TOMATO IMPROVEMENT FOR FRUIT QUALITY AND DISEASE RESISTANCE WITH INTEGRATED APPROACH AT NCSU: AN OVERVIEW

Dilip R. Panthee

Department of Horticultural Science, North Carolina State University, USA

ABSTRACT

Tomato (Solanum lycopersicum L.) is considered as the model plant for genetic analysis associated with root, shoot and fruit development. This is also the second most economically important crop after potato as shown by the estimated global production of about 164 million mt contributing $2.36B to the world economy. The USA ranks second in the world production after China. Efforts to improve the fresh market tomato for fruit quality and disease resistance is continued to pyramid the resistance genes and add quality traits by using conventional and molecular breeding approach. From replicated trials conducted at Mountain Horticultural Crops Research and Extension Center, Mills River, NC over the years, several hybrids and breeding lines have been released and there are more in the pipeline. Important genes conferring resistance to Fusarium wilt race 3 (I-3), late blight (Ph-2 and Ph-3) Verticillium wilt (Ve), tomato spotted wilt virus (Sw-5), and root knot nematode (Mi) are introgressed into the breeding lines improving the disease resistance. In this presentation, some of the breeding efforts combining these disease resistance and fruit quality related traits will be presented. Seed production approach after the development and release of hybrid variety is discussed.

Introduction

The tomato (Solanum lycopersicum L.) is one of the most widely consumed vegetables throughout the world. It is consumed as fresh and processed product such as juice, soup, ketchup, sauces, puree and paste. While China is the leading producer of tomato, with the total production of 1.68 million mt fresh market tomato, the U.S. is second in the total tomato production producing 1.67 million mt in the world (FAOSTAT, 2014).

As an economically important crop, significant research attention and breeding efforts from both private and public sectors have led to the development of a considerable number of improved varieties. Major emphasis of these programs has been on fruit quality characteristics including firmness and size, disease resistance and toward increasing yield. Significant improvement in these areas has been realized. However, tomato flavor has not been of major concern of breeding programs and consequently, many of the modern varieties lack the distinctive flavor that consumers associate with fresh garden-grown heirloom varieties. Flavor is one of the most highly demanded consumer traits of tomato at present, and the lack of good flavor is one of the most commonly heard complaints associated with modern varieties of tomato. In this presentation, we present some of the on-going tomato improvement efforts on various aspects using conventional and molecular approaches that have practical application.Overall breeding objectives consist of improving tomato for disease resistance, fruit quality and heat stress tolerance at North Carolina State University. We will discuss some of the improvement efforts on diseases and fruit quality in this presentation.

Breeding for Disease Resistance

Fusarium Wilt

Fusarium wilt is a vascular disease of tomato caused by a soil-borne fungal pathogen Fusarium oxysporum f. sp. lycopersici. (Fol). The disease is initiated with yellowing of a leaflet or shoot, followed by wilting and yellowing of more leaves, dropping of wilted leaves, and ultimately death of plants before maturation.Three physiological races of this pathogen have been reported as of now i.e. race 1, 2, and 3 to cause fusarium wilt in tomato. Corresponding this, three fusarium wilt resistance genesI, I-2, and I-3have beenidentified in PI 79532, PI 126915, and LA716, respectively (Alexander and Tucker, 1945).Although I and I-2 have been used most often for breeding purposes (Scott, 2008), currently I-3 is a preferred resistance gene because race 3 is becoming more common than before and also few tomato cultivars are available with I-3 resistance gene. In most of the NC State University breeding materials, we have already introgressed I and I-2 genes. Molecular markers have been utilized along with conventional screening to introgress the I-3 gene. Mountain Honey as well as Mountain Vineyard are the hybrid grape tomato varieties developed from NC State University recently(Panthee and Gardner, 2013a; Panthee and Gardner, 2013b). Mountain Honey was screened with race 3 of fusarium wilt in growth chamber and the results were verified with molecular markers, which were found consistent (Fig 1 and 2).

