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# Marker-assisted biopiracy
## *Ex situ* wild tomato collections, genetic breeding techniques and patent claims

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Where people rely upon supermarkets for their fruits and vegetables, the tomato is a food that sometimes loses its attraction. An often-heard complaint is that supermarket tomatoes are unripe, hard and unpalatable. It seems that traits that lend themselves to processing (for canning and fresh sale) simply don't make for good fresh eating.

Much of the blame lies with industrial agriculture, particularly mechanically harvested tomato varieties and large-scale hothouses. These homogenized operations are an important source for table tomatoes in urban (and some rural) areas, and can be found in the US, Canada, Mexico, the Netherlands, Turkey, Egypt, Kenya, China and many other places, producing and shipping tomatoes worldwide. While some may still savour tomatoes produced by more traditional methods, for most of the world, the tomato is increasingly an industrial product.

The expansion of industrial tomato farming has been accompanied by a rise in patent claims over tomato traits and genes. The eight patent applications discussed in this report include claims over seedless tomatoes, disease resistance, growth habits, higher yields and harder fruit (a desirable trait in industry). Other claims cover tomato genes that yield precursor molecules for the pharmaceutical and chemical industries.

The cases underscore difficulties in achieving equity in the use of biodiversity when *ex situ* collections are the source of patented materials. They also exemplify unsettled issues of access and benefit-sharing for the range of agricultural biodiversity that is *not* included in the Multilateral System of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). In addition, in one case, the origin of tomato genes claimed cannot be determined from the patent documents, underscoring the need for patent applicants to be obligated to divulge the geographic origin of materials they claim.

Finally, this case study of patent claims on tomatoes shows how genetic breeding techniques related to marker-assisted selection (MAS), a biotechnology that (unusually) is generally positively regarded by industry and NGOs alike, can have a darker side when put in the context of biopiracy. It is through these techniques, applied to wild relatives of tomatoes, that the patented tomato genes discussed in this report were identified. With the combination of these genetic techniques and adroit patent lawyering, it has become possible in some cases for patent applicants to reach over the top of national access laws and, in effect, claim biodiversity that has never left its country of origin.

**Background**

A search of the World Intellectual Property Organization's Patentscope database was performed to identify claims on tomato (*Solanum lycopersicum*) filed since 2007. Eight patent applications were selected for further investigation (see chart, page 4). In seven of these cases, the materials claimed come from a wild relative of the cultivated tomato obtained from a seed bank. Genes from these related species can be introduced into commercial tomato varieties and, in some cases, other organisms. In the eighth case, the patent application contains insufficient data to determine the origin of genes claimed.

Since Europeans took them from the Americas, cultivated tomatoes have spread across the world. The tomato has approximately 17 species of wild relatives,[1] however, and these remain critical sources of traits for plant breeders. The centre of genetic diversity of these wild relative species of tomatoes is in Andean South America, especially Peru and Ecuador, and to a lesser extent nearby countries, particularly northern Chile.

These wild tomato species have yielded, and continue to provide, important traits for the cultivated tomato. For example, endemic tomato species from Ecuador's Galapagos Islands (*S. galapagense* and *S. cheesmaniae*) have provided salt tolerance and a growth habit trait ("jointless fruit stalk") that is widely used in the tomato industry today.

**Tomato genebanks**

Despite being cultivated globally, large *ex situ* tomato collections available to commercial plant breeders and allied academics are relatively few. Tomatoes are not among the crops in the ITPGRFA Multilateral System, meaning that new collections of tomato seeds are (generally) governed by the access and benefit-sharing provisions of the Convention on Biological Diversity (CBD), and by national regulations.

| **Largest Tomato Producers, 2009**[2] | |
|---|---|
| **Country** | **Production** (1000 MT) |
| China | 46366 |
| USA | 14142 |
| India | 11149 |
| Turkey | 10746 |
| Egypt | 10000 |
| Italy | 6877 |
| Iran | 5888 |
| Spain | 4604 |
| Brazil | 4311 |

Major *ex situ* collections of tomato seeds, including wild relatives, can be found in the US and Europe. A US Department of Agriculture (USDA) facility in Geneva, New York holds the US government's collection,[3] while the University of California at Davis' Tomato Genetic Resources Centre (TGRC) is the genebank with greatest emphasis on diversity of tomato wild relatives.[4] The latter is a legacy of Charles Rick, a geneticist whose 1940s-1980s studies not only contributed to many new tomatoes (including, indirectly, some of the patent claims discussed in this paper), but also pioneered molecular selection and breeding techniques that are now widely applied to other crops.

