

# Tomato Crop Germplasm Committee Report, 2004

## I. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) ranks as a leading fresh and processed vegetable crop in the U.S. with approximately 177 thousand hectares (436,700 acres) planted for commercial production in 2002 (USDA-NASS 2004 agricultural statistics, [http://www.usda.gov/nass/pubs/agr04/04\\_ch4.pdf](http://www.usda.gov/nass/pubs/agr04/04_ch4.pdf)). World production, which exceeded 108.5 million metric tons in 2002, occupied approximately 4.0 million hectares (FAO 2003 agricultural statistics, <http://apps.fao.org/page/collections?subset=agriculture>).

World volume has increased approximately 10% since 1985, reflecting a substantial increase in dietary use of the tomato. Nutritionally, tomato is a significant dietary source of Pro-vitamin A and C. Furthermore, recent studies have shown the importance of lycopene, a major component of red tomatoes, to have important antioxidant properties which reduce the incidence of several cancers.

## II. PRESENT GERMPLASM ACTIVITIES

United States Department of Agriculture (USDA) supports public tomato germplasm collections in the U.S. are currently held at two locations. The P.I. collection is maintained at Geneva, N.Y. This collection contains 5,802 accessions with 89.2% being *L. esculentum* and the rest wild species. Presently, 2.9% of the collection is unavailable for distribution. In 2002, 1377 samples of 1,180 accessions were requested. Since 1996 an average of 813 accessions were requested per year which amounts to 14% of the collection. The C.M. Rick Tomato Genetics Resource Center (TGRC) at Davis, California presently maintains approximately 3,553 accessions which include 1,131 wild tomato species, 1,007 monogenic mutants and 1,415 miscellaneous genetic or cytogenetic stocks. In 2002, 5,372 seed samples representing 1,656 unique accessions were sent in response to 362 requests from 230 investigators. The relatively heavy use of TGRC accessions relative to the PI collection may reflect its large number of wild species populations which are used as a source of disease resistance and other economic traits. In addition, the mutant and cytogenetic stocks are well defined and have many uses for basic research involving tomato. The National Seed Storage Laboratory (NSSL) maintains back up stocks of approx. 95% of the TGRC stocks and 89% of the P.I. Collection.

In addition to these two supported collections, there are still collections including tomato lines as well breeding stocks that include wild introgressions in breeding collections in state supported programs. Preventing the loss of valuable materials from these collections is discussed in section IV. A below.

## III. STATUS OF CROP VULNERABILITY

Use of germplasm in breeding. The present germplasm collections have been extensively used as genetic resources for tomato improvement, with a major focus on disease

resistance. Resistances to at least 44 major diseases have been reported in present world collections and 23 of these resistances have been incorporated into adapted cultivars. New diseases or new strains of some previously known diseases appear on a regular basis and frequently the germplasm collections serve as the only source of resistance to these new pathogens.

Although the available genetic resources in *Lycopersicon* and related *Solanum* species are intensively utilized for many research purposes, their use in breeding programs has been somewhat deterred by a variety of factors which include: a tendency to rely on chemical controls for pathogens and insect pests, the time and effort required to introgress genes from exotics, a reduction in the number of tomato breeding programs at public institutions, the absence of systematic variety evaluation data, and a lack of reliable screening criteria for some traits of interest (e.g. insect resistance and fruit flavor).

Crop Uniformity. As a result of these influences on tomato breeding, genetic comparisons of modern tomato cultivars have generally shown them to be highly uniform. Some, if not most, of the genetic variation that can be detected with markers is attributable to the segments of DNA surrounding disease resistance genes bred into cultivated tomato from the wild species. Within individual market classes of commercial tomatoes, the diversity of varieties grown is even narrower. For example, the modern processing tomatoes are closely related genetically and represent a very narrow sampling of the gene pool available in tomato. Despite the narrow genetic base, introgression and breeding have increased the genetic diversity of modern cultivated tomatoes relative to those released prior to 1960. Commercial cultivars remain vulnerable to epidemics of diseases for which little resistance has been bred into the widely grown varieties.

Crop genetic diversity that could be mobilized in an emergency. Genetic improvement and release of modern varieties is too slow a process to solve emergency disease outbreaks. One more likely scenario is to shift production to other regions, or grow varieties adapted to regions in which specific diseases have been widespread for a long period of time, hence resistant varieties have been developed. For example, varieties resistant to the virus TYLCV from the Middle East could be accessed if an outbreak occurred in the U.S. However, these may not be well adapted or high yielding under growing conditions in the U.S.A. Another scenario for emergencies due to diseases/pests already targeted by current programs, would be to support cooperative work and accelerated generation cycling, using partially bred materials in these existing programs.