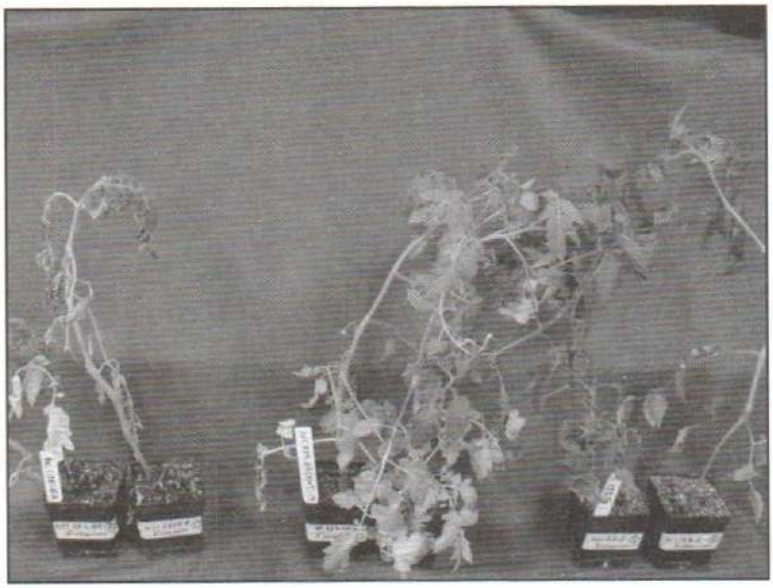

Fig. 1: Screening of Mountain Honey (NC 10242) for Fusarium wilt race 3 resistance in the growth chamber at MHCREC, Mills River, NC. In the picture, Mountain Honey (center) was not different from NC 123S (resistant control on right) whereas NC 1CELBR (susceptible control on left) was almost dead.

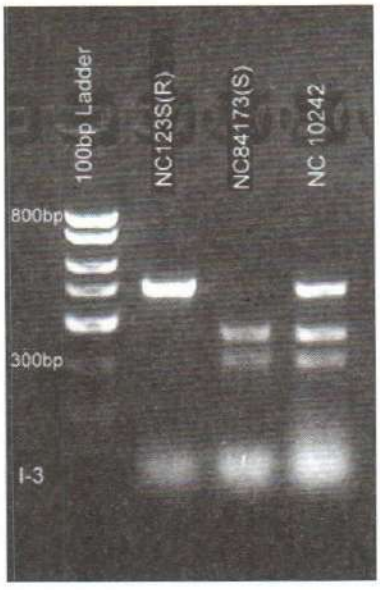

Fig. 2: Molecular screening of Mountain Honey (NC 10242) for Fusarium wilt race 3. In the picture, NC 123S is homozygous resistant for I-3, NC84173 is susceptible and Mountain Honey is heterozygous showing double bands.

Late Blight

Late blight (LB), a serious and destructive disease of tomato is caused by oomycete Phytophthora infestans (Mont.) de Bary. It is believed to have been originated from the Andean region, which is also the center of origin of tomatoes and potatoes (Foolad et al., 2008) and then distributed throughout the world.

Three major genes, Ph-1, Ph-2 and Ph-3, conferring resistance to the late blight, have been identified and mapped to tomato chromosomes 7 (Peirce, 1971), 10 (Moreau et al., 1998), and 9 (Chunwongse et al., 2002), respectively. Ph-1 provides resistance only to race T-0, which is currently not of importance. Ph-2, a single incompletely-dominant gene provides partial resistance to several isolates of race T-1, however, it often fails in the presence of more aggressive isolates. Ph-3 is a much stronger resistance gene, conferring incompletely-dominant resistance to a wide range of P. infestans isolates of tomato, including those that overcome Ph-1 and Ph-2(Chunwongse et al., 2002).

There were co-dominant cleaved amplified polymorphic sequence (CAPS) markers in use for marker-assisted breeding for Ph-2 and Ph-3(Robbins et al., 2010). Recently, we developed new molecular markers associated with Ph-3for the screening of late blight resistance (Panthee et al., 2015). Overall, the availability of useful PCR-based markers for Ph-2 and Ph-3 greatly improves breeders' ability to select and breed for late blight resistance in tomato by facilitating marker-assisted pyramiding of these two resistance genes in new tomato breeding lines. These markers are quite useful to select the right plants at early stage based on its phenotye (Fig. 3 and 4). Mountain Honey has Ph-2 at heterozygous conditions whereas NC 1CELBR has both Ph-2 as well as Ph-3 at homozygous conditions(Gardner and Panthee, 2010). If both genes are present in a genotype, the level of LB resistance is better as we can see in Fig. 3. However, if none of these genes are present in a genotype, it is completely susceptible such as NC 123S. Mountain Magic, Mountain Merit and Mountain Rouge are late blight resistant hybrids tomato released from NC State University (Gardner and Panthee, 2012a; Panthee and Gardner, 2010; Panthee and Gardner, 2014).

