Series of Crop Specific Biology Documents

BIOLOGY OF *SOLANUM LYCOPERSICUM* (TOMATO)

Phase II Capacity Building Project on Biosafety

सत्यमेव जयते

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Ministry of Environment, Forest and Climate Change
Government of India

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BIOLOGY OF *Solanum lycopersicum* (TOMATO)

Phase II Capacity Building Project on Biosafety

Ministry of Environment, Forest and Climate Change
Government of India

Biology of *Solanum lycopersicum* (Tomato)

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under UNEP/GEF supported Phase II Capacity Building Project on Biosafety

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Message

I am happy to learn that the Ministry of Environment, Forest & Climate Change (MoEFCC) as part of the initiative under the UNEP GEF supported "Phase II Capacity Building Project on Biosafety" has developed eight crop specific biology document on Chickpea, Mustard, Papaya, Pigeon-pea, Potato, Rubber, Sorghum, and Tomato.

I am happy to note that the documents have been prepared with support from seven research institutions namely Indian Institute of Pulses Research, Directorate of Rapeseed and Mustard Research, Indian Institute of Horticulture Research, Central Potato Research Institute, Rubber Research Institute of India, Indian Institute of Millets Research and Indian Institute of Vegetable Research.

While Bt cotton is the only genetically modified (GM) crop approved for commercial cultivation in India, there are several crops under various stages of research, development and field trials. The present set of crop specific biology documents aims to provide scientific baseline information of a particular plant species that can be used as credible source of information for conducting safety assessment of GM plants.

I would like to congratulate all those who were involved in preparing these documents and those involved in steering this initiative.

I am confident that these biology documents will serve as a valuable tool for regulators, scientists, crop developers, policymakers, academicians and other stakeholders who are involved in the safety assessment of GM plants. I am also hopeful that baseline information provided in the biology document would further enhance awareness on biosafety aspects of GM crops.

(Prakash Javadekar)

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सत्यमेव जयते

PREFACE

India is an agriculture based economy with abundance of genetic base, diverse agro-climatic zones and highly qualified manpower which provides a rich scope for technological advances in agricultural biotechnology. The shortage of healthy seeds/planting material, lack of disease resistant clones, crop damage by insects, pests etc. have often affected the Indian agricultural economy adversely and therefore the role of new technologies assumes significant importance for Indian economy.

With significant advances in the field of agricultural biotechnology the regulatory system has to deal with multiple crops integrated with multiple traits. In order to streamline the process of safety assessment, the Ministry of Environment, Forest & Climate Change (MoEF&CC) under the UNEP-GEF supported "Phase II Capacity Building Project on Biosafety" has prepared a set of crop specific biology documents namely Chickpea, Mustard, Papaya, Pigeon-Pea, Potato, Rubber, Sorghum, Tomato with support from six Indian Council of Agriculture Research (ICAR) institutions and Rubber Research Institute of India.

The biology documents provides an overview of baseline biological information of a particular plant species such as taxonomy, the centres of origin, its related species including wild relatives, general description of their morphology, reproductive biology, biochemistry, potential for gene introgression, biotic and abiotic interactions. Such species specific information is expected to serve as a guiding tool for use in risk assessment of genetically modified (GM) plants.

The documents has been prepared through a consultative approach and comments received from several organizations have been extremely useful in validating this

document. I express my deep appreciation for the support provided by Indian Institute of Pulses Research, Directorate of Rapeseed and Mustard Research, Indian Institute of Horticulture Research, Central Potato Research Institute, Rubber Research Institute of India, Indian Institute of Millets Research and Indian Institute of Vegetable Research in preparing these documents. I would also like congratulate Dr. Ranjini Warrier, Advisor, (MoEFCC) and Dr O.P Govila (Former Professor, Department of Genetics, IARI) for their sincere efforts and the consultative approach adopted in finalizing the biology documents.

I am confident that these crop specific biology documents would be of immense value for researchers, regulators and industry in planning for the safety assessment of GM crops.

Hem Pande

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BIOLOGY OF *Solanum lycopersicum* L. (TOMATO)

1. INTRODUCTION

1.1 General Description

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop. At present, about 160 million tonnes of fresh tomatoes are produced from 4.7 million ha(FAOSTAT, 2011).Tomatoes are native to South America, but were brought to Europe sometime in the 15th century, where they soon became popular and were exported around the world. For a long time tomatoes were known by the name *Lycopersicon esculentum*, but recent work has shown that they are part of the genus *Solanum* - as Linnaeus recognized when he first described the species. The tomato is consumed in diverse forms, including raw, as an ingredient in several dishes, sauces, salads, and drinks. While it is botanically a berry fruit, it is considered avegetable for culinary purposes. The fruit is rich in lycopene, which has beneficial health effects. The plants typically grow to 1–3 meters in height and have a weak stem that often sprawls over the ground. It is a perennial in its native habitat, although often grown outdoors in temperate climates as an annual. Most cultivars produce red fruits, but a number of genotypes with yellow, orange, pink, purple, green, black, or white fruit are also available. Multi-colored and striped fruitsare also quite striking. Tomatoes grown forcanning and sauces are known as plum tomatoes, which typically have lower water contentwith elongated fruits.

China, the largest producer, accounts for about one quarter of the global output followed by India and United States (Table1). Currently tomato has a higher consumption rate in more developed countries and is often referred to as a luxury crop. In Israel, for example, tomato is a major part of the food basket, which is used when calculating the consumer price index (CPI). In other words, a scarcity of tomatoes can cause the CPI to rise and influence the inflation rate. In developing countries, tomato is becoming more important part of the food basket; hence the farmersaim to increase quantity than quality of the produce. As improved varieties and new cultivars with better resistance to various biotic and abiotic stresses are developed, it will become easier to grow the crops in more marginal conditions and the tomato will become an important part of the diet in poorer countries as well.

Table 1: Top producers of tomatoes in world (source: FAOSTAT, 2011)

Rank	Country	Production (MT)
1	China	48,572,921
2	India	16,826,000
3	United States	12,526,070
4	Turkey	11,003,433
5	Egypt	8,105,263
6	Iran	6,824,298
7	Italy	5,950,215
8	Brazil	4,416,652
9	Spain	3,864,120
10	Uzbekistan	2,585,000

1.2 Nomenclature and Classification

The cultivated tomato, *Solanum lycopersicum*, is grown for its popular fleshy fruits and is known by different names worldwide like tomate (German), tomaatti (Finish), pomidoro (Italian), kamalis (Malay), jitomate (Spanish), pomidor (Russian) and tamatar (Hindi). Linnaeus (1753) classified tomatoes in the genus *Solanum* and described *S. lycopersicum* (the cultivated tomato) and *S. peruvianum* (Table 2). The very next year Miller (1754) followed Tournefort (1694) and formally described the genus *Lycopersicon*. Miller did not approve of Linnaeus's binomial system, and he continued to use polynomial phrase names for all plants until 1768. Miller's circumscription of the genus *Lycopersicon* also included potatoes as "Lycopersiconradice tuberose, esculentum".

Later, Miller (1768) began to use Linnaeus binomial system and published descriptions under *Lycopersicon* for several species.It included *L. esculentum*, *L. peruvianum, L. pimpinellifolium* and *L. tuberosum* (potatoes). In the posthumously published edition of '*The gardener's and botanist's dictionary*' (Miller, 1807) the editor, Thomas Martyn, followed binomial system of Linnaeus and merged *Lycopersicon* back into *Solanum*. Following Miller's early work, a number of classical and modern authors recognized tomatoes under *Lycopersicon*, but other taxonomists included tomatoes in *Solanum*.Today, based on evidence from phylogenetic studies using DNA sequences and more in-depth studies of plant morphology and distribution, there is general acceptance of the treatment of tomatoes in the genus *Solanum* by both taxonomists and breeders alike. Theuse of *Solanum* names has gained wide acceptance by the breeding and genomics community such as the Solanaceae Genomics Network (SGN) and the International SOL Project (http://www.sgn.cornell.edu/). These names in *Solanum* are being incorporated in germplasm bank databases as in the C.M. Rick Tomato Genetic Resources Center (http://tgrc.ucdavis.edu/).

Table 2: Scientific classification of cultivated Tomato (*Solanum lycopersicum*)

Kingdom	Plantae
(Unranked)	Angiosperms
(Unranked)	Eudicots
(Unranked)	Asterids
Order	Solanales
Family	Solanaceae
Genus	*Solanum*
Species	*S. lycopersicum*

1.3 Nutritional Composition of Tomato

The Nutritional composition of tomato is given Table 3 below

Table 3: Nutritional composition of tomato (*Nutrient Data Laboratory, ARS, USDA*)

Content	/100g of Red Tomato	Content	/100g of Red Tomato
Energy	18 K cal	Vit K	7.9 µg
- Carbohydrate	3.9 g	Mg	11 mg
- Sugar	2.6 g	Mn	0.114 mg
Dietary Fiber	1.2 g	Fe	0.3 mg
Fat	0.2 g	Cu	0.19
Protein	0.9 g	S	24 mg
Water	94.5 g	Cl	38 mg
Vit A	833 IU	Na	5 mg
Vit B1 (Thiamine)	0.037 mg	Ca	20 mg
Vit B3 (Niacin)	0.594 mg	P	24 mg
Vit B6	0.08 mg	K	237 mg
Vit C	14 mg	Lycopene	2537 µg
Vit E	0.54 mg	Oxalic acid	2 mg

2. AREA, PRODUCTION AND PRODUCTIVITY

2.1 Distribution in India Including Regions of Cultivation and Existence of Naturalized Populations

Tomato is cultivated in all the states of India from Jammu & Kashmir to Tamil Nadu (North-South) and Arunachal Pradesh to Gujarat (East-West). The major tomato producing states in India are Andhra Pradesh, Karnataka, Madhya Pradesh, Orissa, Gujarat, Bihar and West Bengal (Fig.1; NHB Database 2012). During 2011-12 India produced 186.53 lakh ton of tomato from 907.1 thousand hectare of land. Andhra Pradesh (28.63%) has maximum share to the production followed by Karnataka (10.65%), Orissa (7.39%) and Madhya Pradesh (7.24%). Statewise area, production and productivity from 2010-2013 in given in Table 4.

Pusa-120, Pusa Ruby, HS-101, HS-102, Hisar Lalit and Hisar Arun varieties are recommended for cultivation all over India. Kashi Vishesh is recommended for Jammu & Kashmir, Himachal Pradesh, Uttarakhand, Punjab, Uttar Pradesh, Jharkahand, Chhattisgarh, Odisha, Andhra Pradesh, Karnataka, Tamil Nadu and Kerala. Besides, several varieties *viz.*, Kashi Aman, Kashi Abhiman,

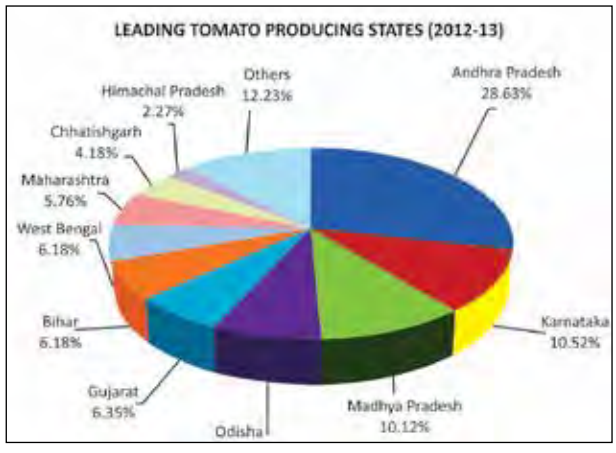


Fig.1: Leading tomato producing states in India 2012-13 (**Source:**NHB Database 2013)

Arka Ananya, Arka Vardhan, Arka Vishal, Arka Abhijit, Arka Vikash, Arka Abha, Arka Alok, Kashi Amrit, Kashi Anupam, Kashi Sharad, Pusa Sheetal, Pusa Gaurav, Pusa Rohini, Pusa Hybrid-2, Pusa Hybrid-4 and Pusa Hybrid-8 are adopted for cultivation in different parts of the country (NHB Database 13).

Table 4: State wise Area, Production and productivity of tomato during last 3 years (NHB Database 2013).

