similar gene diversity values were reported by other studies. Rao et al. (2012) used 48 SSR markers on 322 accessions of *Solanum pimpinellifolium* and reported an overall gene diversity of 0.7122; while, Aguirre et al., (2017) measured a gene diversity of 0.6946 among 30 wild tomato accessions, using 36 SSR markers.

PIC is regarded as a valuable tool to evaluate the differentiation ability of markers within the population. It is a measure of informativeness related to expected heterozygosity and is calculated from allele frequencies (Osei et al., 2012). The results of our study imply that the loci have high polymorphism as PIC values ranged from 0.3074 to 0.7780. Thus, SSR markers used in this study were efficient in discriminating the species. The value of PIC is a function of detected alleles and the distribution of their frequency. Therefore, markers with more alleles and low allele frequency had larger PIC as found in SLR19 (6 alleles and the highest PIC of 0.7778) indicating a better distinction of the accessions. These results confirm the utility of PIC as a measure of the capacity of a marker to discriminate among closely related individuals as also reported by Prevost et al., (1999) and Escandon et al., (2007). Marker SLR19 had highest PIC value of 0.7778 whereas SLR21 had the lowest value of 0.3074. PIC values showcased that the markers used in the study were highly informative. The average PIC value in this study was 0.6289 which was in the range from 0.31 to 0.78 reported by Korir et al. 2014, Benor et al. 2008 and Garcia-Martinez et al. 2006.

Cluster analysis

Cluster analysis based on SSR markers divided tomato accessions into six groups and several sub-groups according to genetic relatedness, however, none of the groupings were highly associated with herbicide tolerance trait (Figure 4.1), thus indicating that the markers used in this study were not strongly related to herbicide tolerance trait. Moreover, the 18 SSR loci used in this study were distributed over 10 chromosomes with only 1-2 loci on each chromosome, thus resulting in a low probability of association with herbicide tolerance. As of today, no specific markers have been reported in tomato, associated with tolerance to the herbicide. Markers used in the present study were primarily selected from genetic diversity studies hoping to find associating with herbicide tolerant phenotypes. Results from STRUCTURE (Figure 4.2) also indicate that all three herbicide tolerant groups (susceptible, intermediate tolerant, and tolerant) have a similar genetic background.

Cluster 1 and 2 comprised of all wild species expect POP 21 in cluster 1 and POP22 in cluster 2, both belonging to same species as cultivated tomato. All wild accessions in cluster 1 belong to the same place of origin, Peru, and the majority of these accessions belong to *S. pennelli*, one of the most stress tolerant wild species of tomato (Bolger et al., 2014). Similarly, all wild accessions in the cluster 2 also originate from Peru but are of *S. pimpinellifolium* species. All accessions in Cluster 3 consists of cultivated tomato (*S. lycopersicum)* and are highly susceptible to herbicide drift (80-100 % injury). Cluster 4 contained all wild accessions belonging to different species such as *S. galapagense, S. cheesmaniae, S. chilense* and *S. chmielewskii*; while cluster 5 and 6 contained a mixture of wild and cultivated tomato. All of the cultivated tomato accessions

in cluster 6 were that of cherry tomato biotype (*S. lycopersicum var. cerasiforme*), which is considered to be an admixture of wild and cultivated tomato rather than a cultivated tomato (Ruiz et al., 2005). Cluster groupings were therefore primarily based on the species composition. Similar results were reported by Zhou et al. (2015) and Frary et al. (2005) using the markers included in our study. Wild and cultivated tomato separated into different clusters and indicated high genetic variation with respect to markers; gene diversity for wild accessions was 0.722 whereas for the cultivated tomatoes it was 0.611 (Zhou et al. 2015). The germplasm included in this study can act as a genetic source of novel abiotic or biotic stress tolerant genes. Moreover, the wild germplasm used in the study can be used to enhance the genetic diversity in cultivated tomatoes.