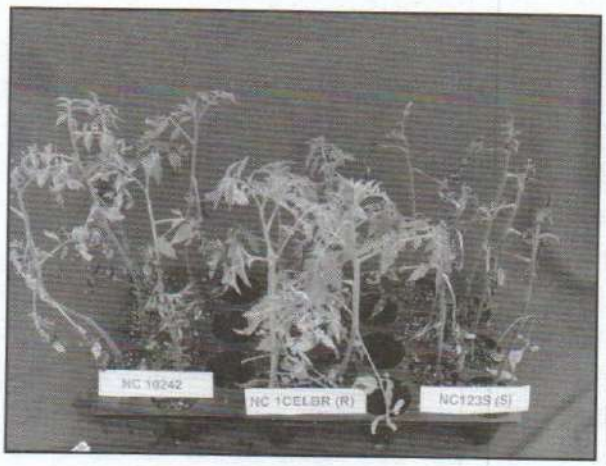

Fig. 3: Screening of Mountain Honey (NC 10242) for late blight resistance in the growth chamber at MHCREC, Mills River, NC. In the picture, Mountain Honey (left) was partially infected with late blight whereas NC 1CELBR (resistant control on the center) was clean whereas NC 123S (susceptible control on left) was completely dead.

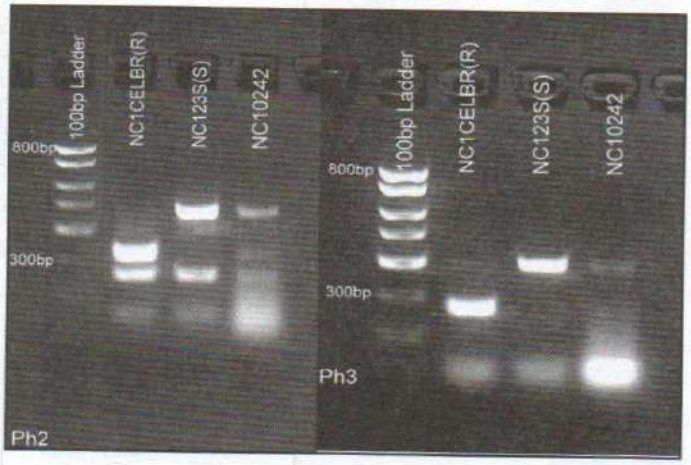

Fig. 4: Molecular screening of Mountain Honey (NC 10242) for Ph-2 and Ph-3 genes conferring late blight resistance in tomato. Results indicated that it was heterozygous only for Ph-2 gene whereas it did not produce a double band for Ph-3 gene indicating that it may not have this gene.

Tomato Mosaic Virus

Tomato mosaic virus (ToMV), a Tobamovirus closely related to tobacco mosaic virus (TMV), is a RNA-virus that is highly stable under natural conditions and conducive to mechanical transmission, such as routine plant handling in field or greenhouse operations (Sacristan et al., 2011). Leaves of ToMV infected plants display light green or yellow mottling, with

rough downturned edges, and a shoestring-like elongation on young growth (Fig.5). Plant growth may be stunted, with poor fruit set and small, brown-streaked fruit.(Jung et al., 2002). Often, it is difficult to distinguish between symptoms of nutrient deficiency and ToMV on younger leaves.

Various sources of resistance to tomato mosaic virus (ToMV) have been identified, which have led to the identification and mapping of three major dominant genes, Tm-1, Tm-2 and Tm-22 (Tm-2a) (Ohmori et al., 1996). These resistance genes have been used for the development and release of several ToMV-resistant cultivars through conventional and molecular breeding approach. In an experiment conducted in NC, ToMV symptoms appeared three weeks after inoculation. Phenotypic observations were taken three weeks after the first symptoms were identified. The resistant parent, Vendor VFT was symptom free whereas susceptible parents, NC 514-1(2006) were severely affected (Fig. 5). The phenotypic observations as well as Agdia ImmunoStrip kit results were recorded as either resistant (R) or susceptible (S), which was segregating in an F2 population derived from 514-1(2006) x Vendor VFT (population NC1089) and NC714 x Vendor VFT (population NC10143). This observation was found useful for molecular marker development (Fig. 6). There are several potential hybrids resistant to ToMV in the pipeline, which have already been evaluated multiple environments.