Statewise Area, Production and Productivity of Tomato
Area in '000 HA, Production in '000 MT and Productity = MT/HA

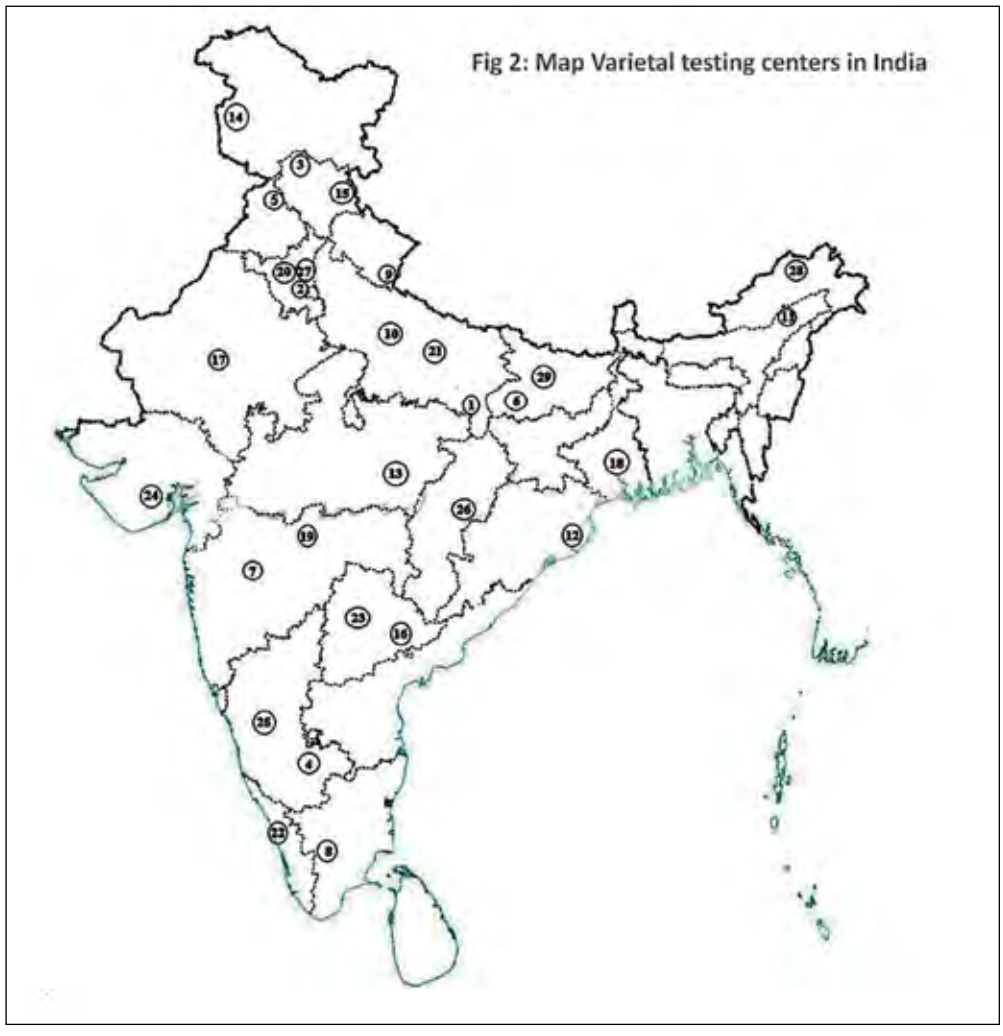
State	2010 - 11			2011 - 12			2012 - 13		
	Area	Production	PDY.	Area	Production	PDY.	Area	Production	PDY.
Andhra Pradesh	296.3	5926.2	20.0	300.8	6015.1	20.1	260.91	5218.10	20.0
Karnatka	51.2	1756.7	34.3	56.6	1986.5	35.1	57.80	1916.60	33.2
Madhya Pradesh	27.8	346.9	12.5	55.3	1349.6	24.4	62.59	1845.00	29.5
Odisha	96.6	1367.2	14.1	96.7	1378.4	14.3	96.55	1382.78	14.3
Gujrat	38.8	978.4	25.2	42.1	1092.5	26.0	44.00	1156.72	26.3
Bihar	46.8	1056.2	22.6	47.2	1104.8	23.4	47.80	1126.25	23.6
West Bengal	54.1	1063.7	19.6	55.2	1104.5	20.0	56.00	1125.60	20.1
Maharashtra	52.0	738.0	14.2	48.0	1007.0	21.0	50.00	1050.00	21.0
Chhatishgarh	42.9	627.9	14.6	44.6	718.5	16.1	47.97	762.22	15.9
Himachal Pradesh	9.9	388.4	39.1	10.0	400.0	40.0	9.93	413.71	41.7
Others	148.4	2576.8	17.4	150.59	2496.44	16.6	146.1	2229.7	15.3
Total	**864.9**	**16826.4**	**19.5**	**9.7.1**	**18653.3**	**20.6**	**879.6**	**18226.6**	**20.7**

2.2 Zonalization of Varietal Testing System

In India, tomato varieties are tested under the umbrella of All India Coordinated Research Project (Vegetable Crops), situated at Indian Institute of Vegetable Research (IIVR), Varanasi. For varietal testing, AICRP-VC has grouped India into 8 vegetable growing zones. Based on the performance, varieties are identified and released for specific zones. Following are the 8 vegetable growing zones for varietal testing system:

- Humid Western Himalayan Region- Jammu and Kashmir, Himachal Pradesh and part of Uttarakhand (Zone-I).
- Humid Bengal Assam Basin- West Bengal and Assam (Zone-II).
- Humid Eastern Himalayan Region and Bay Islands- Arunachal Pradesh, Nagaland, Manipur, Mizoram, Tripura, Meghalaya and Andaman and Nicobar Island (Zone-III).
- Sub-humid Sutlej Ganga Alluvial Plains- Punjab, Uttar Pradesh and Bihar (Zone-IV).
- Humid Eastern and South Eastern Upland- East Madhya Pradesh, Orissa and Andhra Pradesh (Zone-V).
- Arid Western Plains- Haryana, Rajasthan and Gujarat (Zone-VI).
- Semi-arid Lava Plateau and Central High Land- Maharashtra and rest of Madhya Pradesh (Zone-VII).

- Humid to Semi-Arid Western Ghats and Karnataka Plateau- Karnataka, Tamil Nadu, Kerala and Lakshadweep Islands (Zone-VIII).



Head Quarter

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4. Indian Institute of Horticultural Research (IIHR), Bangalore, Karnataka
5. Punjab Agricultural University (PAU), Ludhiana, Punjab
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11. Assam Agricultural University (AAU), Jorhat, Assam
12. Orissa University of Agriculture & Technology (OUAT), Bhubaneshwar, Orissa
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14. Department of Horticulture, Jammu & Kashmir, Srinagar, Jammu &Kashmir
15. Himachal Pradesh Agricultural University (HPAU), Palampur, Himachal Pradesh
16. Andhra Pradesh Agricultural University (APAU), Lam, Guntur, Andhra Pradesh
17. Rajasthan Agricultural University, Durgapura, Jaipur, Rajasthan
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23. Andhra Pradesh Agricultural University (APAU), Hyderabad, Andhra Pradesh
24. Gujarat Agricultural University (GAU), Junagarh, Gujarat
25. University of Agricultural Science (UAS), Dharwad, Karnataka
26. Indira Gandhi Krishi Vishwavidyalaya (IGKV) , Raipur, Chhattisgarh
27. IARI (Reg. Station), Karnal, Haryana (After the merger of AICVIP with NSP)
28. CAU, Pasighat, Arunachal Pradesh
29. Rajendra Agricultural University, Samastipur, Bihar

3. GEOGRAPHIC ORIGIN, GENOMIC EVOLUTION AND CHROMOSOME NUMBER

3.1 Centers of Origin and Diversity

India is neither the centre of origin of tomato nor has genetic diversity of tomato. Wild tomatoes are native of western South America, distributed from Ecuador to northern Chile (Darwin *et al.*, 2003; Peralta and Spooner, 2005). They grow in variety of habitats, from near sea level to over 3,300 m in elevation, in arid coastal lowlands and adjacent regions where the pacific winds drop scarce rainfall and humidity; in isolated valleys in the high Andes, and in deserts like the severe Atacama Desert in northern Chile. Andean topography, diverse ecological habitats and different climates have contributed significantly to wild tomato diversity (Fig 3).

Based on morphological characters, phylogenetic relationships, and geographic distribution, scientists proposed the segregation of four species within the highly polymorphic green-fruited species *S.peruvianum* sensulato (sensulato refers to a broad concept of a species): *S. arcanum, S. huaylasense, S. peruvianum*, and *S. corneliomulleri*. The first two have been described as new species (Peralta et al., 2005) from Perú, while the latter two had already been named by Linnaeus (1753) and MacBride (1962), respectively. Another new yellow- to orange-fruited tomato species, *S. galapagense*, segregated from *S. cheesmaniae*, have been recognized; both are endemic to the Galápagos Islands (Darwin *et al.*, 2003). In total, 13 species of tomatoes, including the cultivated tomato (*Solanum lycopersicum*) and its weedy escaped forms are distributed worldwide (Table 5) . This is an increase from the nine species of tomatoes traditionally recognized (Rick *et al.*, 1990).

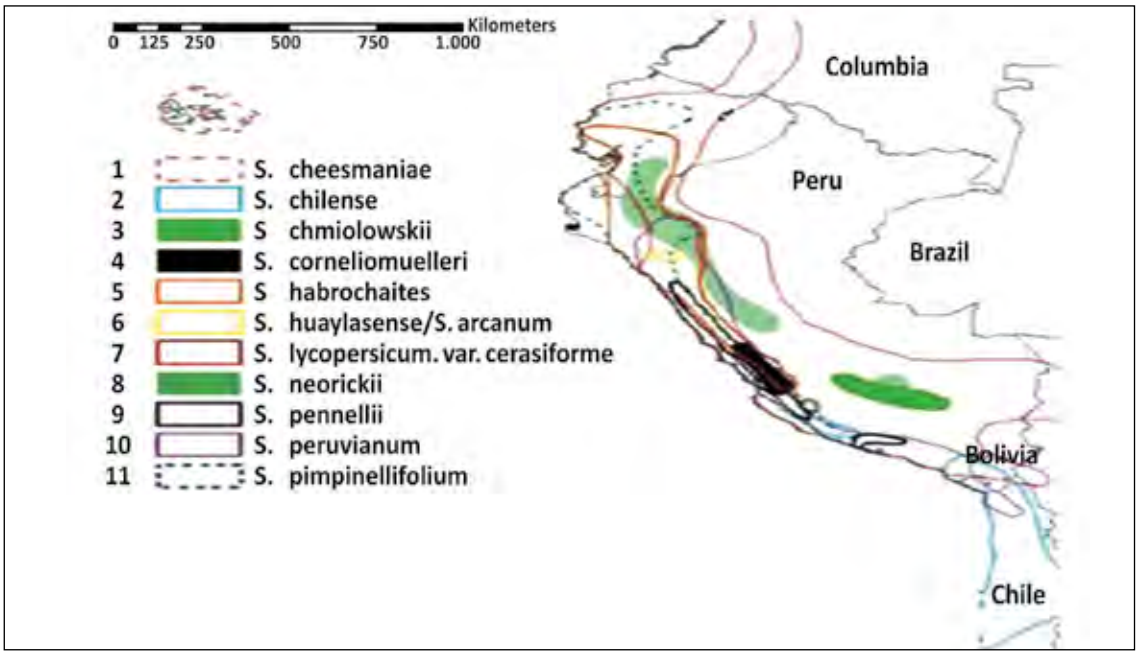

Fig.3: Wild tomato species geographical distribution in South America. Inset: Environmental variation of wetness based on distribution of populations location (**Source:** Moyle, 2008)

Table 5: Species list for *Solanumsection Lycopersicon*, with equivalents in the previously recognized genus *Lycopersicon,* now part of a monophyletic genus *Solanum* (Peralta et al. 2005)