In California's central valley, the TGRC is located in close proximity to the seed production, farming and processing operations of some of the world's largest tomato growing companies, such as Campbell's Soup and Heinz. Many of these companies produce their own processing tomato varieties, tailored for specific products such as soups, juices and salsas. As these companies have tomato breeding programmes and have enjoyed a longstanding relationship with the TGRC, it is quite likely that their privately held collections are substantial; however, no public catalogue of these holdings is available.

Since 2006, the European Commission, through a project based in the Netherlands, has brought some of its agricultural research resources to bear on tomatoes. The "EU-SOL" programme, which also works on potatoes, aims to "*extract the under-exploited natural biodiversity present in* [tomatoes] *to improve consumer-driven and environmentally-directed quality of tomato fruits…*" A key aim of the project is to create "*new elite genotypes to boost our knowledge and provide a blueprint for novel high quality varieties to be developed by EU breeding companies*."[5]

The seeds branch of the EC programme is a group of Dutch, Italian and Israeli scientists from the private and public sectors. They have assembled a core collection of 7,000 tomato seed types, and are working to create a comprehensive set of research and breeding lines (introgression lines) to enable the systematic identification and transfer of genes from wild relatives into cultivated tomatoes.[6]

The EU-SOL programme's collection is mainly composed of accessions acquired from genebanks around the developed world. These include seeds from the USDA, IPK Gatersleben (Germany), and Dutch and Italian collections, among others. For tomato wild relatives,[7] EU-SOL has overwhelmingly relied on seeds acquired from two related US sources: the TGRC in California, and Steven Tanksley, a professor at Cornell University and a former student of Charles Rick, the TGRC founder.

One international agricultural research centre, the Asian Vegetable Research and Development Centre (AVRDC) in Taiwan, possesses a significant tomato collection. The tomato wild relatives held by AVRDC, however, are relatively few and mainly come from California's TGRC. In addition to the TGRC material, AVRDC has a small number of other accessions, mainly from Mexico and Chile (the latter via Kew Gardens, UK).[8]

**Few recent collections publicly available**

The major international tomato genebanks distribute very few recent collections from the tomato's Andean centre of diversity. Most seeds of wild relatives in genebanks were collected from the 1930s through the 1980s. On the one hand, this may be attributable to the fact that, in comparison to some crops, tomato specialists feel that existing collections are relatively good (except a few remote Andean areas that remain relatively unexplored).

On the other hand, it appears that collections have also slowed in part due to changes in national law and policies in the countries of origin. Foreign scientists may feel these policies discourage collections, while governments would likely assert that the drop-off in collections reflects unwillingness (or institutional inability) on the part of some scientists to collect under modern, post-CBD access and benefit-sharing terms.

Exceptions to the general state of affairs include 2001 and 2005 collections in northern Chile by TGRC researchers,[9] and a 2009 expedition to Peru by a consortium of US academics funded by the US government. In the latter case, while many tomatoes were collected, it appears that export permits were never granted by the Peruvian authorities. The wild relative samples were left at the International Potato Centre (CIP), the research centre in Lima which facilitated the collecting mission.[10]

The Chilean seeds, however, are being freely distributed by the TGRC with a highly substandard material transfer agreement that simply states that in the event of commercial use of the seeds, the recipient "*should consider*" benefit-sharing with Chile. The MTA does not include any Chilean signatory.[11]

**Wave of new patent claims on tomatoes**

Whether the apparent decline in new collections of tomatoes and wild relatives in South America is the result of new national policies, or of scientists stuck in the political past, or both, the apparent scarcity of newly collected germplasm hasn't stopped US and European companies and universities from filing new claims on tomato wild relatives.

Indeed, developing new tomato genotypes from tomato wild relatives, for use by the private sector, is an explicit goal of the publicly funded EU-SOL programme. Gene giants Monsanto and Syngenta have presented tomato patent applications, along with smaller seed firms and universities. These patent claims are summarized in the chart on page 5 and in the paragraphs below.