Fig. 5: Resistant variety 'Vendor VFT' (left) and susceptible line 'NC 514-1(2006)' (right) six weeks after inoculation with local strain ToMV in North Carolina. The visual phenotypic symptoms were confirmed with AgDia Immunostrip test.

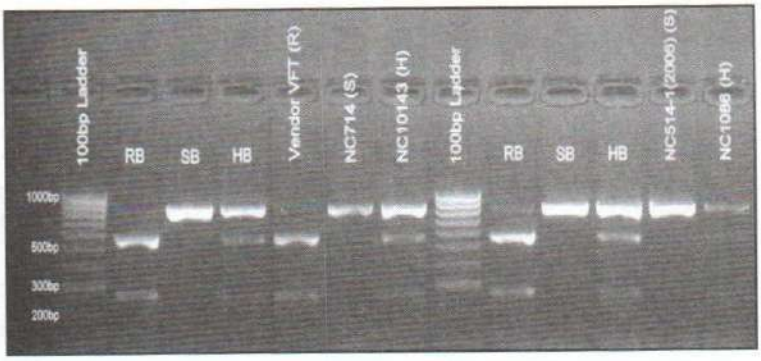

Fig. 6: Bulk segregant analysis (BSA) for Tm2agene conferring resistance to tomato mosaic virus in tomato. The PCR product amplified with NCTm-019 molecular marker and digested with HaeIII restriction enzyme from resistant bulk (RB) and resistant parent (Vendor VFT) was 270 and 600 bp whereas that from susceptible bulk (SB) and susceptible parent (NC514-1(2006) and NC714) was 870 bp. Bulk samples are related to F2 population of NC10143 (left panel) and NC1086 (right panel).

Tomato Spotted Wilt Virus

Tomato spotted wilt virus (TSWV) is a member of the genus Tospovirus, which belongs to the family Bunyaviridae (Gordillo et al., 2008). TSWV has a wide host-range that includes tomato, tobacco, pepper, potato, celery, pea, peanut, dahlia, lettuce, chrysanthemum, and gerbera, among others. TSWV is transmitted by thrips, particularly western flower thrips (WFT: Frankliniellaoccidentalis) (Ullman et al., 1997). Under natural conditions, the magnitude of the TSWV problem is directly proportional to the thrips population. TSWV-infected tomato plants develop chlorotic and necrotic ringspots on their leaves which affects the overall yield and quality of the fruit, becoming unmarketable.

There are eight different genes conferring resistance to TSWV. Among those, Sw-5 has been the most effective resistance gene because it is race non-specific (Stevens et al., 1992). Sw-5has been incorporated into many tomato breeding lines and cultivars around the world mainly by conventional breeding protocols. We released multiples TSWV breeding lines (Gardner and Panthee, 2012b) and hybrids including NC 1CS and Mountain Majesty (Panthee and Gardner, 2011) from North Carolina tomato breeding program. Recently, we developed and deployed the molecular markers to facilitate the screening of the TSWV since field screening for virus is challenging(Panthee and Ibrahem, 2013). Screening results of independent populations with these molecular markers were consistent (Fig. 7).