Solanum name	*Lycopersicon* equivalent	Distribution; habitats
S. arcanum Peralta	Part of *L. peruoianum* (L.) Miller	N Peru, coastal and inland Andean valleys; lomas, dry valleys and dry rocky slopes; 100 to 2800 m.
S. cheesmanie (L. Riley) Fosberg	= *L. cheesmaniae* L. Riley (incorrectly published as *cheesmanii*)	Endemic to the Galápagos Islands, Ecuador; wide variety of habitats; sea ;eve; tp 500 m.
S. chilense (Dunal) Reiche	= *L. chilense* Dunal	S Peru (Tacna) to N Chile (Región II); in hyper-arid rocy plains and coastal deserts; sea level to 3250 m.
S. chmielewskii (C.M. Rick, Kesicki, Fobes & M. Holle) D.M. Spooner, G.J. Anderson & R.K. Jansen	= *L. chmeilewskii* C.M. Rick, Kesicki, Fobes & M. Holle	S Peru (Apurimac) to N Bolivia (La Paz); high dry Andean valleys; 1600–3200 m.
S. corneliomuelleri J.F. Macbr.	Part of *L. peruvianum* (L.) Miller; also known as *L. glandulosum* C.F. Mull.	Central to S. Peru, W slops of the Andes; landslides and rocky slopes; (40)200–3300 m.
S. galapagense S. Darwin & Peralta	Part of *L. cheesmaniae* L. Riley (previously known as forma or var. *minor*)	Endemic to the Galápagos Islands; mostly occurring on coastal lava to within 1 m of high tide mark within range of sea spray, but occasionally inland; sea-level to 50 m.
S. habrochaites S. Knapp & D.M Spooner	= *L. hirsutum* Dunal	Central Ecuador to Central Peru, on the western slopes of the Andes; in a variety of forest types from premontane forests to dry forests; (40)200–3300 m.
S. huaylasense Peralta	Part of *L. peruvianum* (L.) Miller	N Peru (Ancash); rocky slopes of the Callejón de Huaylas along the Río Santa and in the adjacent Río Fortaleza drainage; (940)1700–3000 m.
S. lycopersicum L.	= *L. esculentum* Miller	Known only from cultivation or escapes; world-wide in a variety of habitats, many escaped plants have smaller fruits ("cerasiforme"); sea level to 4000 m.
S. neorickii (C.M. Rick, Kesicki, Fobes & M. Holle) D.M. Spooner, G.J. Anderson & R.K. Jansen	= *L. parviflorum* C.M. Rick, Kesicki, Fobes & M. Holle	S Ecuador (Azuay) to S Peru (Apurimac); dry inter-Andean valleys, often found trailing over rocky banks and roadsides; (920)1950–2600 m.
Solanum pennellii Correll	= *Lycopersicon pennellii* (Correll)D'Arcy	N Peru (Piura) to N Chile (Tarapacá); dry rocky hillsides and sandy areas; sea level to 2300 m.
S. peruvianum L.	= *L. peruvianum* (L.) Miller	Central Peru (Ancash) to N Chile (Región) II); coastal lomas formations field edges in coastal river valleys; sea level to 600 m.
S. pimpinellifolium L.	= *L. pimpinellifolium* (L.) Miller	Central Ecuador to central Chile; dry coastal habitats; 0–500 m, but exceptionally up to 1400 m.

3.2 Genomic Evolution

The genus *Solanum* includes the cultivated tomato (*Solanum lycopersicum*) together with its wild relativesbearinga wealth of genetic variability. Less than 10% of the total genetic diversity in the *Solanum* gene pool is found in *S. lycopersicum* (Miller and Tanksley, 1990). The primary center of diversity for tomato is located in western South America, and the cherry tomato *S. lycopersicumvar. cerasiforme* is considered as the most immediate ancestor of cultivated tomatoes. Karyotypes of the *Solanum* species are very similar with little or no structural difference among species. As a crop plant, tomato is one of the best-characterized plant model systems. It has a relatively small genome of 0.95pg or 950Mb per haploid nucleus (Arumuganathan and Earle, 1991), and features such as diploidy, self-pollination, and a relatively short generation time make it amenable to genetic analysis.

The tomato clade of Solanum (*Solanum* sect. *Lycopersicon*) includes 12 species and subspecies. All are diploid (2n = 2x = 24), except 2 natural tetraploid populations of S. chilense (2n = 4x = 48) (Chetelat and Ji, 2007), and share the same number of acrocentric to metacentric chromosomes with large blocks of pericentric heterochromatin and distal euchromatic arms (Brown, 1949; Barton, 1950). The only exception to this generalization is chromosome 2 with a completely heterochromatic short arm including a distal nucleolus organizing region (NOR) (Barton, 1950).

Classical genetics has created one of the largest stocks of morphological mutations induced by radiation (X-rays, UV-light, neutrons) and chemical mutagenesis. An interesting example of induced mutagenesis was the directed manipulation of fruit size of *S. lycopersicum* and *S. pimpinellifolium*. A considerable proportion of these mutations have been mapped into the classical genetic map. By 1988, the classical linkage map of the tomato genome comprised of 233 morphological and isozyme loci. An additional 86 have been assigned to their respective chromosomes via two-point or trisomic tests. The number of mapped genes in the form of cDNAs has increased considerably with the introduction and application of RFLP markers. The current tomato RFLP map was constructed using an F2 population of the interspecific cross *S. lycopersicum* x *S. pennellii* and contains more than 1030 markers distributed over 1276 cM (Tanksley et al.1992). A number of morphological and isozyme markers have also been mapped with respect to RFLP markers orienting the molecular linkage map with both the classical morphological and cytological maps of tomato. An integrated high-density RFLP-AFLP map of tomato based on two independent *S. lycopersicumx L. pennellii* F_2 populations has been constructedspanning 1482 cMwith 67 RFLP and 1175 AFLP markers. Both RFLP and AFLP maps show clusters of markers associated with almost all centromeres and some telomeres indicating that recombination is suppressed in those regions.

The current tomato map is considered to be complete in that all molecular and classical markers could be mapped to one of the 12 linkage groups indicating that no loci failed to link up with the map. The average relationship between genetic and physical distance in tomato is about 750 kb per cM. The actual ratio of genetic and physical distance varies considerably depending on the chromosomal region. High-resolution genetic and physical mapping around the *Tm-2a* region, which is located close to the centromere of chromosome 9, indicates that one cM in this area corresponds to more than five million base pairs, approximately a sevenfold suppression of recombination over the expected value based on the estimated physical size of the region. In contrast, map-based cloning of the chloronerva gene, which is involved in iron uptake and located in euchromatin of chromosome 1, demonstrated that the ratio of genetic to physical distance in the chloronerva region is 160 kb per 1 cM, suggesting much higher levels of recombination in this area of the genome. By determining frequency and distribution of recombination nodules on tomato synaptonemal complexes, it was observed that frequency of recombination nodules in heterochromatic regions around the centromeres is much lower compared to euchromatin. Suppression of recombination near the centromeres and higher values of recombination in distal chromosomal regions were also observed in potato (Tanksley *et al.*, 1992) and many other plant and animal species.

The tomato genome at the DNA level comprises of approximately 78% single copy sequences, as evaluated under high stringency hybridization conditions. In other plant species with large genome sizes, such as wheat or pea, the single copy fraction is less than 20%, and in barley and rye, it is less than 50%. The remaining part of the tomato sequences is repetitive DNA of which four major classes have been characterized. Ribosomal

DNA represents the most abundant repetitive DNA family and comprises approximately 3% of the tomato genome. Both 5S and 45S rRNA genes are tandemly repeated with 1,000 and 2,300 copies and map to single loci on chromosome 1 and 2, respectively. As confirmed by in-situ hybridization, a 162 bp tandem repeat, TGRI, with 77,000 copies in the genome is localized within a few hundred kb of the terminal 7 bptelomeric repeat TT(T/A)AGGG at 20 of 24 chromosome ends; and, in addition, it is also found at a few centromeric and interstitial sites (Ganal *et al.*, 1991). Two other tomato genomic repeats, TGRII and TGRIII, are less abundant with 4,200 and 2,100 copies, respectively. TGRII is apparently randomly distributed with an average spacing of 133 kb, and TGRIII is predominantly clustered in the centromeric regions of chromosomes. Except TGRIII, these repeats are only present in *Solanum* species. (Ganal *et al.*, 1991).

The approximate map position of the centromere is now known for each tomato chromosome. For chromosomes 1 and 2, the centromere positions have been identified by RFLP mapping and by in-situ hybridization with 5S rDNA and 45S rDNA respectively. The centromeres of chromosomes 3 and 6 have been located on the integrated molecular-classical map and by deletion mapping. Since there is evidence that the potato/tomato inversions on chromosomes 5, 10, 11 and 12 involve entire chromosome arms, the respective centromeres are most likely located at the inversion breakpoints (Tanksley *et al.*, 1992). Map positions of the centromeres of chromosomes 4 and 8 were predicted based on the relationship among the cytological, genetic and molecular tomato maps. Despite their functional importance, the molecular characteristics of the centromeres of higher eukaryotes remain ill-defined. The most extensively studied DNA sequence is the 171bpα-satellite sequence, which is located exclusively at the primary constriction of human chromosomes and thought to play a major structural and/or functional role at human centromeres. So far, no plant DNA sequences essential for centromere function have been identified.

Microsatellite polymorphism and genomic distribution were studied by fingerprinting the tomato genome using labeled oligonucleotide probes complementary to GATA or GACA microsatellites. The copy number and the size of microsatellite containing restriction fragments were highly variable among tomato cultivars. The mapping of individual fingerprint bands containing GATA or GACA microsatellites showed predominant association of these repeats with tomato centromeres. GATA clusters are now known to be located in most if not all of the centromeric regions of the tomato chromosomes. A number of polymorphic microsatellite markers generated from database sequences have been used successfully for genotyping tomato cultivars and accessions but their map positions have not been published to date.

3.3 Genetic Diversity of Indian Germplasm

There is no naturally growing wild species of tomato in India. Although a large number of germplasm lines of tomato are available at various institutes including NBPGR, New Delhi and IIVR, Varanasi. These lines also include several potential wild species like *S. pimpinellifolium, S. lycopersicumvar. cerasiforme, S. peruvianum, S. habrochites, S. pimpinellifoliumetc*. NBPGR in collaboration with several institute and state

agricultural university has successfully completed several exploration trips in different parts of country. Fruit weight, plant height and number of fruits per plant are the potent factors in differentiating the germplasm of tomato in India contributed 92.40% to the total divergence (Reddy *et al.*, 2013). High pair-wise similarity has been observed among tomato varieties bred by both public and private sector institutions demonstrating the narrow genetic base among Indian tomato cultivars irrespective of the source of breeding (Patil *et al.*, 2010). Interestingly, old introductions and locally developed cultivars of the 1970s exhibited significantly greater genetic variation than the ones released during the 1990s (Archak *et al.*, 2002). Reduction in the genetic diversity among modern tomato cultivars (Vishwanath *et al.*, 2013) may be attributed to the recent trend towards breeding for similar plant and fruit characteristics. Further, difference in genetic relationship inferred by morphological /protein/ RAPD can be attributed solely to difference in level of polymorphism detected by each marker system, but rather than that they reflect the complexity of the inheritance of different quantitative and qualitative characters. Although, reduction in the genetic diversity among the modern Indian tomato cultivars may be attributed to the trend towards breeding for similar ideotypes in achieving higher productivity, this drift is ominous for the breeders from the perspectives of maintenance of genetic diversity and acquiring breeders' rights over novel varieties.

Donor germplasm lines have been identified with definite source of resistance, i.e. ability to tolerate temperature stress and drought conditions. These can be used to develop lines possessing resistance one or more stresses. There was a wide variation among genotypes for fruit set under high temperature conditions, and a review of the evaluation studies (at Delhi with temperature of 38-40^{0}C) reveals that 43 accessions set fruit at high temperature. Varieties HS-101 and HS-102 had the ability to set fruit in April, when temperature was 35-39^{0}C in May. EC 130042, *S. cheesmaniiand* EC-162935 set fruits at high temperatures due to the stigma exertion of less than 1 mm, whereas other sensitive genotypes produced more than 1 mm of stigma exertion. At IIVR, genotype EC-528380, VRT 101-A & EC-538148 performed better in open field conditions in February to May, 2012 (>45^{0}C), while genotypes, EC- 620447, EC- 439542, F-7-1, EC- 620567, EC- 625645, EC- 620561, EC- 620443, DT-10, EC- 620535, EC- 620540, EC- 620504, EC- 620541, EC-620648, EC- 538155 and EC- 620570 showed more than 90% pollen viability in same high temperature conditions. The most serious problem of high temperature is the reduced size of fruits. Generally there is fruit set in heat tolerant lines but development of such fruit is very slow and poor, with the result that fruits remain smaller. Wild species show remarkable variation in their inherent adaptation to drought stress. *S. pennelliiand S. lycopersicumvar. cerasiformeare* drought tolerant genotypes. *S. habrochaites* species is tolerant to cold and drought. EC-130042, Sel-28, EC-65992 and *S. pimpinellifoloium* (PI-205009) require more days to express wilting, thus showing tolerance to drought conditions. At IIVR, genotypes Co-3, D-3-2, I-4-4, Kashmiriya, G-4-5 performed better under severe moisture stress condition. Sel-28, *S. cheesmanii*, K-14, EC-104395, *S. pimpinellifolium* (EC-65992) and *S. pimpinellifollium* (Pan-American) are the best potential source for drought tolerance.