In its patent application WO2009021545, **Enza Zaden B.V.**, a Dutch breeding company, has claimed higher-yielding tomato plants. The source gene is a growth trait identified in LA0716, a seed bank accession from the TGRC in California. LA0716 is an *S. pennellii* accession that was collected in 1959 near the town of Atico in the Arequipa Province of southern Peru.[12] Enza Zaden is a participant in the EU-SOL programme, with responsibilities in evaluating wild relatives' potential for the private sector.[13]

In an overlapping patent application (WO2010147467), **Monsanto** has claimed the same gene from LA0716 because it imparts a growth habit (a specific form of a trait called sympodial index) that results in more and heavier fruit per square metre in hothouse tomatoes. Monsanto's subsidiary De Ruiter also participates in the EU-SOL programme.

A frequently studied tomato type, LA0716 (also written LA716) was also the subject of a patent claim by Cornell University in 2000 (US patent 6,066,482). One reason why LA0716 has been the source of so many claims is that it is self-fertile,[14] an unusual trait for the species that makes this particular seed easier to use in research. It has also been used in genetics and breeding tools called introgression lines, explained below.[15]

| PATENT PUBLICATION | TITLE | OWNER | SUBJECT/CLAIMS | COMMENT |
|---|---|---|---|---|
| **WO2007123407** | Tomato plants having a high level of resistance to Botrytis | Monsanto (US) | Transfer of botrytis resistance genes from *S. habrochaites* (LYC 4/78) to cultivated tomatoes, and related genetic regions/markers. | Claims cover any source of *S. habrochaites*, but LYC 4/78 in particular. LYC 4 is a seed from the IPK Gatersleben in Germany. |
| **WO2009005343** | Parthenocarpy genes in tomato | Western Seed Intl (Netherlands) | Cultivated tomatoes with genes from *S. habrochaites* coding for seedlessness. | Gene source is also LYC 4. This species typically comes from Ecuador and Peru. Old name: *Lycopersicon hirsutum* Dunal. |
| **WO2009021545** | Promoter sequence and gene construct for increasing crop yield in tomato | Enza Zaden B.V. (Netherlands) | SP3D sequence inserted into cultivated tomatoes in order to boost yield. | The preferred form of the SP3D gene, whose sequence is claimed, comes from LA0716, a TGRC tomato collected 1959, at Atico, Arequipa, Peru. |
| **WO2009117423** | High lycopene content tomato plants and markers for use in breeding for same | Pennsylvania State University (US) | Claims high-lycopene tomato plants and ways of breeding them. Lycopene is thought to have human health benefits. | Specific source is *S. pimpinellifolium* LA2093 from California's TGRC. LA2093 was collected in 1980 at La Union, El Oro, Ecuador. |
| **WO2010147467** | Tomato plants resulting from the introgression of a trait from *S. pennellii* into *S. lycopersicum* … | Monsanto (US) | Patents use of SP3D gene coding for a trait ("average sympodial index of 2"), and related breeding methods to use the gene in hothouse tomato varieties. | Source is LA0716. (See Enza Zaden above.) |
| **WO2011020797** | Disease resistant tomato plants | Syngenta (Switzerland) | Claims tomato plants resistant to botrytis and related genetic markers and DNA primers. | Resistance identified in *S. habrochaites* 04TEP990312. This seed cannot be identified in any genebank and the patent application does not explain its origin. |
| **WO2011038244** | Methylketone synthase, production of methylketones in plants and bacteria | Univ. of Michigan (US), Hebrew Univ. (Israel), Salk Institute (US) | Methylketones are used in industrial chemical and pharmaceutical manufacture. Claims methylketone-related gene sequences and their use, and similar sequences in other tomatoes and other species. | The key original sequence in this patent application comes from PI 126449, an *S. habrochaites* accession collected in 1937 near Yaso, Peru. |
| **WO2011051120** | Tomato fruit having increased firmness | Syngenta (Switzerland) | Genes for firmer tomatoes, their use in breeding, and resulting tomato plants/seeds. | Claimed genes originate in LA0716, the same TGRC tomato claimed above. LA0716 has been used in introgression lines to identify genes and to cross wild relatives with cultivated tomatoes. |
| | | | | |

In patent application WO2007123407, **Monsanto** has claimed tomato genes and plants resistant to botrytis, a mould infecting many fruits and vegetables. The resistance comes from *S. habrochaites*, another tomato wild relative. Monsanto's source for the resistance is LYC 4,[16] a seed from the IPK Gatersleben seed bank in Germany. Although IPK Gatersleben's seed database lists LYC 4 as being of unknown origin, native populations of *S. habrochaites* are found in Peru and Ecuador.