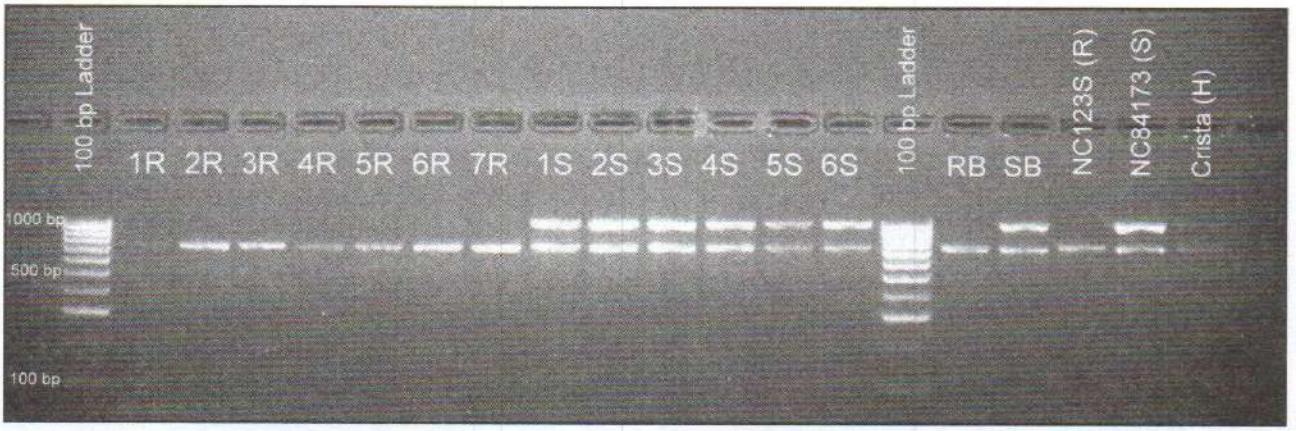

Figure 7. Sequence characterized amplified region(SCAR) markers associated with theSw-5 gene in tomato. NCSw-012, a dominant SCAR marker associated with theSw-5 gene in tomato. The size of the PCR product of this marker from the resistant genotypes (1R - 7R, RB, and NC 123S) was 1000 bp. 'Crista' is an F1 hybrid and produced a band similar to the resistant genotypes.

Root Knot Nematodes

Root-knot nematodes affects many crop species, and six species are pathogenic on Solanaceous crops-- Meloidogyne arenaria, M. incognita, M. javanica in more tropical regions and M. hapla, M. fallax, and M. chitwoodi in more temperate regions . The tropical species are the typical pathogens of tomato (Williamson and Roberts, 2009). There are several species of Meloidogyne, including M. incognita, M. javanica M. hapla and M. enterolobii (syn. M. mayaguensis) ,however, M. incognita is the dominant species causing damages (Agrios, 2004).Sources of resistance to root knot nematode (RKN) in tomato were originally identified in S. peruvianum in the 1940s, and subsequently a dominant resistance gene, Mi, was detected in a S. lycopersicum ×S. peruvianum population .In most of the cases, this gene is still effective against M. incognita. Mountain Merit and Mountain Rouge are resistant to RKN (Panthee and Gardner, 2010; Panthee and Gardner, 2014).

Breeding for Fruit Quality

Fruit quality consists of fruit composition, which has health benefits and morphological traits, which may improve not only overall yield but also marketability of the fruits. Components of the fruit quality includes total titratable acids (TTA), total soluble solids (TSS), firmness, flavor-contributing components, lycopene, fruit shape, size,blossom end scar size, cracking, smoothness and color. Emphasis has been placed to improve these traits overtime and a significant achievement has been made.

Evaluation of Tomato Germplasm for Flavor and Firmness

In an effort to improve the flavor in tomato, we evaluated 173 tomato germplasms from USDA collection for flavor-contributing components by subjective methods as has been described by Sinesio et al. (2010) with some modifications. Details of the methodology is described in Panthee et al. (2013). There were highly significant differences ($p<0.01$) among tomato genotypes for overall flavor and flavor-contributing components. Fruit firmness has beenreported to be associated with fruit flavor (Wang et al., 2009). For that reason, firmness was measured in this core collection and average firmness was found to be 10.9 N ranging from 5.8 to 35.8 N, which was a wide range. Average TTA was 0.3% ranging from 0.15 to 0.64%. However, average TSS was 4.9 ranging from 3.4 to 9%, which is a very good level of variation. Average ratio of TSS to TTA was 16.4 ranging from 8.6 to 33.1 - the wider the ratio, the sweeter the tomato (Auerswald et al., 1999). Fruity odor of tomato ranged from 0.9 to 4.0 when measured on a scale from 0 to 5, 5 being an excellent fruity odor. With a similar ranking, sweet flavor ranged from 0.8 to 4.2 whereas acidic flavor ranged from 0.7 to 4.2. Fruity flavor ranged from 0.6 to 4.0 whereas melon flavor was virtually absent in this collection of tomato genotypes. Juicy texture ranged from 1.1 to 4.5 whereas mealy texture ranged from 0.9 to 3.8. Overall flavor rating, which is due to the interactions of individual components, ranged from 0.8 to 4.1 with an average of 2.6. All these traits varied broadly, revealing the potential to utilize the available germplasm for fruit quality improvement.