A survey of the tomato crop germplasm committee identified a series of tomato problems that could be resolved (or minimized) by genetic methods (Table 1). In this Table, some factors may be more, or solely important, for processing vs. freshmarket tomato, or the reverse. Some factors may be important for some production regions, and not others. Either of these situations might result in the overall importance being lower than a trait affecting both types of crops or across regions. (That is, a factor that is of high importance for one of the types of crops or in a limited, though major, regions may have an overall rating of medium or low).

**Table 1. Tomato problems where genetic improvements would benefit U.S. production.**

<b>TYPE</b>	<b>PRIORITY</b>	<b>DESCRIPTION</b>
<b>DISEASES</b>		
<b>Bacterial</b>	<b>High</b>	Bacterial canker
		Bacterial spot
		Bacterial speck race 1
	<b>Low</b>	Bacterial speck race 2
<b>Fungal</b>	<b>High</b>	Verticillium wilt race 2
	<b>Medium</b>	Late blight
		Corky root
		Phytophthora root rot
		Fruit rots
	<b>Low</b>	Target spot
		Powdery mildew
<b>Viral</b>	<b>High</b>	TYLCV & other begomoviruses
		Pepino mosaic virus
		Tomato spotted wilt virus
		Tomato bushy stunt virus
	<b>Medium</b>	Cucumber Mosaic Virus
		Beet curly top virus
		Tomato infectious chlorosis virus
		Tomato chlorosis virus
	<b>Low</b>	PVY
		Tobacco etch virus
<b>INSECTS</b>	<b>High</b>	New/improved screening protocols
		Silverleaf whitefly
		Nematodes, heat stable
	<b>Medium</b>	Aphids
		Greenhouse whitefly
	<b>Low</b>	Lepidopterous pests, esp. those on fruit
	Leaf Miner	
<b>STRESS</b>	<b>High</b>	Cold tolerance
	<b>Medium</b>	Heat tolerance
		Salinity tolerance
		Color disorders
<b>HORTICULTURAL</b>	<b>High</b>	Soluble solids
		Flavor (need to define components)
	<b>Medium</b>	Antioxidants/nutritional content
		Color
		Sugar type
		Peelability/dicing
	<b>Low</b>	Pectin chemistry
		Blossom-end smoothness

## IV. GERMPLASM NEEDS

### A. Collection

Together, the USDA and TGRC maintain approximately 9,355 accessions of 9 *Lycopersicon* and 4 related *Solanum* species, with minimal overlap between the collections. Each *Lycopersicon* species is represented by many accessions, which are generally well distributed over their respective geographic ranges. The collections are, however, deficient in certain restricted areas -- mostly in territory that is difficult to access. Collecting expeditions were sponsored by the International Board for Plant Genetic Resources (IBPGR) in 1980, 1984, 1986, 1987, and 1988. Additional wild/primitive germplasm was collected in 1985, 1995, 1996 and 2001 on trips sponsored by the TGRC, the USDA Plant Exchange Office, and other agencies or institutions. These forays have covered areas that were poorly represented in existing collections. Drs. Cuartero and Diaz of the "La Mayora" Experiment Station near Malaga, Spain also collected valuable populations in Peru in 1983, and Dr. Nuez and colleagues at the Univ. de Valencia have collected in Ecuador, including the Galapagos Islands. The TGRC assumed responsibility of increasing and maintaining some of the stocks collected by these expeditions.

In view of these circumstances, the status of wild tomato germplasm is considered good and vastly better than that of many other crop plants. Certain remote areas of Peru, notably in the watershed of the Rio Maranon, are known to harbor populations of *L. peruvianum* and possibly other species. Therefore, Dr. Chetelet of the TGRC identified the areas where addition collecting would be justified as:

1. Chile: Coastal populations of *L. chilense*, which are poorly represented in ex situ collections and which we have found to be genetically distinct from montane populations of the species. A proposal to collect in Chile in 2005 has been submitted.
2. Peru: Germplasm from the Maranon drainage, thought to be mostly *L. peruvianum*. The TGRC has only a handful of collections from this drainage, due to its physical inaccessibility. Yet, these populations are quite distinct. A monograph of the tomatoes currently in preparation split *L. peruvianum* into 4 species. The authors consider the differences between the northern races (of which Maranon is one) and the southern races to be sufficient for separate species status. It is important that adequate collections be made of each of the 4 groups. Also, *L. peruvianum* has been a rich source of resistance genes bred into cultivated tomatoes.