**Pennsylvania State University** (PSU) has claimed high-lycopene tomatoes in its patent application WO2009117423. Lycopene is the red pigment in tomato fruit, and is thought to be an "antioxidant" when consumed by humans. Studies have linked antioxidants to protection against cancer and improved cardiovascular health. PSU's high-lycopene trait was found in LA2093, a wild relative from the TGRC collection. LA2093 is a *S. pimpinellifolium* seed that was collected in 1980 on a roadside in southwestern Ecuador in the town of La Union in El Oro Province.[17]

**Western Seed**, a Dutch company, has claimed seedless tomatoes in patent application WO2009005343. The seedless trait, called parthenocarpy, comes from a set of genes found in LYC 4, the same *S. habrochaites* accession from which Monsanto has patented mould resistance. Some cooks prefer to remove tomato seeds before cooking or serving tomato dishes; thus, Western Seed is seeking a market advantage in selling a tomato that already comes with few or no seeds.

In patent application WO2011038244, the **University of Michigan** (US), **Hebrew University** (Israel) and the **Salk Institute** (US) have together claimed genes that code for chemicals called methylketones. Members of this class of chemicals are naturally produced in tomato wild relatives where, scientists speculate, one of their roles is to help repel pests. Methylketones have potential as insecticides and can be used as an "ingredient" in chemical manufacturing processes ranging from pharmaceuticals to industrial coatings.

Salk and the universities' claim primarily focuses on PI 126449, an *S. habrochaites* accession from the US Department of Agriculture collection. According to USDA, the wild relative was collected in 1937 in a Peruvian town named Yaso.[18] Also claimed is a similar gene found in LA1708, a *S. peruvianum* accession from the TGRC that was collected in 1977 near the town of Jaén, in Peru's northeastern Andes. The patent claims the methylketone genes as matter, their insertion into bacteria and other microorganisms and plants, and the resulting organisms. One use the inventors clearly have in mind is production of methylketones in bacteria genetically engineered with tomato genes.

Not to be left out of the patenting fray, Switzerland-based giant **Syngenta** has two claims of its own. In patent application WO2011020797, Syngenta claims botrytis-resistant tomatoes (the same moulds targeted by Monsanto). The source of the resistance is a *S. habrochaites* accession identified as "04TEP990312". No *S. habrochaites* accession with this name (or anything similar) could be identified in a major seed bank.

The Syngenta patent application is not helpful with respect to the seed's origin. No information is provided on where 04TEP990312 comes from, when and how it was collected, or how it came to be part of the Syngenta research programme. (As previously noted, however, native populations of *S. habrochaites* are found in Peru and Ecuador.)

In another patent application (WO2011051120), **Syngenta** claims harder tomatoes (it says "increased firmness"). The gene for harder fruit was identified in *S. pennellii* LA0716, the same source of genes patented by Monsanto and Enza Zaden. "Firmer" tomatoes have a variety of uses in industrial tomato production and processing.

**Introgression lines and patent claims**

At a glance it seems as if two specific tomato wild relatives – LA0716 and LYC 4 – have specific characteristics that lend themselves to commercial interest. The frequent citation of these seeds in patent-related research, however, mainly has to do with their use in tomato introgression lines. These lines are crosses between wild tomatoes and their cultivated cousins (see below) that are particularly useful for identifying and isolating genes and loci of interest.

Having found a useful trait, and identified its gene(s) and locus (the place where it occurs on a chromosome) in a specific introgression line, companies then have patent attorneys draft claims that seek to cover not only the specific gene sequence found in the introgression line, but also other, similar sequences and similarly useful forms of the gene. Thus, in many cases, even if the examples of the patent application refer to one wild relative, for example, LA0716, the language of the patent claims will often attempt to cover similar genes that might be found in other accessions of tomato wild relatives.