Correlation and Regression Analysis

Subjective rating of overall flavor and its correlations with individual components was the major focus of this study. As others have reported (Causse et al., 2003), firmness was negatively correlated with overall flavor ($r=-0.10$, $p>0.05$) but it was non-significant in the present study. However, when correlation was determined by fruit-type including large-fruited, plum (roma) and grape (cherry); firmness was positively correlated with overall flavor in grape tomatoes ($r=0.35$, $p<0.05$) whereas it was negative ($r=-0.20$, $p<0.05$) in large-fruited and there was no correlation in plum ($r=0.07$, $p>0.05$) tomatoes (data not shown). Acid taste was also found to be negatively correlated with overall flavor but it was non-significant. However, TSS, TTA and other subjective components including fruity odor and sweet taste were positively correlated with overall flavor.

Scatter plots of overall flavor and its regression on individual components is presented in Fig. 8. Linear regression analysis revealed that every single change in fruity flavor contributed to the overall flavor by a unit since the regression slope was 1.03 (Fig. 8B). Regression analysis of flavor on fruity odor had fit the equation with R2-value of 33.5%. However, juicy texture produced much better linear fit with a slope of 0.97 and R2-value of 61.7% (Fig. 8C). Mealy texture, which is believed to be a good predictor of flavor, produced not only a negative slope ($b=-0.54$) but also a low R2-value of 15.8%. However, sweet taste produced the equation with R2-value of 76.3% (Fig. 8D). Both TSS and TTA had poor regression equation with R2-value of 13.9% and 10.8%, respectively. None of the multiple as well as polynomial regression equations were better than linear equations to predict the overall flavor value using its individual components. Utilizing this information, we developed mapping population, and we are in the process of mapping QTL and developing molecular markers associated with quality traits.