Another aspect of collection is the acquisition of germplasm for research projects that have terminated or are anticipated to terminate in the near future. A great wealth of tomato germplasm exists in such holdings, consisting of cultivars, breeding lines, genic and chromosomal variants, and other stocks. In the recent past it has been possible to acquire the valuable items from certain collections (e.g. mutant lines donated by Kerr or Darby to the TGRC); unfortunately, in others, collections were discarded before useful germplasm could be salvaged. It is suggested that the Tomato CGC survey collections

that could be in jeopardy now or in the near future and request that caretakers assist the NPGS in acquiring the most valuable items. Sufficient funds should be budgeted for such activities to cover costs of correspondence, publicity, labor, and acquisition. Guidelines to evaluate the “value” of such material should be developed.

Of particular interest from both terminated and active programs are enhanced stocks from wild species. This is especially true for material bred from *L. peruvianum* or *L. chilense*, since it is more difficult to obtain hybrids and the first backcross with these species, but also for other wild species such as *L. hirsutum* and *L. pennellii*, which also cause considerable sexual barriers in interspecific populations. A number of useful prebred populations representing the genomes of specific wild species accessions in the genetic background of cultivated tomato have been developed and accessioned by the TGRC. The first such resource was a library of *L. pennellii* introgression lines (ILs), synthesized by Eshed and Zamir, that capture the entire donor genome in 50-75 lines. Similar, though less complete populations are also available for the genomes of *L. hirsutum*, *L. pimpinellifolium* and *S. lycopersicoides*. In the process of selecting for genes or traits of interest, breeders necessarily discard much genetic variation in early generations, some of which may be of value in other circumstances. When the prebred lines were derived from species or accessions of particular value, making such prebred lines available for other researchers could save breeders years of time and considerable work. The collection and maintenance of these prebred or introgression stocks could be a major input to the Geneva center and may require additional funding.

## **B. Maintenance and Preservation**

### **1. PI Collections:**

The PI collection contains nearly 6000 accessions. All accessions that needed to be re-grown because of poor germination or insufficient seed quantity on hand are currently under regeneration, either in the field or greenhouse. The seed of the Geneva collection is maintained at -20° after drying the seed to approximately 6% relative humidity. Field increases with no pollination control are suitable for *L. esculentum* accessions, all *L. parviflorum* and *L. cheesmanii*, and about half of the *L. pimpinellifolium* accessions. The other species must be protected against outcrossing by isolation or hand-pollinations in the greenhouse.

Twenty-four plants are used for regeneration of the self-pollinating accessions, up to 50 for the outcrossing lines.

### **2. Tomato Genetics Resource Center**

As mentioned, the TGRC at Davis, CA, has 3,553 accessions of wild species, monogenic mutants, and miscellaneous genetic/cytogenetic stocks, in approximately equal proportions.

**a. Wild species.** The TGRC has extensive experience in managing various collections. This experience served to instruct how regeneration and maintenance operations can be best conducted. An understanding of the genetic composition and natural mating system of a given accession is vitally important to proper management of increasing seed stocks. The wild species most closely related to the tomato are classified as follows in respect to their mating systems:

Autogamous	<i>L. cheesmanii</i> , <i>L. esculentum</i> , <i>L. parviflorum</i>
Facultative self-compatible:	<i>L. chmielewskii</i> , <i>L. pimpinellifolium</i>
Self-compatible or self-incompatible	<i>L. chilense</i> , <i>L. hirsutum</i> , <i>L. pennellii</i> , <i>L. peruvianum</i>
Allogamous (all self-incompatible)	<i>S. juglandifolium</i> , <i>S. lycopersicoides</i> , <i>S. ochranthum</i> , <i>S. sitiens</i>

A further breakdown is necessary within the facultative species because their subspecific groups can differ radically in their maintenance requirements. In *L. pimpinellifolium* the situation varies from the extremes of nearly complete autogamy at the margins of the distribution to high levels of outcrossing in the central region, intermediate situations being found in parts of the intervening territory. The extent of outcrossing very closely parallels the extent of genetic variation within accessions. *L. hirsutum* is an example of a facultative species in which populations of the central area are self-incompatible, hence obligatorily allogamous and highly polymorphic, whilst the northern and southern peripheries are populated by self-compatible, highly uniform colonies.