The way introgression lines work is that a wild tomato species is crossed and then backcrossed, often over several generations, with a domesticated tomato variety. From these crosses, a number of lines are selected, each incorporating some chromosomes, or partial chromosomes, from the wild genome (which breeders can identify using molecular markers). The goal of the interspecies effort is to produce a set of lines that together include all of the wild type's genes expressed in the domesticated tomato's "genetic background". These introgression lines then facilitate gene identification and marker-assisted breeding.

Several tomato introgression lines have been created, and LA0716 (*S. pennellii*) and LYC 4 (*S. habrochaites)* have each been used as a representative of their species in the crosses. As a result, LA0716 and LYC 4 are frequently cited in scientific and patent documents.

But many patent claims that refer to wild relatives used in introgression lines also claim the same or similar genes when found elsewhere. For example, a growth trait first found in LA0716 introgression lines may also be present in other *S. pennellii* seeds different than LA0716, including genetic variants that may prove equally or better suited to the purposes of the patent claim.

The companies (and universities) lodging patent claims are aware of this, and may take measures to try to prevent their patent claims from being dodged by somebody discovering a slightly divergent gene in another wild relative. **So, companies write patent claims to not only cover the specific diversity they have identified in the introgression lines, but to also try to claim other forms of the same gene and/or trait that are in genebanks or the wild, but which have yet to be specifically described.**

For example, in patent application WO2010147467, after claiming the growth habit found in the Peruvian LA0716 accession, Monsanto proceeds to attempt to claim any other red tomato breeding line with that same growth habit, whether it comes from LA0716, any other *S. pennellii* seed or, in fact, any other plant in the *Solanum* genus.[19]

Similarly, Western Seed's patent application WO2009005343 describes seedless tomato genes isolated from introgression lines made with *S. habrochaites* LYC 4. In claims, however, the company asserts that any low- or no-seeded cultivated tomato with a combination of those genes (even if from a different source) is its intellectual property. Thus, other *S. habrochaites* types or other wild relatives with functionally equivalent genes in the same chromosomal location are encompassed. Interestingly, the international patentability search under the Patent Cooperation Treaty (administered by the World Intellectual Property Organization) raised questions about the novelty of most of Western's claims. Nevertheless, the patent has been issued in Canada with the original claims intact, and is pending in many other jurisdictions.

**Conclusion**

Seed bank collections of tomato wild relatives are of strong interest to major agriculture multinational corporations as well as universities and smaller companies involved in developing proprietary tomatoes. Despite the new Nagoya Protocol on Access and Benefit-Sharing that was adopted in October 2010, discussions at the Convention on Biological Diversity have yielded little concrete progress on the thorny issue of *ex situ* collections. Tomatoes in particular show how *ex situ* agricultural germplasm that is not covered by the ITPGRFA Multilateral System may be appropriated for private gain without benefits accruing to countries of origin. The issue of benefit-sharing from new uses of germplasm collected before the entry into force of the CBD was also unresolved during the Nagoya Protocol negotiations and again the tomato cases reinforce the continuing injustice on countries of origin.

Patent claims on tomato wild relatives are accelerating, despite the fact that new collections of these seeds in their centres of diversity appear to have been limited since the entry into force of the CBD. Increased patents on old seeds might seem paradoxical, but can be explained by the economics of the expansion of industrial tomato production and, most importantly, by newer genetic and breeding technology that makes it easier to identify valuable genes in seed banks.

With tomatoes, the combination of genes and loci identified through introgression lines and crafty drafting of patent claims over them is allowing companies and universities to remotely reach into centres of origin by identifying genes in one wild relative collected years ago, and then using the language of patent claims to try to also control the same (or similar) genes found in as yet uncollected, or unstudied, wild seeds (i.e., controlling the relevant loci insofar as possible under law).

Thus, while marker-assisted selection and related biotechnological breeding techniques have escaped the wrath of environmentalists because they don't necessarily result in genetically modified crops, the situation with tomato wild relatives shows that these techniques can add to the potential for biopiracy. This is particularly the case for crops like tomatoes, in which combining domesticated types with related wild species is relatively easy.