Conclusions

Plant breeders often have to deal with the arduous tasks of genetic improvement in crops for tolerance to biotic/abiotic stress when the detailed mechanisms are not well characterized. One of the most commonly used approaches in molecular breeding is the selection with the help of molecular markers linked to the QTLs underlying physiological or agronomical performance under stress when candidate gene(s) are not available. The QTLs controlling abiotic stress tolerance such as salt tolerance have been identified in tomato using molecular markers (Breto et al., 1994). Although this approach remains promising, its application to complicated traits such as herbicide tolerance in terms of physiological characteristics may be limited due to large sample size required for screening in segregating populations, and possible significant interactions between genotype and environment for QTL analysis. In our present study, the selected genotypes did not classify into different herbicide tolerant categories, using the markers selected for

this study. However, we observed an association of these 18 SSR markers with wild and cultivated tomato. Additionally, all markers in this study were informative according to their PIC values. These markers may be useful in screening for herbicide tolerance in tomato germplasm, but the number of genotypes used for microsatellite clustering was relatively small. Thus, there is need to use a bigger population and larger number of markers to increase our chances of identifying markers related to herbicide tolerance trait.

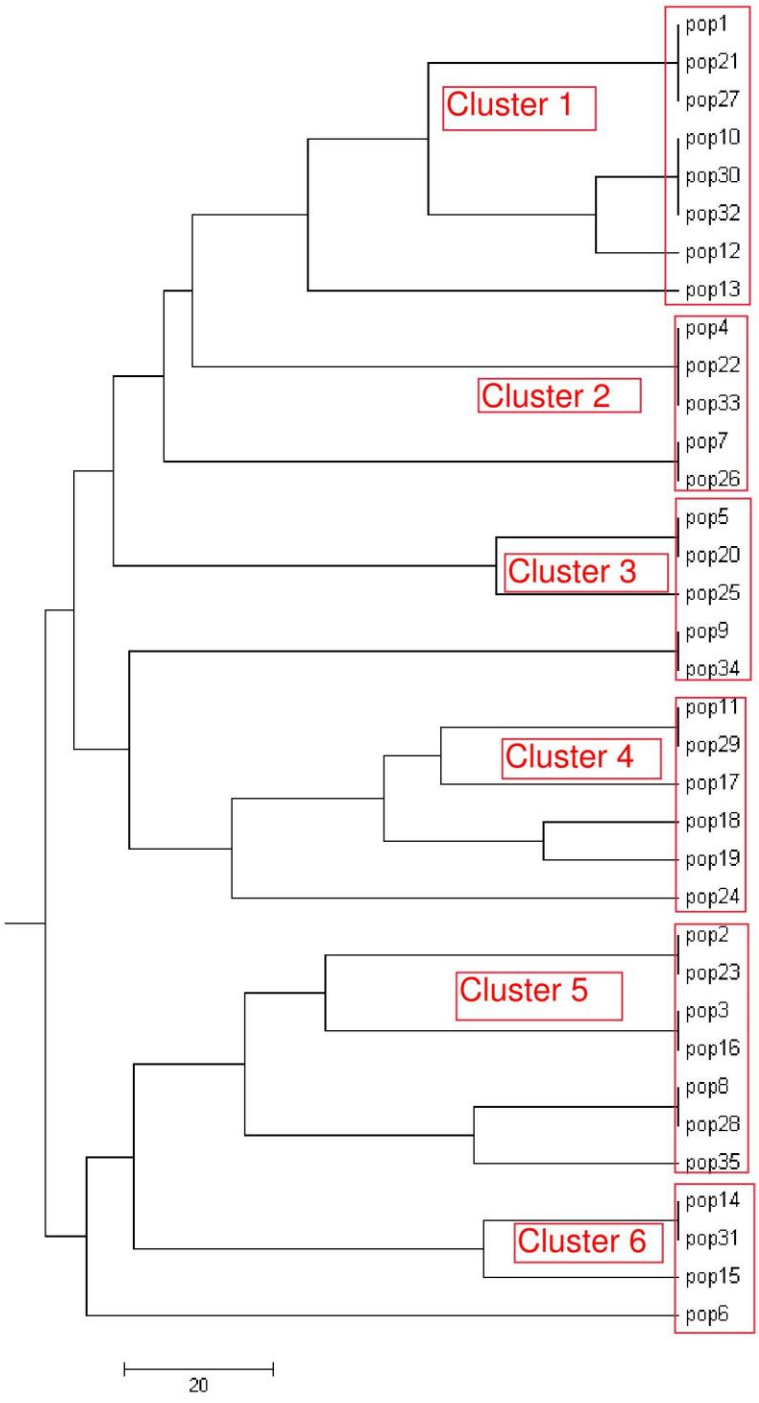


Figure 4.1 UPGMA-based dendrogram of 35 tomato accessions based on polymorphisms of 18 SSR markers, using Neis genetic distance