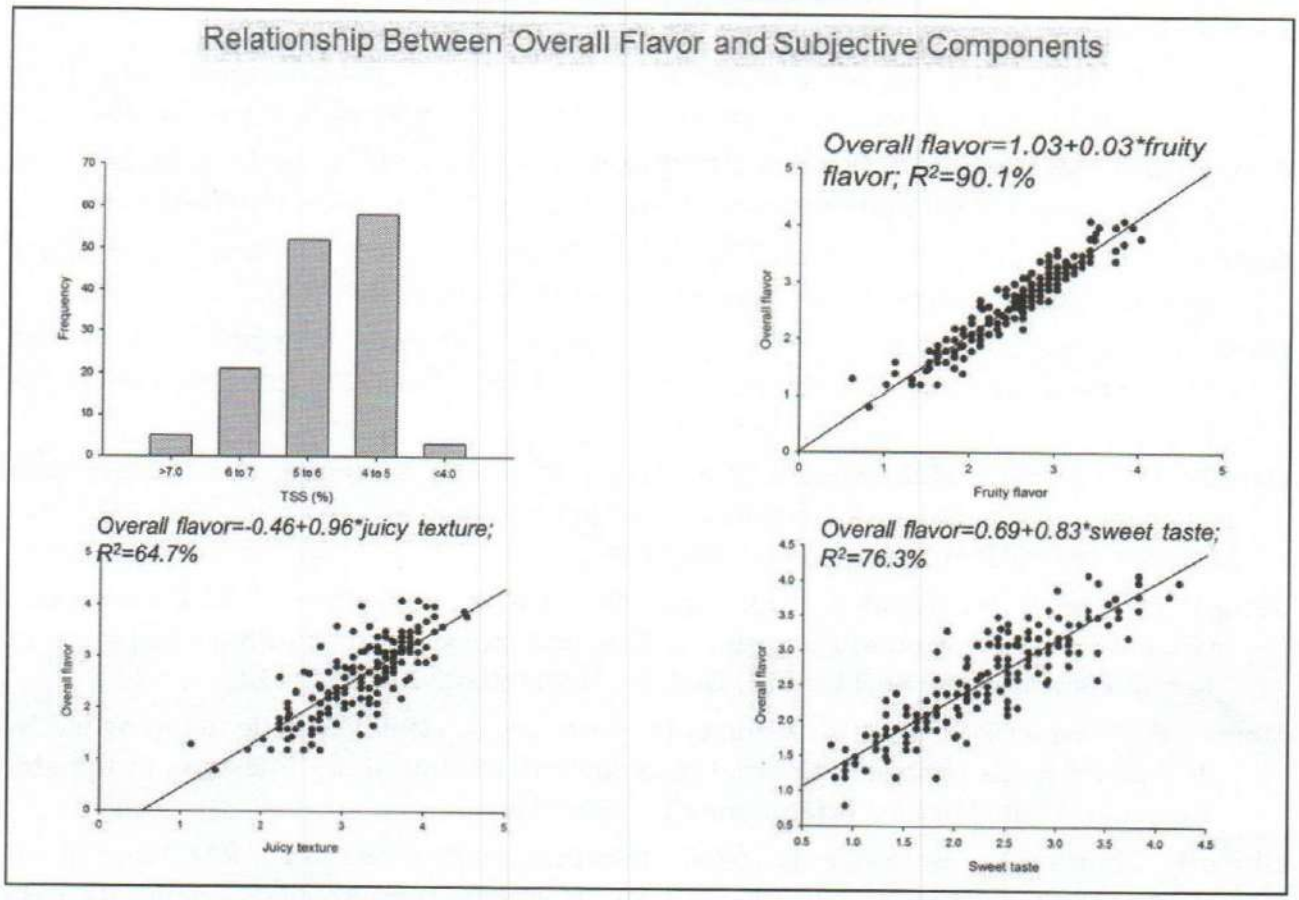

Figure 8. Relationship between overall flavor and flavor components in tomato lines.

Summary

Tomato varieties have been improved for disease resistance and fruit quality. Molecular markers associated with disease resistance genes have been useful for the selection of the varieties. Marker-assisted selection is useful to advance the genetic materials from early generation, and when there is limited opportunity to select the genotypes under enough inoculum pressure, for instance ToMV or TSWV. Development of suitable molecular markers associated with quality traits can minimize the time involved in assessing lines for phenotypic traits for selection.

References

Agrios G.N. (2004) Plant pathology. Fifth ed. Elsevier, New York.

Alexander L., Tucker C. (1945) Physiological specialization in the tomato wilt fungus Fusarium oxysporum f. sp. lycopersici. Journal of Agricultural Research 70:303-313.

Auerswald H., Peters P., Bruckner B., Krumbein A., Kuchenbuch R. (1999) Sensory analysis and instrumental measurements of short-term stored tomatoes (Lycopersicon esculentum Mill.). Postharvest Biology and Technology 15:323-334.

Causse M., Buret M., Robini K., Verschave P. (2003) Inheritance of nutritional and sensory quality traits in fresh market tomato and relation to consumer preferences. Journal of Food Science 68:2342-2350. DOI: 10.1111/j.1365-2621.2003.tb05770.x.

Chunwongse J., Chunwongse C., Black L., Hanson P. (2002) Molecular mapping of the Ph-3 gene for late blight resistance in tomato. Journal of Horticultural Science & Biotechnology 77:281-286.

FAOSTAT. (2014) World Production (Tonnes) of Tomatoes in 2013.

Foolad M.R., Merk H.L., Ashrafi H. (2008) Genetics, genomics and breeding of late blight and early blight resistance in tomato. Critical Reviews in Plant Sciences 27:75-107.

Gardner R.G., Panthee D.R. (2010) NC 1 CELBR and NC 2 CELBR: Early blight and late blight resistant fresh market tomato breeding lines. HortScience 45:975-976.

Gardner R.G., Panthee D.R. (2012a) 'Mountain Magic': An early blight and late blight resistant specialty type F1 hybrid tomato. HortScience 47:299-300.

Gardner R.G., Panthee D.R. (2012b) Tomato spotted wilt virus resistant fresh-market tomato breeding lines: NC 58S, NC 123S, NC 127S and NC 132S. HortScience 47:531-532.

Gordillo L.F., Stevens M.R., Millard M.A., Geary B. (2008) Screening two Lycopersicon peruvianum collections for resistance to Tomato spotted wilt virus. Plant Disease 92:694-704. DOI: 10.1094/pdis-92-5-0694.

Jung H.W., Yun W.S., Hahm Y.I., Kim K.H. (2002) Characterization of Tobacco mosaic virus isolated from potato showing yellow leaf mosaic and stunting symptoms in Korea. Plant Disease 86:112-117. DOI: 10.1094/pdis.2002.86.2.112.