In addition, whether from a new collection or a seed bank, Syngenta's patent application on a mould-resistant tomato, in which the origin of the claimed gene is not identified, shows that patents continue to be possible without revealing the origin of materials made proprietary. This emphasizes the urgency for an unequivocal international solution to close this crucial gap, again something that was rejected by developed countries in the Nagoya Protocol negotiations. The current provision is for the following:[20]

*Parties shall consider the need for and modalities of a global multilateral benefit-sharing mechanism to address the fair and equitable sharing of benefits derived from the utilisation of genetic resources and traditional knowledge associated with genetic resources that occur in transboundary situations or for which it is not possible to grant or obtain prior informed consent. The benefits shared by users of genetic resources and traditional knowledge associated with genetic resources through this mechanism shall be used to support the conservation of biological diversity and the sustainable use of its components globally.*

However, there is no time frame for this to be established; the issue will be taken up at the second meeting of the preparatory Intergovernmental Committee for the Protocol in April 2012. It can be expected that there will be more years of negotiations starting with the "need" issue.

Countries of origin will need to speedily put in place comprehensive and strong national access and benefit-sharing regulations and work internationally to press user countries to legislate benefit-sharing obligations with effective compliance systems. One aspect of the compliance system must be patent offices requiring the mandatory disclosure of the origin of a claimed gene, evidence of prior informed consent and evidence of a fair and equitable benefit-sharing agreement(s).

**Endnotes**

[1] There is scientific disagreement on an exact number.
[2] Source: FAOSTAT, http://faostat.fao.org
[3] See USDA Plant Genetic Resources Division at http://www.ars.usda.gov/Aboutus/docs.htm?docid=6452
[4] See Tomato Genetic Resources Centre (TGRC) at http://tgrc.ucdavis.edu/
[5] EU-SOL 2011. About the Project (website). http://www.eu-sol.net/science/about-the-project
[6] EU-SOL 2011. Module 4 – Tomato Genetic Resources. http://www.eu-sol.net/science/about-the-project/module-4
[7] Counted among these are Latin American-origin cerasiforme types of *L. esculentum*, some of which are not 'wild', as they were domesticated in Mexico prior to the arrival of Europeans to the Americas.
[8] AVRDC 2011. AVGRIS Database. http://203.64.245.173/avgris/
[9] Chetelat R 2001. Plant Exploration in Northern Chile to Collect Wild Tomato Species, With Emphasis on *Solanum lycopersicoides* and *S. sitiens*. Final Report. And Chetelat R & R Petuze 2005. Plant Exploration in Northern Chile to Collect Wild Tomato Species, With Emphasis on Coastal Populations of *Lycopersicon chilense*. Final Report.
[10] Chetelat R 2001. Personal communication, 29 September.
[11] TGRC n.d. Material Transfer Agreement [for recently collected Chilean germplasm]. See: http://tgrc.ucdavis.edu/MTA/mta-chile.pdf (accessed 4 October 2011).

[12] TGRC 2011. Database Entry for LA0716. http://tgrc.ucdavis.edu

[13] Keygene, partially owned by Enza Zaden, also works in the EU-SOL programme, as do Monsanto and other companies. See the EU-SOL website at http://www.eu-sol.net/public/eu-sol/partners/

[14] Meaning that the plant can pollinate itself and produce fruit with viable seeds genetically identical (or near-identical) to the parent plant.

[15] Rick CM. *Genetics* 128: 1-5 (May 1991).

[16] Finkers R et al. *Theor Appl Genet* 114(6): 1071-1080 (April 2007).

[17] TGRC 2011. Database Entry for LA2093. http://tgrc.ucdavis.edu

[18] USDA 2011. ARS-GRIN Database Entry for PI 126449. http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1133106. There are at least two places named Yaso in Peru. One is northeast of the capital, Lima, and the other is in the more southerly Arequipa Province. Information available from USDA is insufficient to be certain of the collection location.

[19] Monsanto 2011. PCT Publication WO2010147467. See, especially, Claims 8, 9 and 10.

[20] Nagoya Protocol, Article 10.

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