Methods of maintenance must obviously be regulated according to mating system and extent of genetic variation in order to maintain integrity of the accessions. The highly autogamous accessions (nearly always genetically uniform) need to be increased from no more individuals than necessary to produce the desired seed quantity (generally 6-12 plants). At the other extreme, the obligate outcrossing accessions with very high levels of genetic variability need to be increased from as many individual plants as possible (in practice, usually 50).

Seed stocks are increased and placed under optimal storage conditions as soon as possible after the original accession is received because seeds collected in the field may be of uncertain quality, immature at harvest, or exposed to harmful conditions in transit. The planting for increase is made by sampling seeds from each wild plant in order to obtain as much of the original variation as possible. The general practice with this group is to crowd as many plants per container as possible without interfering with individual seed producing capacity. The allogamous and facultative species are mostly grown in the fall-winter greenhouse to prevent cross-contamination and to have appropriate conditions (photoperiod, light intensity) for seed production. Pollen is collected by a mechanical vibrator after all plants are in flower and used for hybridizing flowers of all plants. Several such pollinations at weekly intervals are usually required to produce adequate seed supplies. When ripe, fruits are harvested from each plant and seeds extracted and thoroughly blended to provide as adequate as possible representation of the original variation of the accession in question.

**b. Genic and chromosomal stocks.** Maintenance procedures vary considerably between subcategories: many items reproduce well by automatic selfing under field conditions; male steriles and other sterile or lethal types must be propagated via heterozygotes; autotetraploids and many genic variants require hand pollination; trisomics must be selected from mixed populations and are mated with diploids; weak genotypes must be maintained in greenhouses and may require special feeding, grafting, or other care; and stocks of certain other accessions that produce defective seeds must be replenished frequently. In certain instances, even with optimal care, very low seed yields are obtained. Otherwise, maintenance procedures do not differ from the bulk of the tomato accessions.

### **C. Evaluation:**

A list of minimal descriptors with high heritability was approved by the Tomato Crop Germplasm Committee in 2001 (Table 2). These descriptors are now recorded during regeneration of the tomato collection at Geneva. Digital imaging of fruits and leaves of the PI collection has begun and images are now available on GRIN. A core collection representing the early historical record for cultivated tomato breeding was approved by the Tomato CGC in 2003. This core collection provides a starting point for new evaluations of older varietal accessions in the Geneva collection.

Limited evaluation of tomato germplasm at the TGRC and Geneva has been supported by USDA's NPGS plant germplasm evaluation program. Since 1996, funding has been awarded for screening subsets of the collections for resistance to late blight, early blight, foot rot, and tomato spotted wilt. Identification of germplasm to address the genetic improvement of tomato for additional priority needs listed in Table 1 has been hampered due to the lack of sufficient funding for germplasm evaluation.

Many quantitative traits such as soluble solids necessitate evaluation in important tomato production areas, possibly more than one, to lend credence to the results. This may be more expensive and more cumbersome to handle but would be of more value to the user community. It is also important that evaluation for disease resistances that do not have a proven artificial screening technique be carried out in regions where there is adequate disease pressure so accurate data can be obtained.

Often data are obtained by researchers on traits of interest, but reports are not given directly to the germplasm centers. Users could often provide data on traits if they were aware such traits were of interest. Efforts should be made to facilitate this by correspondence from the germplasm centers. Possibly the data sheets outlining desired characteristics could be distributed along with requested seed. At the same time, efforts should be made by the Geneva station to search the literature and enter data on P.I. lines into the GRIN system.

Efforts should also be made to develop controlled vocabularies and trait ontologies for descriptor lists. These efforts would bring NPGS descriptions in line with fundamental research do describe genomes and gene function.

**Table 2. Minimal descriptor list for tomato**

<b>I. Plant characteristics</b>	
A. Plant growth type	1. Miniature Dwarf
	2. Dwarf
	3. Determinate
	4. Semi-determinate
	5. Indeterminate
B. Canopy size	Small [Red Rock]
	Intermediate [Florida MH-1]
	Large [Mountain Pride-determinate; Tropic-indeterminate]
C. Leaf type	1. Rugose
	2. Potato leaf
	3. Standard
	4. Curled
	5. Others [Specify in Notes]
D. Flowers per inflorescences	1. Low
	2. Medium
	3. High
E. Type of inflorescence	1. Simple
	2. Forked
	3. Compound
F. Number of fruit set on second truss	
G. Number of days to maturity from sowing until 50% of the plants have at least one fruit ripened	
<b>II. Fruit Descriptors</b>	
A. Exterior color of immature fruit	1. Light
	2. Medium
	3. Dark
B. Exterior color of mature fruit	1. White
	2. Green
	3. Yellow
	4. Gold
	5. Orange
	6. Pink
	7. Red
	8. Other [Specify in Notes]
C. Exterior mature fruit appearance	1. Dull
	2. Medium
	3. Glossy