4. REPRODUCTIVE BIOLOGY

4.1 Reproduction

The cultivated tomato plants generally reproduce by means of self-pollination. The reproduction of the tomato plant involves the stamen and the carpels. The stamen produces pollen that can fertilize the carpels. The carpel gets fertilized once a pollen grain enters its pollen tube. Rarely cross pollinations may occur with the help of an insect or animal bringing the pollen to another tomato plant. After the ovule is fertilized, it develops into an embryo which in turn matures into a seed. The seed is wrapped with flesh within a mature fruit. The fruit can then be spread by being eaten and digested by an animal.

4.2 Floral Biology of Tomato

Tomato plants have yellow flowers that, in full bloom, are generally less than an inch in diameter. The flowers can occur in a simple or a complex inflorescence. There are different types of inflorescences (Fig 3). A raceme inflorescence is one in which the flowers branch off laterally from a main shoot that grows indefinitely. The number of flowers that occur in an inflorescence is dependent upon environmental factors such as temperature.

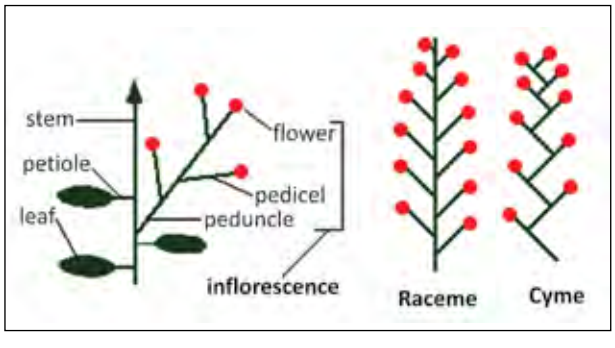

Fig.4: Inflorescence in tomato

In a cyme inflorescence, the shoot apex differentiates into a flower, subsequent growth occurs due to activity in an axillary branch which will eventually terminate in a flower. The tomato flower occurs in the three organizational patterns drawn below: simple flowers, simple cymes and branched cymes (Fig 4).

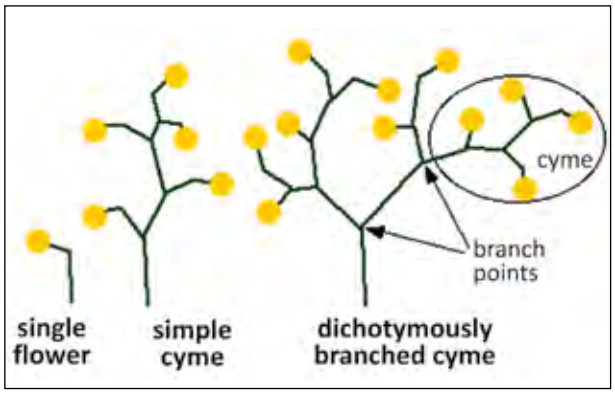

Fig.5: Types of cyme inflorescence in tomato

The pedicel is the stem that supports the flower. The outermost whorl consists of the sepals. Collectively, the sepals are called the calyx. The next whorl, the bright yellow petals, serves to attract pollinators. Together, the petals are called the corolla (Fig 5).

The male reproductive organs (stamens), which house pollen production, sit inside the petals. A single tomato stamen consists of two elongated compartments. The individual stamens are fused together to form a yellow cylinder that surrounds the carpels. The tomato carpels are green. They vary in number from cultivar to cultivar, but they are invariably fused together into a single bulb-like structure. The number of carpels in the tomato flower corresponds to the number of locules found in the fruits. Fertilization takes place in the carpels. The ovules which develop into seeds are protected in the carpel.

Fig.6: Typical flower structure of tomato

4.3 Methods of Pollination, Known Pollinators and Pollen Viability

Most plants in the wild are self-sterile as nature dislikes incestuous propagation and prefers cross pollination, where the pollen is transferred between two genetically different plants. Cross pollination is achieved for most vegetables by an agent (called a pollinator), that physically moves the pollen. Usually this is a bee, because they are specially equipped to efficiently carry pollen; they are brawny, fuzzy, and have a static charge, so they acquire and transfer many grains of pollen. In the wild, original state, tomatoes required cross-pollination; they were much more self-incompatible than domestic cultivars. As a floral device to reduce selfing, the pistil of wild tomatoes extends farther out of the flower than in present day cultivars. The stamens remain entirely within the closed corolla.

As tomatoes were moved from their native areas, their traditional pollinators, (probably a species of halictid bee) did not move with them. The trait of self-fertility became an advantage, and domestic cultivars of tomato have been selected to maximize this trait. This is not the same as self-pollination, despite the common claim that tomatoes do so. That tomatoes pollinate themselves poorly without outside aid is clearly shown in greenhouse situations, where pollination must be aided by artificial wind, vibration of the plants (one brand of vibrator is a wand called an "electric bee" that is used manually), or more often today, by cultured bumblebees. The anther of a tomato flower is shaped like a hollow tube, with the pollen produced within the structure, rather than on the surface, as in most species. The pollens move through pores in the anther, but very few pollens shed without some kind of outside motion. The best source of outside motion is a sonicating bee, such as a bumblebee, or the original wild halictid pollinator. In an outside setting, wind or animals provide sufficient motion to produce commercially viable crops.

Pollen viability is measured as percent of flowers that set fruit, and as number of seed per fruit. High temperature is most critical factor which affects the pollen production, pollen viability and other reproductive mechanisms in tomato because reproductive structures of the tomato plants are highly responsive to temperature.

4.4 Seed Production and Dispersal

Tomato is a warm season crop and requires frost free period of about four months for seed production. Optimum temperature for seed production is

16-29oC and for growth and fruit set between 20-25oC. Below 15oC and above 32oC, the pollen germination is very poor. Hence, very high and very low temperature and drought adversely affect fruit setting. High temperature combined with dry winds causes blossom drop. A warm and sunny weather is most suited for proper fruit set and seed development which results in higher seed yield. To maintain the purity of variety, a minimum isolation distance of 50m for foundation seed and 25m for certified seed should be provided all around the field to check the contamination from other varieties of tomato (Vegetable seed Production, CCSHAU, Hisar). Another study conducted by Singh *et. al.* (2012) projected the isolation distance for certified seed production of tomato for open pollinated and hybrid seeds to be 25 and 100 m, respectively.

In order to maintain the biological diversity of tomato, the seeds need to be distributed at some distance from the parent plant. This is true for all plants, and the means of dispersal varies widely among the different species. Some are spread by the wind, some by water, some with sticky coatings or spikes get stuck to the fur of passing animals, and some get eaten by animals/birds to be later passed, undigested, onto the ground great distances away.Tomatoes, along with many other fruits such as strawberries, raspberries, grapes and plums contain seeds surrounded by gelatinous coats which allow the seeds to pass through an animal's digestive system without damage to the seed itself. This is a biological mechanism for seed dispersal is called endozoochory. The fruit is consumed by a bird, or perhaps a mammal, along with the seeds. The seeds themselves are later excreted without being digested and are thereby scattered. In time, through the action of natural processes in the soil, the outer coating dissolves away, leaving the seed free to germinate and produce a new plant.

4.5 Potential for Vegetative Propagation

Though tomato is universally and commercially propagated through seed but asexual propagation techniques like grafting and micro propagation have been successfully attempted by several researchers across the globe. Extensive research has been carried out for grafting mechanism, selection of rootstock, time of grafting and other related aspects in tomato. Potato rootstock 'KufriSinduri' was used to graft scion of a popular tomato variety'Pusa Ruby' to find out the effect of time of grafting on yield and maximum yield was reported from the plants grafted in December month. High humidity and low light intensity prevented wilting of scion and favouring healing of the graft union. For the micropropagation of tomato, direct regeneration from different explants like shoot tips, cotyledon, hypocotyl, leaves and seeds as well as generation via callogenesis of the explants has been reported. Till date, there are no reports of survival and regeneration of any vegetative plant parts in the soil from tomato plant.

4.6 Growth Stages of Tomato

Tomatoes undergo four main stages of growth during their life:

4.6.1 Seed germination

The first stage is of course the seed sprouting. If we grow tomatoes from seed, we can expect our seed to germinate and sprout within eight days. Tomatoes need damp to moist soil for germination and 24-29° Celsius. The sprouts will unfurl seed leaves which are not true leaves. During this stage the tomato sprout will begin developing its root

system, using the cotyledons or seed leaves to begin the process of photosynthesis. We can speed up this stage somewhat by giving your sprouts about twelve to fifteen hours of light each day.

4.6.2 Vegetative stage

After a few weeks the tomato plant will enter its second stage of growth. Here the plant will begin to make its first set of true leaves. During this

stage most of the tomato plant's energy is directed toward forming strong roots and leaves. The stem will get stronger to support the increasing weight of the leaves. At this stage, tomatoes need at least six hours of full sun every day.

4.6.3 Flowering stage

In the third stage of growth the tomato plant will begin to develop its first set of flowers. Determinate tomato plants are ones where the

plant bears all of its fruit at once. Following this third stage of growth, these plants will generally stop growing new leaves. Such plant will not get taller anymore. Indeterminate tomato varieties will continue to grow taller, produce new leaves, and set additional flowers even after this stage of growth.

4.6.4 Fruiting stage

The fourth stage of tomato growth begins when the

flower petals die back and the tomato fruit begins to swell. Tomato fruit starts out with green color and will retain this color until it reaches full size. When the fruitattains full size,it will begin to ripen. The first sign of ripening occurs at the bottom of the fruit or the blossom end. Indeterminate varieties will stay longer in this stage of growth than determinate ones. Semi-determinate tomato varieties will fall somewhere in between. After fruit ripening, the tomato will begin to die back.

5. HYBRIDIZATION AND INTROGRESSION

5.1 Naturally Occurring Interspecific Crosses

Wild tomato species are useful for studies of Interspecific Reproductive Barriers (IRBs) (Bedinger *et al.* 2010). Wild tomatoes display significant differences in morphology, mating systems, and habitat preferences. There are 12 wild species related to the domesticated tomato according to recent taxonomic studies (Figure 3). These wild species are endemic to South America and range from central Ecuador through Peru to northern Chile on the western Andean Slope (Peralta and Spooner 2005; Moyle 2008; Darwin et al. 2003). All of species of *Solanumhave* the same chromosome number and are diploid (2n=24). There are no major differences in chromosome structure among the wild tomato species, and they share a high degree of genomic synteny, although some chromosomes have been detected structural changes such as mismatched kinetochores or inversion loops in F1 hybrids. In addition to its diploid genome, there are many genetic resources that make the tomato clade a good study system. These include genomic resources, extensive collections of wild species, collections of expressed sequenced tags, and mutants (Moyle 2008; Bedinger *et al.* 2010).

Species in the tomato clade exhibit three types of mating systems (Fig 6). Autogamous self-compatible species that accept self-pollen include *S. lycopersicum, S. galapagense, S. cheesmaniae, S. pimpinellifolium,* and *S. neorickii*. Facultative self-compatible species such as S. chmielewskiiis self-fertile but has floral morphology characters to promote outcrossing. Allogamous self-incompatible species reject self-pollen, which forces outcrossing. Species that are mostly SI but have some SC populations include *S. arcanum, S. habrochaites, and S. pennellii* (Peralta and Spooner, 2005; Moyle 2008; Bedinger 2010). These mating systems are correlated with floral morphology characters. Self-compatible species in the tomato clade, including the domesticated tomato, *S. lycopersicumhave* smaller flower size and less stigma exsertion. In contrast, self-incompatible species have larger flower size and longer exserted stigmas both of which promote outcrossing. None of these wild species are naturally occurring in tomato growing regions of India. Hence, there is no chance of gene flow from cultivated tomato to any wild species in Indian conditions. Studies conducted by Ilardi and Barba (2002) and Iwasaki *et al.*, (2005) on transgenic tomato plants confirmed that there is no gene/transgene flow from transgenic tomato line to the other tomato plants/environment.

Interspecific Reproductive Barriers (IRBs) preserve species identity by preventing interspecific hybridization, an essential facet of the biological species concept. Wild tomato species (*Solanum* sect.