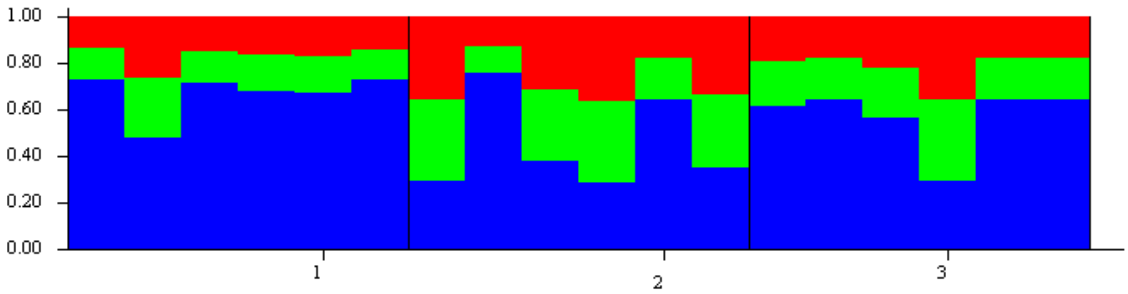
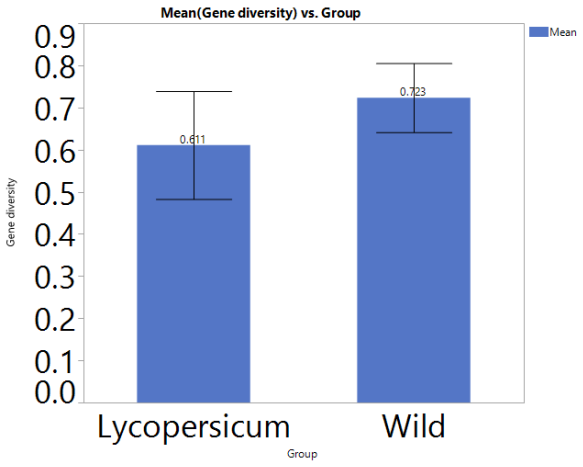


Figure 4.2 Population structure of 35 tomato lines when divided into three sub-populations.

Where 1 is for susceptible, 2 for intermediate tolerant and 3 for tolerant using the model-based program STRUCTURE. Results shown are for K=3 and 3 subpopulations. *Y*-axis in figure indicates the estimated membership coefficients for each individual



New

Figure 4.3 Variation in genetic diversity among wild and cultivated tomatoes presented by different gene diversity values