<b>Fruit Descriptors (Continued)</b>	
D. Shoulder Color	1. Green
	2. Gray Green (light green)
	3. Uniform
E. Mature fruit interior flesh color	1. White
	2. Green
	3. Yellow
	4. Orange
	5. Pink
	6. Red
	7. Other (Specify)
F. Mature fruit interior flesh color intensity	1. Pale
	2. Intermediate
	3. Deep
H. Fruit shape	1. Flattened (oblate)
	2. Slightly flattened (deep oblate)
	3. Globe
	4. Deep Globe
	5. Blocky (square round)
	6. Heart-shaped
	7. Ellipsoid
	8. Cylindrical
	9. Pyriform
H. Pistil scar	1. Dot
	2. Stellate
	3. Linear
	4. Irregular
I. Fruit weight (g, average of 10 fruits)	Checks, of increasing size, [Sweet 100], [Red Cherry Large], [Roma or New Yorker], [Flora-Dade], [Tropic], [Florida 7060]
J. Uniformity of fruit size	3. Low
	5. Intermediate
	7. High
K. Fruit firmness	3. Soft
	5. Medium
	7. Hard
L. Nippled fruit [mature fruits]	1. Absent
	2. Present
	3. Inverted

<b>Fruit Descriptors (Continued)</b>	
M. Presence/absence of jointless pedicel	1. Absent
	2. Present
	3. Arthritic (not complete)
N. Radial cracking	0. Absent
	3. Slight
	5. Intermediate
	7. Severe
P. Concentric cracking	0. Absent
	3. Slight
	5. Intermediate
	7. Severe
Q. Cuticle cracking	0. Absent
	3. Slight
	5. Intermediate
	7. Severe
<b>III. Chemical composition</b>	
A. Soluble solids Measured with refractometer from 4 fruits	

#### **D. Enhancement**

The role of the public sector in tomato improvement has declined substantially in the past two decades. At least 12 breeding positions have been eliminated. At the same time private companies have consolidated and several regions of the U.S. that produce tomatoes are orphaned with respect to active breeding efforts by the private sector. In view of the long term nature of tomato improvement efforts which entail use of germplasm collections, it would appear likely that expanded use of these collections would be encouraged by expanded funding of public germplasm enhancement efforts. Certainly enhancement efforts are needed for difficult areas such as insect resistance and salt tolerance where wild species have been known to have tolerance for years but little in the way of improved germplasm has been developed.

## **V. RECOMMENDATIONS:**

### **A. FUNDING PRIORITIES**

1. The current Specific Cooperative agreement between the USDA and the TGRC runs through 2008. The current annual allocation is \$61,000 and has varied between \$40,000 and \$60,000, dependent upon the availability of funds at Geneva (PGRU). This agreement should be continued and the allocation increased as evidenced by the high usage of TGRC accessions.
2. Evaluation. The current collections should be evaluated for the traits listed in Table 1 with emphasis placed according to the priorities listed. This should include studies on research methods where appropriate.
3. Access materials available in terminated public and private programs and add this material into the Geneva collection.

### **B. SUGGESTED FUTURE TOMATO CGC ACTIVITIES**

2. Refine the *L. esculentum* core collection at Geneva to improve geographic representation of core accessions (core subsets are already in place for the wild material at Davis).
3. Consider the various uses of a core collection from representing the genetic diversity of a crop to hypothesis testing regarding geographical, market, and ecological diversity.
4. Continue efforts to access core materials from terminated public programs while retired personnel are available to assist.
5. Access early generation lines introgressed from wild species in both terminated and active programs. This could be done on a worldwide basis.
6. Improve coordination between the TGRC database and the GRIN system.
7. Transfer all active collection accessions from the NCGRP to Geneva, after elimination of duplicate accessions.
8. Update evaluation procedures so scientists can report existing information and so any future funding will be utilized with maximum efficiency. Increase use and integration of controlled vocabularies and trait ontologies.

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