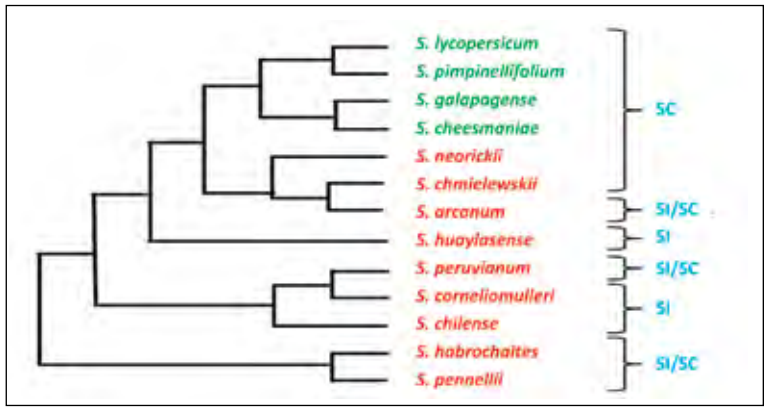

Fig.7: Phylogenetic tree of tomato species. Red colored species: red-fruited species, green colored species: green-fruited species. SC= self-compatible, SI=self-incompatible, SI/SC= both mating system exhibit (**Source:** Bedinger *et al.* 2010)

lycopersicum) are useful for studying interspecific reproductive barriers. Within the tomato clade, there are 13 closely related species possessing diverse mating systems and complex IRBs. IRBs can be divided into two types: those occurring before mating (premating barriers) and, those operating after mating (postmating barriers). Premating barriers include a variety of floral morphological characters correlated with a diversity of mating systems. Postmating barriers can be subdivided into prezygotic, those acting after mating but before fertilization, and postzygotic, those acting after fertilization. In the tomato clade, regulation of pollen tube growth in pistils constitutes postmating-prezygotic barriers that are known to be important for preventing hybridization. Unilateral incongruity/incompatibility (UI), which prevents hybridization in one direction of an interspecific cross by inhibiting pollen tube growth in the pistil, is common in the tomato clade. Postzygotic barriers are also important as genetic isolating mechanisms resulting in failure of fruit or viable seed production in cases where prezygotic barriers are absent.

5.2 Experimental Interspecific Crosses

The wild taxa of tomato possess a large reservoir of economic attributes, particularly resistance to biotic and abiotic stresses and quality attributes. Wide crosses have been attempted to develop resistant, high quality and high-yielding cultivars, to produce new genetic variation and to generate information on cytogenetical investigations. The wild taxa are more important for the development of resistant cultivars. In several biotic and abiotic stress areas, the survival of tomato cultivars is largely due to the presence of resistance gene(s) in the cultivars derived from the wild species (Kalloo 1991). Thirteen closely related species of tomato along with four closely allied *Solanum* species, give a distinct group with diverse mating systems that display complex interspecific reproductive barriers. Different types of pre- and postzygotic barriers have already been recognized within the genus *Solanum*. Well-developed genetic maps, introgression lines, interspecific bridging lines, and the newly available draft genome sequence of the domesticated tomato

Table 6: Crossing behavior within the tomato clade (Mutschler and Liedl 1994)

Female	Male									
	S. lyc	*S. pim*	*S. che*	*S. neo*	*S. chm*	*S. hab g*	*S. hab*	*S. pen*	*S. chi*	*S.per*
S. lyc	SC	C	C	C	C	C	C	C	C*	C*
S. pim	C	SC	C	C	C	C	C	C	F	F
S. che	C	C	SC	C	C	C	C	C	F	F
S. neo	I	I	I	SC	C	C	C	C	F	F
S. chm	I	I	I	C	SC	I		I	F	F
S. hab	I	I	I	I	I	I	SI/SC	C	F	F
S. pen	I	I	I	I	I	I	I	SI/SC	I	I
S. chi	I	I	I	I	I	I	I	I	SI	var
S.per	I	I	I	I	I	I	I	var	var	SI/SC

S. lyc, S. lycopersicum; S. pim, S. pimpinellifolium; S. che, S. cheesmaniae (includes data from both *S. cheesmaniae* and *S. galapagense*); *S. neo, S. neorickii; S. chm, S. chmielewskii; S. hab, S. habrochaites; S. pen, S. pennellii; S. chi, S. chilense; S. per, S. peruvianum* (SC: self-compatible, SI: self-incompatible, C: successful cross, I: unsuccessful cross, C*: embryo rescue needed, F: fruit with no viable seed, var: variable)

(*Solanum lycopersicum*) are valuable tools for the genetic analysis of interspecific reproductive barriers. The wild relatives of the cultivated tomato provide a great diversity in mating systems and reproductive biology (Rick 1988).

Interspecific crosses have been performed among the members of the tomato clade, and fruit set assessed, to determine compatibility among them (Bedinger *et al.* 2010). Crosses are successful when the self-compatible red fruited species (*S. lycopersicum, S. pimpinellifolium*, and the two closely related *S. cheesmaniae and S. galapagense*) are used as female parents with pollen from the self-incompatible green-fruited species; on the other hand, the reciprocal crosses are not successful. Additionally, crosses between the self-compatible green fruited species *S. neorickii* as female parent with self-incompatible species as pollen parents are successful but not the reciprocal crosses. The phenomenon of one-way success in interspecific crosses is known as unilateral incompatibility or incongruity (UI). Unilateral incompatibility often follows the "SI × SC" rule wherein SI species reject pollen of SC species but not vice-versa. However, pollen rejection in the self-compatible species *S. chmielewskii* shows an exception to the SI × SC rule, because pistils of this species reject pollen from both self-compatible and self-incompatible species, accepting only self-pollen and of that of *S. neorickii*. Other exceptions to the SI × SC rule include the rejection of *S. lycopersicum* pollen on pistils of SC accessions of *S. pennellii* (Hardon 1967; Liedl *et al.* 1996) and S. habrochaites (Martin 1961), and cases of rejection of pollen from SI species on pistils of other SI species (e.g., *SI S. pennellii* × *SI S. habrochaites*). Despite these exceptions, the SI × SC rule is still a useful generalization within the tomato clade, as it is in other Solanaceae (Table 6) (Lewis and Crowe 1958).

5.3 Genetic Introgression

Different traits including disease or abiotic stress resistance have been transferred into cultivated crops through distant hybridization with their wild relatives. The thirteen recognized species of tomato which are closely related to each other have been extensively used for improvement of the tomato crop. In addition, the lack of geographical barriers has permitted natural hybridization between *S. lycopersicum* and its closest wild relative *S.pimpinellifolium* in Ecuador, Peru and northern Chile. In order to better understand patterns of *S. lycopersicum* diversity, 47 markers ranging in length from 130 to 1200 bp (total of 24 kb), were sequenced in genotypes of S. lycopersicum and wild tomato species (*S. pimpinellifolium*, *S.arcanum, S.peruvianum, S.pennellii* and *S.habrochaites*).

Several of the markers had previously been hypothesized as carrying wild species alleles within *S. lycopersicum* (Labate and Robertson, 2012) and they reported that each marker was mapped with high confidence to a single genomic location using BLASTN against tomato whole genome shotgun chromosomes (SL2.40) database. Hybridization and parsimony splits networks, genomic map positions of markers relative to documented introgressions, and historical origins of accessions were used to interpret evolutionary patterns at nine markers with putatively introgressed alleles. Out of 47 genetic markers surveyed, four were involved in linkage drag on chromosome 9 during introgression breeding, while alleles at five markers apparently originated from natural hybridization with S. pimpinellifolium and were associated with primitive genotypes of *S. lycopersicum*. The positive identification of introgressed genes within crop species such as *S. lycopersicum* will help inform conservation and utilization of crop germplasm diversity (Labate and Robertson, 2012).

Eshed andZamir (1995) reported a novel population consisting of 50 introgression lines (ILs) originating from a cross between the green-fruited species *S.pennellii* and the cultivated tomato. Each of the lines contains a single homozygous restriction fragment length polymorphism-defined *S. pennellii* chromosome segment, and together the lines provide complete coverage of the genome and a set of lines nearly isogenic to cultivated tomato variety. Chitwood *et al.*(2013) genotyped an IL population derived from the wild desert tomato (*S.pennellii*) at ultrahigh density, providing the exact gene content harbored by each line. To take advantage of this information, they determine IL phenotypes for a suite of vegetative traits, ranging from leaf complexity, shape, and size to cellular traits, such as stomatal density and epidermal cell phenotypes. Elliptical Fourier descriptors on leaflet outlines provide a global analysis of highly heritable, intricate aspects of leaf morphology. They also demonstrate constraints between leaflet size and leaf complexity, pavement cell size, and stomatal density and show independent segregation of traits previously assumed to be genetically co-regulated. Meta-analysis of previously measured traits in the ILs shows an unexpected relationship between leaf morphology and fruit sugar levels, which RNA-Seq data suggest may be attributable to genetically co-regulated changes in fruit morphology or the impact of leaf shape on photosynthesis.

5.4 Gene Flow to Other Organisms

The only means by which genes could be transferred from non-plant organism is by horizontal gene transfer (HGT). Such transfers have not been demonstrated under natural conditions and deliberate attempts to introduce them have failed so far. Thus, gene transfer from tomato to other organisms (other than plants) is extremely unlikely.

6. KNOWN INTERACTIONS WITH OTHER ORGANISMS IN MANAGED AND UNMANAGED ECOSYSTEMS

6.1 Interactions in Unmanaged and Managed Ecosystems

In tomato, bumble bee, honey bee and solitary bee are reported pollinators. However, there is no specific study/report on ecological interaction of the tomato within natural and managed ecosystems.

6.1.1 Free living populations of tomato

The term free living is assigned to plant populations that are able to survive, without direct human assistance, over the long term in competition with the native flora. This is a general ecological category that includes plants that colonize in open, disturbed prime habitat that is either under human control (weedy populations) or natural disturbed areas such as river banks and sand bars (wild populations). There are no such free living populations of tomato in India.

6.1.2 Weediness of tomato

Tomato has been grown for centuries throughout the world without any reports that it is a serious weed pest. But, no tomato spp. are recognized as problematic weeds, either agriculturally or environmentally in India. Tomato has no relatives that are problematic weeds.

6.2 Important Insect Pests

6.2.1 Fruit Borer (*Helicoverpa armigera* Hubner)

The adult is stout and medium-sized moth and has a dark circular spot in the centre on the forewing.

Fig.8: Borer damaged tomato fruit

They lay small, single, and whitish round eggs on the trifoliate leaves beneath the topmost flower cluster. Eggs hatch in about 3-4 days and the first instars larvae initially feed on the leaves and migrate to the developing green fruit later. The larvae bore into the fruits with the posterior end outside the hole (Fig. 8). Full grown caterpillars show characteristic whitish and dark brown longitudinal stripes.

6.2.2 Serpentine Leaf Miner (*Liromyza trifolii* Burgess)

The tiny, metallic fly punctures the leaf lamina and feeds on the oozing sap. It lays eggs on the outer margin of leaves. Within 2-3 days, whitish maggots hatch out of these eggs and start mining the leaves and pupate in 6-10 days. Pupation takes place in the soil and occasionally on the leaf surface itself. Typical serpentine shaped tunnels are formed in the leaf lamina indicating the path of feeding by the maggots.

Fig.9: Leaf minor affected tomato leaf

6.2.3 Whitefly (*Bemisia tabacii*)

Whitefly is a well-known vector, which transmits tomato leaf curl virus (Fig 10). It has piercing and sucking mouthpart and both nymphs and adults feed on lower surface of the leaves causing deformation of young leaves. Whiteflies also excrete honeydew, causing sooty mold.Under protected conditions whiteflies become more persistent, which require extensive management practices.

Fig.10: Tomato leaf showing whitefly attack

6.2.4 Tobacco Caterpillar (*Spodoptera litura*)

This is a minor pest under open conditions and assumes severe form under protected cultivation particularly in ill managed playhouses (Fig. 11). Eggs are laid in clusters on foliage. Young larvae feed gregariously on leaves. Mature larvae, migrate and cause extensive damage to leaves and fruits. They hide in soil and crop debris during the day time.

Fig.11: Tomato caterpillar

6.2.5 Red Spider Mite (*Tetranychus urticae*)

Red spider mites thrive under high temperature, dry weather and are more serious under protected conditions. They are generally found on the lower

Fig.12: Red spider mite of tomato

surface of older leaves (Fig.12). However, when the infestation is very high they attack all parts of the plant and are observed in colonies covered by white-silky webs. Adults and nymphs lacerate the leaves causing yellowing and discoloration.

6.2.6 Aphid (*Macrosiphum euphorbiae*)

Potato aphids infest a wide range of host plants. Some important cultivated hosts include potato, tomato, eggplant, sunflower, pepper, pea, bean, apple, turnip, corn, sweet potato, asparagus, clover and rose. Sporadic in occurrence, aphid infestations

Fig.13: Aphid attack on tomato

are rarely severe enough to kill plants. Aphids pierce veins, stems, growing tips and blossoms with their needlelike mouthparts (Fig.13). As a result, blossoms are shed and yield is reduced. New growth becomes stunted and curled. Heavily infested plants turn brown and die from the top down. Aphids tend to spread rapidly from field to field transmitting a number of viral diseases. These include various mosaics, leaf roll, spindle tuber and un-mottled curly dwarf.