Table 4.2 Dendrogram coding, accession, taxon and place of origin

Dendrogram coding	Accession	Taxon	Place
POP11	TOM60	*S. galapagense*	Ecuador
POP1	TOM53	*S. peruvianum*	Peru
POP27	TOM54	*S. arcanum*	Peru
POP29	TOM59	*S. cheesmaniae*	Ecuador
POP17	TOM19	*S. chilense*	Peru
POP18	TOM64	*S. chmielewskii*	Peru
POP19	TOM66	*S. chmielewskii*	Peru
POP13	TOM108	*S. corneliomulleri*	Peru
POP24	TOM107	*S. corneliomulleri*	Peru
POP14	TOM45	*S. habrochites*	Ecuador
POP15	TOM70	*S. huaylasense*	Peru
POP2	TOM69	*S. huaylasense*	Peru
POP16	TOM92	*S. lycopersicum*	Hawaii
POP20	TOM38	*S. lycopersicum*	Edkawi
POP21	TOM6	*S. lycopersicum*	U.S.A
POP22	TOM94	*S. lycopersicum*	Venezuela
POP23	TOM30	*S. lycopersicum*	USA
POP25	TOM95	*S. lycopersicum*	Hawaii
POP28	TOM82	*S. lycopersicum*	India
POP3	TOM96	*S. lycopersicum*	Hawaii
POP34	TOM9	*S. lycopersicum*	Ecuador
POP5	TOM81	*S. lycopersicum*	India
POP9	TOM72	*S. lycopersicum*	Mexico
POP31	TOM129	*S. lycopersicum var. cerasiforme*	Poipu
POP6	TOM44	*S. lycopersicum var. cerasiforme*	Malintka 101
POP10	TOM17	*S. pennellii*	Peru
POP30	TOM63	*S. pennellii*	Peru
POP32	TOM3	*S. pennellii*	Peru
POP12	TOM12	*S. pimpinellifolium*	Peru
POP26	TOM14	*S. pimpinellifolium*	Peru
POP33	TOM15	*S. pimpinellifolium*	Peru
POP35	TOM51	*S. pimpinellifolium*	Peru
POP4	TOM49	*S. pimpinellifolium*	Peru
POP7	TOM39	*S. pimpinellifolium*	Peru
POP8	TOM13	*S. pimpinellifolium*	Peru

Table 4.3 Enlist all the 35 accessions according to the herbicide tolerance level for seven different herbicides,

Accession	2,4-D	Dicamba	Quinclorac	Glyphosate	Aminocycloparachlor	Aminopyralid	Picloram
TOM81	I	S	I	S	S	S	S
TOM38	I	I	I	I	S	S	S
TOM12	T	I	I	S	S	S	S
TOM14	T	T	S	S	S	S	S
TOM94	S	S	S	S	S	S	S
TOM13	T	T	I	S	I	I	I
TOM45	T	I	I	T	I	I	I
TOM6	S	S	S	S	S	S	S
TOM53	S	S	S	S	S	S	S
TOM51	I	I	I	I	S	S	S
TOM95	S	S	S	S	S	S	S
TOM108	I	I	I	T	I	I	S
TOM72	S	S	S	S	S	S	S
TOM70	S	S	S	S	S	S	S
TOM15	S	S	S	S	S	S	S
TOM39	I	I	I	I	I	I	I
TOM69	S	S	S	S	I	S	S
TOM9	I	I	I	S	I	S	S
TOM49	S	S	S	S	I	S	S
TOM59	S	S	S	S	S	S	S

Table 4.3 (Continued)

TOM30	S	S	S	S	S	S	S
TOM129	S	I	T	S	I	I	I
TOM64	S	S	I	T	I	S	S
TOM96	S	S	S	S	S	S	S
TOM17	T	T	T	S	I	T	I
TOM19	S	S	S	S	S	S	S
TOM66	I	I	T	S	I	I	S
TOM54	I	I	I	I	I	I	I
TOM60	I	T	I	S	S	I	S
TOM92	S	S	S	S	S	S	S
TOM63	I	I	S	S	S	S	S
TOM3	I	T	I	S	I	I	S
TOM107	S	S	S	T	S	S	S
TOM82	I	I	I	S	T	S	S
TOM44	I	T	I	S	T	S	I

*where T=tolerant, I= Intermediate tolerant and S= susceptible

Table 4.4 PCR reaction components for all the 18 SSR markers

S. No.	Reagents	Concentration	Quantity
1.	DNA template	200ng/µL	1 µL
2.	Nuclease free water	---------	9.5 µL
3.	Taq polymerase	25U/ml	12.5 µL
4.	Forward Primer	1µM	1 µL
5.	Reverse primer	1µM	1 µL
		Total	25 µL

Table 4.5 New PCR temperature profile

Steps	Cycles	Temperature	Duration
Initial Denaturation	1	94°C	5 minutes
Denaturation	35	94°C	40 seconds
Annealing	35	55-60°C	1 minutes
Extension	35	72°C	1 minutes
Final Extension	1	72°C	10 minutes

Table 4.6 List of all the prime sequences along with their annealing Temperature (°C)