Moreau P., Thoquet P., Olivier J., Laterrot H., Grimsley N. (1998) Genetic mapping of Ph-2, a single locus controlling partial resistance to Phytophthora infestans in tomato. Molecular Plant-Microbe Interactions 11:259-269.

Ohmori T., Murata M., Motoyoshi F. (1996) Molecular characterization of RAPD and SCAR markers linked to the Tm-1 locus in tomato. Theoretical and Applied Genetics 92:151-156.

Panthee D.R., Gardner R.G. (2010) 'Mountain Merit': A late blight-resistant large-fruited tomato hybrid. HortScience 45:1547-1548.

Panthee D.R., Gardner R.G. (2011) 'Mountain Majesty': A Tomato Spotted Wilt Virus-resistant Fresh-market Hybrid Tomato and Its Parents NC 714 and NC 1CS. Hortscience 46:1321-1323.

Panthee D.R., Gardner R.G. (2013a) 'Mountain Honey' Hybrid Grape Tomato and Its Parent NC 6 Grape Breeding Line. Hortscience 48:1192-1194.

Panthee D.R., Gardner R.G. (2013b) 'Mountain Vineyard' Hybrid Grape Tomato and its Parents: NC 4 Grape and NC 5 Grape Tomato Breeding Lines. HortScience 48:1189-1191.

Panthee D.R., Gardner R.G. (2014) 'Mountain Rouge': A Pink-fruited, Heirloom-type Hybrid Tomato and Its Parent Line NC 161L. Hortscience 49:1463-1464.

Panthee D.R., Gardner R.G., Ibrahem R., Anderson C. (2015) Molecular markers associated with Ph-3 gene conferring late blight resistance in tomato. American Journal of Plant Sciences 6:2144-2150.

Panthee D.R., Ibrahem R. (2013) New molecular markers associated with the Sw-5 gene conferring resistance to Tomato spotted wilt virus in tomato. Journal of Horticultural Science & Biotechnology 88:129-134.

Panthee D.R., Labate J.A., Robertson L.D. (2013) Evaluation of tomato accessions for flavour and flavour-contributing components. Plant Genetic Resources-Characterization and Utilization 11:106-113. DOI: 10.1017/s1479262112000421.

Peirce L.C. (1971) Linkage tests with Ph conditioning resistance to race 0, Phytophthora infestans. Rep. Tomato Genet. Coop. 21:30.

Robbins M.D., Masud M.A.T., Panthee D.R., Gardner C.O., Francis D., Stevens M.A. (2010) Marker-assisted selection for coupling phase resistance to tomato spotted wilt virus and Phytophthora infestans (late blight) in tomato. HortScience 45:1424-1428.

Sacristan S., Diaz M., Fraile A., Garcia-Arenal F. (2011) Contact transmission of tobacco mosaic virus: A quantitative analysis of parameters relevant for virus evolution. Journal of Virology 85:4974-4981. DOI: 10.1128/jvi.00057-11.

Scott J.W. (2008) Fresh market tomato breeding in the USA. Acta Hort. 789:21-25.

Sinesio F., Cammareri M., Moneta E., Navez B., Peparaio M., Causse M., Grandillo S. (2010) Sensory Quality of Fresh French and Dutch Market Tomatoes: A Preference Mapping Study with Italian Consumers. Journal of Food Science 75:S55-S67.

Stevens M.R., Scott S.J., Gergerich R.C. (1992) Inheritance of gene for resistance to tomato spotted wilt virus (TSWV) from Lycopersicon Peruvianum Mill. Euphytica 59:9-17.

Ullman D.E., Sherwood J.L., German T.L. (1997) Thrips as vectors of plant pathogens, in: T. Lewis (Ed.), Thrips as Crop Pests, CAB International, Wallingford, UK pp. 539–565.

Wang Y.T., Liu R.L., Huang S.W., Jin J.Y. (2009) Effects of Potassium Application on Flavor Compounds of Cherry Tomato Fruits. Journal of Plant Nutrition 32:1451-1468. DOI: 10.1080/01904160903092663.

Williamson V.M., Roberts P.A. (2009) Mechanisms and genetics of resistance, in: R. N. Perry, et al. (Eds.), Root-knot Nematodes, CAB International, Wallingford, UK. pp. 301-325.