6.2.7 Predators of Tomato insects

A natural predator of the tomato hornworm/ caterpillar is a tiny beneficial insect called the braconid wasp. This wasp lays its eggs inside the hornworm caterpillar where they hatch into larvae

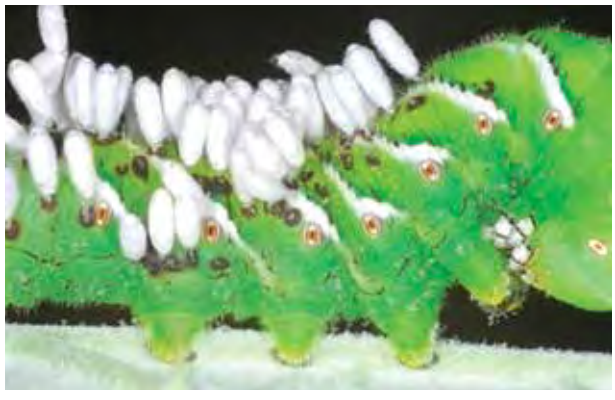

Fig.14: hornworm/caterpillar being attacked by braconid wasp

that feed on the hornworm's muscle tissues, while leaving its heart and other essential organs intact until the larvae mature. This largely paralyzes the hornworm, which becomes merely a living fresh food vessel that sustains the wasp larvae. Once the braconid larvae mature, which takes about a week, they then exit through a hole they make in the hornworm's skin and build a silken cocoon on the outside (as shown in the photograph) within which, like butterflies, they transform into adult braconid wasps that then fly off to infect other tomato hornworms (Fig.14). Different species of braconid wasps parasitize aphids and many other harmful insects. Of course, those tomato hornworm caterpillars that survive produce the magnificent Sphinx moths, one of our largest and most beautiful geometrids, so completely eradicating them is not entirely desirable for aesthetic reasons, and braconid wasps provide an ecologically sound method of keeping the population of tomato hornworms under control without leading to the complete loss of this wonderful lepidopteran.

Fig.15: Ants and aphids on tomato leaf

Aphids and ants have a mutual love affair with tomatoes (Fig.15). Aphids love to suck on tomato stems and consume the sap from these plants. Ants also love honeydew that is produced by the aphids when they cannot consume all the sap from the tomato plant. Ants also provide a long-term babysitting service for the aphids by moving the aphids eggs to their nests to over winter. They carry aphids eggs out and place them on host plants in the garden. They guard and protect these eggs until they hatch and begin to provide honeydew again. A never-ending love story that has lasted eons in an insect paranoid world: a love story between ants and aphids. This love story does not have to end in tragedy where the lovers die but instead it can end in relocation and insect sprawl. A simple solution to aphid infestation is just spraying the tomato plants with water. This spray will dislodge the aphids from the tomato plants and reduce any damage caused by them. Another approach is to release beneficial insects into the garden environment. Ladybugs and lacewings love aphids. Once aphids are gone ant numbers will decrease and the love story of the ant and the aphid will come to an end without environmental destruction.

6.2.8 Root-knot Nematodes (*Meloidogyne incognita, M. javanica*)

Root-knot nematodes cause root galls on the feeder roots and sometimes affect the entire root system

Fig.16: Tomato roots infected by root knot nematode

showing heavy galling (Fig.16). This affects the uptake of nutrition and water and the plants show wilting during warmer part of the day. This causes stunted plants with yellow foliage resulting in yield reduction.

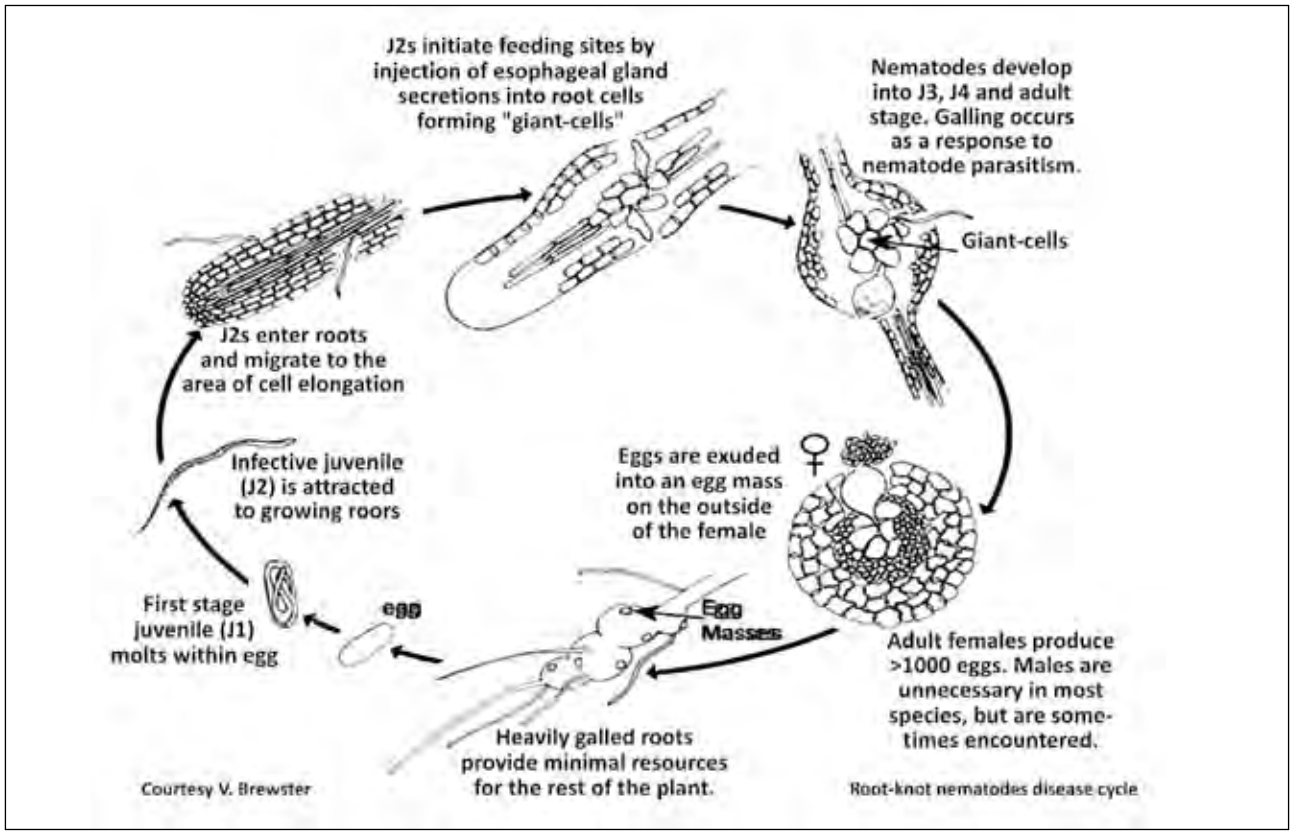

Fig.17: Disease cycle of root knot nematode (**Source:** Mitkowski and Abawi 2003)

6.3 Important Diseases

6.3.1 Damping Off (*Pythium aphanidermatum*)

This is one of the worst diseases of tomato occurring in the nursery. Damping off of tomato occurs in two stages, i.e. the pre-emergence and the post-emergence phase. In the pre-emergence phase the seedlings are killed just before they reach the soil surface. The young radical and the plumule are killed and there is complete rotting of the seedlings. The post-emergence phase is characterized by the infection of the young, juvenile tissues of the collar at the ground level. The infected tissues become soft and water soaked. The seedlings topple over or collapse.

6.3.2 Septoria leaf spot (*Septoria lycopersici*)

Fig.18: Tomato leaves infected by *Septoria lycopersici*

The plant may be attacked at any stage of its growth. The disease is characterized by numerous, small, grey, circular leaf spots having dark border.

6.3.3 Early blight (*Alternaria solani*)

This is a common disease of tomato occurring on the foliage at any stage of the growth. The fungus attacks the foliage causing characteristic leaf spots and blight (Fig 19). Early blight is first observed on the plants as small, black lesions mostly on the older foliage. Spots enlarge, and

Fig.19: Tomato leaf showing symptoms of early blight symptoms

by the time they are one-fourth inch in diameter or larger, concentric rings in a bull's eye pattern can be seen in the center of the diseased area. Tissue surrounding the spots may turn yellow. If high temperature and humidity occur at this time, much of the foliage is killed. Lesions on the stems are similar to those on leaves, sometimes girdling the plant if they occur near the soil line. Transplants showing infection by the late blight fungus often die when set in the field. The fungus also infects the fruit, generally through the calyx or stem attachment. Lesions attain considerable size, usually involving nearly the entire fruit; concentric rings are also present on the fruit.

6.3.4 Anthracnose (*Colletotrichum phomoides*)

At first, infected fruit show small, slightly sunken, water soaked spots. These spots enlarge, become

Fig.20: Anthracnose symptom on tomato fruit

darker in color, depressed and have concentric rings (Fig.20). Masses of the pink fruiting fungus can be seen on the surface of the lesions in moist weather. Under warm and humid conditions, the fungus penetrates the fruit, completely destroying it. The fungus persists on infected plant refuse in the soil. Fruit may be infected when green and small, but do not show any marked lesions until they begin to ripen. Fruit becomes more susceptible as they approach maturity.

6.3.5 Bacterial Wilt (*Pseudomonas solanacearum*)

This is one of the most serious diseases of tomato crop. Relatively high soil moisture and soil temperature favour disease development. Characteristic symptoms of bacterial wilt are the rapid and complete wilting of normal grown up plants. Lower leaves may drop before wilting. Pathogen is mostly confined to vascular region; in advancephase, it may invade the cortex and pith and cause yellow-brown discolouration of tissues. When infected plant parts are cut and immersed in clear water, a white streak of bacterial ooze is seen coming out from cut ends.

6.3.6 Fusarium wilt
(*Fusarium oxysporum f. sp. lycopersici*)

This is one of the worst diseases of tomato occurring mostly in the nurseries. The first symptoms of the disease are clearing of the veinlets and chlorosis of

Fig.21: Tomato plants affected by fusarium wilt

the leaves (Fig. 21). The younger leaves may die in succession and the entire may wilt and die in a course of few days. Soon the petiole and the leaves droop and wilt. In young plants, symptom consists of clearing of veinlet and dropping of petioles. In field, yellowing of the lower leaves first and affected leaflets wilt and die. The symptoms continue in subsequent leaves. At later stage, browning of vascular system occurs. Plants become stunted and die.

6.3.7 Late blight (*Phtophthora infestans*)

Late blight occurs when humid conditions coincide with mild temperatures for prolonged periods. If conditions are ideal for disease development, disease development is rapid causing severe economic losses. Lesions produced on the leaves are at first irregular, rather large, greenish-black and water-soaked (Fig. 22). These areas enlarge rapidly, becoming brown, and under humid conditions, develop a white moldy growth near the margins of the diseased area on the lower surface of the leaves or on stems. The disease spreads rapidly under

Fig.22: Tomato leaf infected by *Phytophthora infestans*

humid conditions, destroying quickly large areas of tissue. Lesions produced on the leaves are at first irregular, rather large, greenish-black and water-soaked. These areas enlarge rapidly, becoming brown, and under humid conditions, develop a white moldy growth near the margins of the diseased area on the lower surface of the leaves or on stems. The disease spreads rapidly under humid conditions, destroying quickly large areas of tissue. Fruit lesions occur as large, green to dark brown lesions, mostly on the upper half of the fruit, but they may also occur on other parts. White moldy growth may also appear on fruits under humid conditions. The disease attacks the fruits a well as the leaves of the plant. Symptoms on the fruits usually begin on the shoulders of the fruit because spores land on fruit from above.

6.3.8 Powdery mildew (*Leveillula taurica*)

The disease occurs severely during dry seasons. A

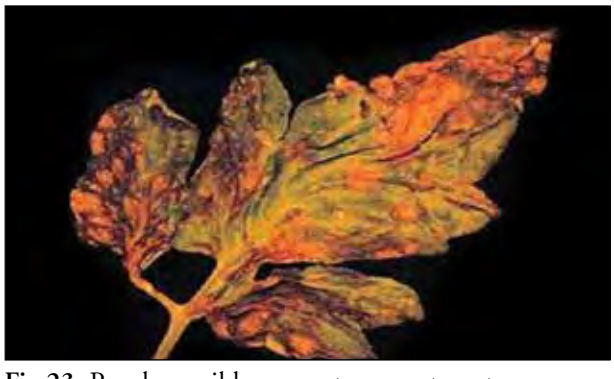

Fig.23: Powdery mildew symptoms on tomato

white powdery coating of the fungal growth appears on the leaf surface. Infected leaves may be dwarfed, stiff, and narrow (Fig. 23). The fungus progressively attacks new leaves, spreading over leaf stems, twigs, and even the fruit. Terminal growth of the affected shoot is stunted or killed. The fruit yield is reduced and the affected fruit are smaller in size.