Primer Name	Primer Sequence (5' to 3')	Annealing Temperature (°C)	Reference
AI773078	F: GAT GGA CAC CCT TCA ATT TAT GGT R: TCC AAG TAT CAG GCA CAC CAG C	55	Solomon et al., 2008
AW034362	F: CCG CCT CTT TCA CTT GAA C R: CCA GCG ATA CGA TTA GAT ACC	55	Solomon et al., 2008
AI895126	F: GCT CTG TCC TTA CAA ATG ATA CCT CC R: CAA TGC TGG GAC AGA AGA TTT AAT G	55	Solomon et al., 2008
SSR50	F: CCG TGA CCC TCT TTA CAA GC R: TTG CTT TCT TCT TCG CCA TT	55	Solomon et al., 2008
Tom236-237	F: GTT TTT TCA ACA TCA AAG AGC T R: TGC AAA GAA CAA AGA CCG TG	55	Solomon et al., 2008
SLR4	F: ACT GCA TTT CAG GTA CAT ACT CTC R: ATA AAC TCG TAG ACC ATA CCC TC	56	Korir et al.,2014
SLR10	F: AGA ATT TTT TCA TGA AAT TGT CC R: TAT TGC GTT CCA CTC CCT CT	58	Korir et al.,2014
SLR13	F: GCC ACG TAG TCA TGA TAT ACA TAG R: GCC TCG GAC AAT GAA TTG	60	Korir et al.,2014
SL1R15	F: GGA TTG TAG AGG TGT TGT TGG R: TTT GTA ATT GAC TTT GTC GAT G	60	Korir et al.,2014

Table 4.6 (Continued)

SL16	F: CGG CGT ATT CAA ACT CTT GG R: GCG GAC CTT TGT TTT GGT AA	58	Korir et al.,2014
SLR18	F: CGA TTA GAG AAT GTC CCA CAG R: TTA CAC ATA CAA ATA TAC ATA GTC TG	58	Korir et al.,2014
SLR19	F: AGC CAC CCA TCA CAA AGA TT R: GTC GCA CTA TCG GTC ACG TA	58	Korir et al.,2014
SLR2	F: TGT TGG TTG GAG AAA CTC CC R: AGG CAT TTA AAC CAA TAG GTA GC	56	Korir et al.,2014
SLR21	F: CCT TGC AGT TGA GGT GAA TT R: TCA AGC ACC TAC AAT CAA TCA	58	Korir et al.,2014
SLR22	F: TTG GTA ATT TAT GTT CGG GA R: TTG AGC CAA TTG ATT AAT AAG TT	52	Korir et al.,2014
SLR23	F: ACA AAC TCA AGA TAA GTA AGA GC R: GTG AAT TGT GTT TTA ACA TGG	54	Korir et al.,2014
SLR26	F: AAC GGT GGA AAC TAT TGA AAG G R: CAC CAC CAA ACC CAT CGT C	60	Korir et al.,2014
SLR 27	F: ATT GCT CAT ACA TAA CCC CC R: GGG ACA AAA TGG TAA TCC AT	60	Korir et al.,2014

Table 4.7 Observed number of alleles, gene diversity, PIC and Shannon Information index for all 18 markers

Locus Name	Observed number of alleles (na)	Gene diversity (H)	PIC	Shannon Information index (I)
SSR15	4	0.6667	0.6071	1.2149
A1895126	4	0.5969	0.5275	1.0575
A1773078	4	0.6667	0.6089	1.2106
SLR13	5	0.7449	0.7036	1.4701
SLR16	4	0.5663	0.5162	1.0372
SLR18	4	0.7041	0.6499	1.2914
AW034362	3	0.5312	0.4683	0.9003
SLR50	6	0.7864	0.7523	1.6184
SLR10	5	0.7701	0.7307	1.5137
SLR19	6	0.8058	0.7778	1.7087
SLR2	4	0.6653	0.5999	1.1840
Tom236-237	6	0.7917	0.7608	1.6569
SLR22	4	0.6600	0.5958	1.1935
SLR23	5	0.7654	0.7272	1.5230
SLR4	5	0.7044	0.6518	1.3435
SLR21	2	0.3750	0.3074	0.5623
SLR26	5	0.6283	0.5871	1.2366
SLR27	5	0.7840	0.7487	1.5671
Mean	4.500	0.6785	0.6289	1.2939

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