6.3.9 Bacterial spot (*Xanthomonas campestris pv. vesicatoria*)

Moist weather and splattering rains are conducive to disease development. Most outbreaks of the disease can be traced back to heavy rainstorms that occur in the area. Infected leaves show small, brown, water soaked, circular spots

Fig.24: Bacterial spot symptoms on tomato

surrounded with yellowish halo. On older plants the leaflet infection is mostly on older leaves and may cause serious defoliation (Fig. 24). The most striking symptoms are on the green fruit. Small, water-soaked spots first appear which later become raised and enlarge until they are one-eighth to one-fourth inch in diameter. Centers of these lesions become irregular, light brown and slightly sunken with a rough, scabby surface. Ripe fruits are not susceptible to the disease. Surface of the seed becomes contaminated with the bacteria, remaining on the seed surface for some time. The organism survives in alternatehosts, on volunteer tomato plants and on infected plant debris.

6.3.10 Bacterial canker (*Clavibacter michiganensis pv. michiganensis*)

Temporary and later on permanent wilting of leaflets of affected plants is observed the disease in the field. Light streaks appear at the juncture of petiole and stem extending down the internode

Fig.25: Tomato leaf and fruit showing symptoms of bacterial canker

and up the petiole (Fig.25). At a later stage canker like opening may appear in stem, petiole and midrib. When the stem of diseased plants is cut longitudinally, a creamy white, yellow or brown line follows the phloem. The disease appears on the green fruit as water soaked spots with a white halo. Halo is the distinguishing character of bacterial leaf spot of tomato.

6.3.11 Tomato Mosaic Virus (*TMV*)

The disease is characterized by light and day green mottling on the leaves often accompanied by wilting of young leaves in sunny days when plants first become infected. The leaflets of affected leaves are usually distorted, puckered and smaller than normal. Sometimes the leaflets become indented resulting in “fern leaf” symptoms. The affected plant appears stunted, pale green and spindly. The virus is spread by contact with clothes, hand of working labour, touching of infected plants with healthy ones, plant debris and implements.

6.3.12 Tomato Leaf Curl Virus (*TLCV*)

This disease is transmitted by whitefly (*Bemisiatabaci*). It is one of the most devastating diseases of tomato. Leaf curl disease is characterized by severe stunting of the plants with downward rolling and crinkling of the leaves. The newly emerging leaves exhibit slight yellow colouration and later they also show curling symptoms. Older leaves become leathery and brittle. The nodes and internodes are significantly reduced in size. The infected plants look pale and produce more lateral branches giving a bushy appearance. The infected plants remain stunted.

6.3.13 Tomato Spotted Wilt Virus (*TSWV*)

The spotted wilt virus is transmitted through thrips (*Thrips tabaci, Frankliniella schultzi and F. occidentalis*). This disease is similar to streak in that it causes streaking of the leaves, stems and fruits. Numerous small, dark, circular spots appear on younger leaves. Leaves may have a bronzed appearance and later turn dark brown and wither. Fruits show numerous spots about one-half inch in diameter withconcentric, circular markings. On ripe fruit, these markings are alternate bands of red and yellow.

6.3.14 Tomato Bunch Top Virus (*TBTV*)

The infected plants show extensive abnormal growth with apical proliferation. The new leaves arising from the axillary buds give closely crowded bunchy appearance. The leaflet margins curl towards the tips and the surface show puckered conditions. Necrosis of leaves and stems are also characteristic symptoms. The diseased plants bear very few flowers and 1-2 very small fruit.

6.4 Physiological Disorders

6.4.1 Blossom end rot (BER)

With blossom end rot, a small water-soaked spot appears near the blossom end of the tomato (Fig.26). As it enlarges, the spot becomes dark brown to black, sunken and leathery. This happens when calcium is not readily available to developing fruit. Calcium imbalance can result from fluctuations in soil moisture caused by improper irrigation

Fig.26: Water-soaked spot (blossom end rot) on tomato fruits

or prolonged dry weather. Other causes are high nitrogen levels from fertilizer, or a disruption of the root system. We can prevent blossom end rot by the correct application of nitrogen, and keeping the plants mulched to maintain moisture. Mulching also helps to control weeds and eliminate the need for cultivation that can damage roots.

6.4.2 Sunscald

The sudden exposure of fruits to direct sunlight in hot, dry weather can cause sunscald (Fig. 27)

Fig.27: Sunscald on tomato fruit

It results in white or yellow patches on the side of the tomato exposed to the sun. To avoid sunscald, limit pruning and keep foliage healthy to provide shade and protection for the ripening fruit.

6.4.3 Catfacing

Catfacing is a term used to describe misshapen fruit with irregular bulges at the blossom end and bands

Fig.28: Irregular bulges on tomato fruit showing catfacing symptoms

of leathery scar tissue (Fig. 28). Cold weather at the time of blossom set distorts and kills certain cells that should develop into fruit, resulting in the deformities. The disorder is most often observed among first-formed fruit. Catfacing is most common in the large-fruited "beefsteak" tomatoes.

6.4.4 Fruit cracking

Fruit cracking occurs as a result of the rapid growth stimulated by wet weather following a dry period. Two types of fruit cracks affect the stem end of tomatoes: concentric and radial. Concentric cracking produces circular cracks around the stem end of the fruit. Radial cracks spread outward from the stem scar.

Fig.29: Fruit cracking in tomato

6.5 Human Health and Biosafety

6.5.1 Medicinal properties of tomato

Tomatoes, aside from being tasty, are very healthy as they are good source of vitamins A and C. Vitamin A is important for bone growth, cell division and differentiation, for helping in the regulation of immune system and maintaining surface linings of eyes, respiratory, urinary and intestinal tracts. Vitamin C is important in forming collagen, a protein that gives structures to bones, cartilage, muscle and blood vessels. It also helps maintain capillaries, bones and teeth and aids in the absorption of iron. Lycopene is a very important antioxidant which can help prevent the development of many forms of cancer (Polivkova et al. 2010, Freedman et al. 2008). Cooked tomatoes and tomato products are the best source of lycopene since the lycopene becomes readily available from the tomato when cooked. A raw tomato has only about 20% of the total lycopene content found in cooked tomatoes. However, raw or cooked, tomatoes are considered the best source for this antioxidant (Rao and Balachandran, 2002). Tomatoes have also been widely used as a natural antiseptic agent, because of its nicotinic acids. This not only has been useful in fighting off viruses and infections, but also helps in clogged arteries and even heart disease, stimulating blood flow and regulating cholesterol levels. Doctors often recommend plenty of tomato in the diet if you are in need of a blood purifier or thinner.

6.5.2 Natural toxicants and common allergenic properties of tomato

There are very few reports on the natural toxicants and common allergenic properties of tomato. Leaves, stems, and green unripe fruit of the tomato plant contain small amounts of the toxic alkaloid tomatine. They also contain solanine, a toxic alkaloid found in potato leaves and other plants in the nightshade family. However, levels of tomatine in foliage and green fruit are generally too small to be dangerous unless large amounts are consumed, for example, as greens. Small amounts of tomato foliage are sometimes used for flavoring without ill effect, and the green fruit is sometimes used for cooking, particularly as fried green tomatoes (Barceloux 2009; Mcgee 2009).

Bassler *et al.* (2009) identified two novel tomato seed allergens, IgE-reactive legumin and vicilin proteins, by multidimensional protein fractionation–mass spectrometry and in silico epitope modelling. IgE-binding LTP (lipid transfer protein) was identified as detectable allergen in peel, pulp and seeds of tomato. In fresh tomato, different LTP isoforms are present as allergens. Industrial tomato derivatives like canned peeled tomatoes, tomato puree and tomato paste also contain LTP in relatively small quantities (Pravettoni *et al.* 2009).

7. AGRONOMIC PRACTICES

7.1 Soil

Tomato is grown in many types of soils from sand to heavy clay. A well-drained, fairly light fertile loam soil with fairly moisture holding capacity is ideal for growing good tomato crop. Good texture of soil is of primary importance. Even poor and medium quality land produce good and early crop. Tomato crop prefers a soil reaction ranging from pH 6.0 to 7.0. In acidic soil liming is required for successful tomato cultivation.

7.2 Sowing Season

In northern India two sowings are done. The autumn-winter crop is raised by sowing the seed in June-July and for the spring-summer crop seeds are sown in November. In parts of the country where frost damage not occurs and where summer sets early only one sowing during July-August is done. Two to three sowing can be done in region with mild climate. In the hills the seed is sown in March-April.

7.3 Raising of Nursery

Tomato seeds are on smooth and well leveled raised beds of 3 x 0.6 m size and 10-15 cm in height. Add sieved FYM @2kg/m2 and fine sand on the seedbed. Raised beds are necessary to avoid problem of water logging in heavy soils. In sandy soils, however, sowing can be taken up in flat beds. About 250-300g seeds are sufficient for raising seedlings for one hectare of land. Prior to sowing seeds are treated with fungal culture of *Trichodermaviride* (5 g/kg of seed) or Carbendazim (2g/kg of seed) to avoid damage from damping-

off disease. Sowing should be done thinly in lines spaced at 10-15 cm distance. Seeds are sown at a depth of 2-3 cm and covered with a fine layer of FYM followed by light watering by water can. The seedlings with 5-6 true leaves are ready for transplanting within 4-5 weeks of sowing.

7.4 Soil Preparation

The field is ploughed to fine tilth by giving four to five ploughing with a sufficient interval between two ploughing. Lavelling should be done for proper irrigation and drainage. Furrows are then opened at the recommended spacing. Well-decomposed FYM (25 t/ha) is thoroughly incorporated at the time of land preparation.

7.5 Spacing and Transplanting

Spacing depends upon the type of variety grown and the season of planting. Normally, the seedlings are transplanted at a spacing of 75-90 x 45-60 cm depending on the growth habit of variety. Seedlings are transplanted in furrows in light soils and on side of the ridges in case of heavy soils. A pre-seedling irrigation is given 3-4 days prior to transplanting. Before planting, seedlings should be dipped in a solution prepared by Imidacloprid (3ml) and Carbendazim or Mencozeb (25g) in 10 liters of water for 1-2 hrs. Plantingshould preferably be done in the evening in order to avoid transplanting shock.

7.6 Manure and Fertilizer Requirement

The fertilizer dose depends upon the fertility of soil and amount of organic manure applied to the crop. For better yield, 20-25tonnes of well-decomposed FYM should be incorporated into the soil. Generally, application of 120 kg N, 80 kg P2O5 and 50 kg K2O per hectare is recommended for getting optimum yield. Half dose of N and full dose of P and K is given at the time of last ploughing. The balance half of N is givenas top dressing 30 days after transplanting. For hybrid varieties, the recommended dose per hectare is 180 kg N, 100 kg P2O5 and 60 kg K2O. 60 kg N and half dose of P and K are given at the time of transplanting. Remaining quantities of P and K and 60 kg N is top dressed 30 days after transplanting. A third dose of 60 kg N is applied 50 days after transplanting.

7.7 Intercultural Operations and Weed Control

Tomato plant requires frequent shallow cultivation, especially during their first month in the field. The surface soil is loosened by hand hoeing as soon as it is dry enough after every irrigation or rainfall. Mulching with straw, polythene and other materials has been found beneficial for soil moisture conservation, weed control and enhancing the quality and yield. Beside these, training and pruning is also advisable for quality yield of tomato, especially in indeterminate varieties of tomato. Due to the tall habit and heavy bearing nature of the hybrids, staking is essential. Staking facilitates intercultural operations and helps in maintaining the quality of the fruits. It is done 2-3 weeks after transplanting. Pre emergence application of Basalin (1kg a.i./ha) or Pendimethalin (1kg a.i./ha), coupled with one hand weeding 45 days after transplanting is effective for control of weeds.

7.8 Harvest and Post-Harvest Practices

Depending on the variety, fruits become ready for first picking in about 60-80 days after transplanting. The stage of harvesting depends upon the purpose to which the fruits are to be used. Fruits are normally harvested early in the morning or evening. The fruits are harvested by twisting motion of hand to separate fruits from the stem. Harvested fruits should be kept in shade. Since all the fruits do not mature at the same time, they are harvested at an interval of 4 days. Generally, there will be 7-11 harvests in a crop life span. The average yield per hectare is 20-25 t/ha for normal varieties and 50-60 t/ha for hybrid varieties.

After harvesting of tomato fruits following post-harvest practices are done to reduce post-harvest losses and to enhance consumer preference and market price:

- Grading- During grading of fruits, damaged, rotten and cracked fruits should be removed. Only healthy, attractive, clean and bright fruits should be selected. The grades are mainly based on the condition and the quality of the fruits. However, on the basis of the size of fruits, three grades are common: small (<100 g), medium (100-255 g) and large (> 255 g). Retailers normally do size grading for the local market. Bureau of Indian Standards has specified 4 grades viz., Super A, Super, Fancy and Commercial for tomato crop.
- Packaging- For local markets, the fruits are packed in bamboo baskets or plastic crates. Plastic crates can be conveniently stacked one on the other and a contoured rim keeps the product safe and natural and allows sufficient air circulation. The packing should ensure careful handling i.e. rigid enough to protect the fruits from being crushed. For exports, the fruits are packed in cardboard telescopic boxes with capacities of not more than 15 kg.
- Storage- The main objective in storage after harvest is to control the rate of ripening to extend the marketing period. As the tomatoes are chilling sensitive, the recommended storage temperatures differ depending on the fruit maturity. A storage temperature of 13^{0}C with 90-95% relative humidity is recommended for slow ripening. At this temperature, most varieties keep good condition for 2-3 weeks and change colour very slowly. In cold storage, unripe tomatoes can be stored for 4 weeks at a temperature of 8-10^{0}C with 85-90 % relative humidity. Fully ripe fruits are stored at 7^{0}C with 90% relative humidity for 1 week.
- Transport- Tomatoes are highly perishable in nature, hence quick means of transportation is necessary. Tomatoes are transported by road through tractors, trucks and also by rail and air to distant markets.

7.9 Seed Production

Tomato is a self-pollinated crop but a certain percentage of cross pollination has also been reported. The percent of out-crossing depends on variety and environment. Safe isolation distance for certified seed production of tomato for open pollinated and hybrid seeds should be 25 and 100 m, respectively. The seed along with the pulp is kept in a container for fermentation for 2-3 days before extraction of seed. The seed is extracted by vigorous stirring and washing. The seed yield varies with the variety. The average seed yield is about 100 to 150 kg per hectare.

8. BREEDING OBJECTIVES

1. Earliness
2. Enhanced fruit yield
3. Fruit quality
   i. High TSS: >5.5%.
   ii. Pericarp thickness: Should be more than 0.5 cm.
   iii. Locules: Minimum number of locules ranging from 2-3.
   iv. Viscosity: Viscosity will improve product yield in processing industry.
   v. Fruit shape: Oblong types are suitable for processing.
   vi. Tomato flavour with a good blend of TSS and acidity
4. Resistance to biotic stresses:

A. Diseases
   i. Fungal diseases: Early blight, late blight, fusarium wilt, and anthracnose.
   ii. Bacterial diseases: Bacterial wilt, bacterial canker, bacterial spot and bacterial speck.
   iii. Viral diseases: Tomato mosaic, leaf curl and bud necrosis.

B. Insect and nematodes
   i. Fruit borer and whitefly
   ii. Nematode : Root knot nematode

5. Tolerance to abiotic stresses:
   i. High temperature tolerance/ Heat stress tolerance
   ii. Moisture tolerance (drought and water logging)
   iii. Salt tolerance
   iv. Chilling injury tolerance
6. Varieties/hybrids suitable for protected cultivation

APPENDIX 1: BIOTECHNOLOGICAL DEVELOPMENTS

The tomato originated from South America and was brought to Europe by the Spanish in the 16th century. Wild tomatoes are small, green and largely unappetizing, but after centuries of breeding there are now thousands of varieties grown worldwide. *Agrobacterium*-mediated genetic engineering techniques were developed in the late 1980s that could successfully transfer genetic material into the nuclear genome of tomatoes. Genetic material can also be inserted into a tomato cell's chloroplast and chromoplast plastomes using biolistic approach. Tomatoes were the first food crop with an edible fruit where this was possible. *Agrobaceriumtumefaciens* is an excellent species of soil dwelling bacteria that can infect plants with a piece of its own DNA. *Agrobacterium* mediated transformation is an effective and widely used approach to introduce foreign DNA into dicotyledons plants. The DNA gets a hold of the plant cellular machinery and uses it to ensure the proliferation of bacterial population. The advantage of this gene is that insecticidal toxin genes or other various genes can be engineered in the bacterial DNA. This bacterium shortens the plant breeding process. Most of all, it allows new genes to be engineered into crops.

In 1994, the *FlavrSavr* became the first commercially grown genetically engineered food to be granted a license for human consumption. A second copy of the tomato gene *Polygalacturonase* was inserted into the tomato genome in the antisense direction. The polygalacturonase enzyme degrades pectin, a component of the tomato cell wall, causing the fruit to soften. When the antisense gene is expressed it interferes with the production of the polygalacturonase enzyme, delaying the ripening process. The *FlavrSavr* failed to achieve commercial success and was withdrawn from the market in 1997. Similar technology, but using a truncated version of the polygalacturonase gene, was used to make a tomato paste. DNA Plant Technology (DNAP), Agritope and Monsanto developed tomatoes that delayed ripening by preventing the production of ethylene, a hormone that triggers ripening of fruit. All three tomatoes inhibited ethylene production by reducing the amount of 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor to ethylene. DNAP's tomato, called *Endless Summer*, inserted a truncated version of the *ACC synthase* gene into the tomato that interfered with the endogenous *ACC synthase*. Monsanto's tomato was engineered with the ACC deaminase gene from the soil bacterium *Pseudomonas chlororaphis* that lowered ethylene levels by breaking down ACC. Agritope introduced an S-adenosylmethionine hydrolase (SAMase) encoding gene derived from the *E. coli* bacteriophage T3, which reduced the levels of S-adenosylmethionine, a precursor to ACC. *Endless Summer* was briefly tested in the marketplace, but patent arguments forced its withdrawal.

Scientists in India have delayed the ripening of tomatoes by silencing two genes encoding N-glycoprotein modifying enzymes, α-mannosidase and β-D-N-acetylhexosaminidase. The fruits produced were not visibly damaged after being stored at room temperature for 45 days,

whereas unmodified tomatoes had gone rotten. In India, where 30% of fruit is wasted before it reaches the market due to a lack of refrigeration and poor road infrastructure, the researchers hope genetic engineering of the tomato may decrease wastage.

Abiotic stresses like frost, drought and increased salinity are a limiting factor to the growth of tomatoes. While no genetically modified stress tolerant plants are currently commercialized, transgenic approaches have been explored. An early tomato was developed that contained an antifreeze gene (afa3) from the winter flounder (fish) with the aim of increasing the tomato's tolerance to frost. The antifreeze protein was found to inhibit ice recrystallization in the flounder's blood, but had no effect when expressed in transgenic tobacco. The resulting tomato was never commercialized, but raised ethical questions over adding genes from one kingdom to another.

Other genes from various species have been inserted into the tomato with the hope of increasing their resistance to various environmental factors. A gene from rice (*Osmyb4*), which codes for a transcription factor, that was shown to increase cold and drought tolerance in transgenic *Arabidopsis thaliana* plants, was inserted into the tomato. This resulted in increased drought tolerance, but did not appear to have any effect on cold tolerance. Overexpressing a vacuolar Na+/H+antiport (*AtNHX1*) from *A. thaliana* lead to salt accumulation in the leaves of the plants, but not in the fruit and allowed them to grow more in salt solutions than wild-type plants. They were the first salt-tolerant, edible plants ever created. Tobacco osmotic genes overexpressed in tomatoes produced plants that held a higher water content than wildtype plants, increasing tolerance to drought and salt stress.

The insecticidal toxin from the bacterium *Bacillus thuringiensis* has been inserted into a tomato plant. When field tested they showed resistance to the tobacco hornworm, tomato fruitworm, the tomato pinworm and the tomato fruit borer. A 91 day feeding trail in rats showed no adverse effects, but the *Bt*-tomato has never been commercialized. Tomatoes resistant to a root knot nematode have been created by inserting a cysteine proteinase inhibitor gene from taro. A chemically synthesised *ceropin B gene*, usually found in the giant silk moth, has been introduced into tomato plants and in vivo studies show significant resistance to bacterial wilt and bacterial spot. When the cell wall proteins, polygalacturonase and expansin are prevented from being produced in fruits, they become less susceptible to the fungus *Botrytis cinerea* than normal tomatoes. Pest resistant tomatoes can reduce the ecological footprint of tomato production while at the same time increase farm income.

Tomatoes have been altered in attempts to improve their flavour or nutritional content. In 2000, the concentration of pro-vitamin A was increased by adding a bacterial gene encoding phytoene desaturase, although the total amount of carotenoids remained equal. The researchers admitted that it had no prospect of being grown commercially due to the anti-GM climate. Sue Meyer of the pressure group Genewatch, told *The Independent* that she believed, "If you change the basic biochemistry, you could alter the levels of other nutrients very important for health". More recently, scientists have increased the production of anthocyanin, an antioxidant in tomatoes in several ways. One group added a transcription factor for the production of anthocyanin from *Arabidopsis thaliana* whereas another used transcription factors

from snapdragon. When the snapdragon genes where used, the fruits had similar anthocyanin concentrations to blackberries and blueberries, and when fed to cancer susceptible mice, extended their life span. Another group has tried to increase the levels of isoflavone, known for its potential cancer preventative properties, by introducing the soybean *isoflavonesynthaseinto* tomatoes.

Contribution of Biotechnology to Root Knot Nematode Control

Root-knot nematodes (*Meloidogyne spp*.) represent a particularly serious pest for tomato crops. These pathogens have evolved a sophisticated interrelationship with the roots of their host where they induce a specific type of nurse cell system, classified as multinucleate giant cells. The structural and physiological transformation of the initial cell to become the nematode feeding site is paralleled by modifications in plant gene expression. The recent characterization of several parasitism genes specifically expressed within oesophageal gland cells of root-knot nematodes suggests that their products can influence the host cellular metabolism. In plants with genetic disease resistance, these secreted molecules might serve as virulence factors for successful parasitism.

The *Mi* gene, which confers resistance to several species of root-knot nematodes, is present in many modern tomato cultivars. Resistance mediated by *Mi* is associated with localized necrosis of host tissue at the nematode feeding site and occurs very early after nematode infection. However, how Mi mediates recognition of and resistance to root-knot nematodes is largely unknown. In parallel with the use of such natural resistance, several biotechnological strategies have been experienced to improve tomato resistance. They are mainly based on the over-expression of anti-nematode and/or anti-giant cell genes placed under the control of specific promoters.

APPENDIX 2: SOMATIC HYBRIDIZATION IN TOMATO

Protoplast fusion can be used to produce somatic hybrids of species that cannot be obtained by sexual hybridization. The possibility to introgress genes from weedy Solanum species into the cultivated tomato species, and to obtain novel cytoplasm-nucleus combinations (cybrids) was considered as an important strategy to extend the genetic variation available for tomato breeding. Somatic hybrids between *S. lycopersicum* and other *Solanum* species, as well as between *S. lycopersicum* and *Nicotiana* species, have been produced. Specific mutants, genotypes with antibiotic resistances, and metabolic inhibition by iodoacetate or iodoacetamide and irradiation are used for the selection of hybrids. In addition, the improvement of protoplast culture techniques and the use of the favourable tissue culture traits derived from species such as *S. peruvianum*, which have been introduced into tomato by classical breeding, allow the efficient recovery of somatic hybrids. However, the occurrence of somatic incongruity in fusion combinations of *S. lycopersicum* and *Solanum* and even more in *S. lycopersicum* and *Nicotiana*, did not allow the production of true cybrids and/or fertile hybrids, indicating the importance of both cytoplasm-nucleus and nucleus-nucleus interactions in somatic incongruity.

Another problem with fusions between distantly related species is the strongly reduced fertility of the hybrids and the very limited homoeologous recombination between chromosomes of the parental species. Partial genome transfer from donor to recipient through microprotoplast (+) protoplast fusion, and the production of monosomic or disomic chromosome addition lines, may overcome some of these problems. In symmetric somatic hybrids between *S. lycopersicum* and *S. tuberosum* the occurrence of limited somatic and meiotic recombination was demonstrated. Fertile progeny plants could be obtained though at a low frequency, when embryo rescue was performed on a large scale after backcrossing hexaploid somatic tomato (+) potato hybrids with a tetraploid potato genotype. The potential value of genomic *in situ* hybridization (GISH) and RFLPs for the analysis of the genome/chromosome composition of the hybrids has been demonstrated for intergeneric somatic hybrids between *Lycopersicon* and *Solanum* (Wolters *et al.* 1994).

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