

# THE SOL NEWSLETTER

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## Community News

### SOL 100

Now that we have almost completed the first phase of the SOL Project in which we aimed to produce a high quality draft of the tomato genome, it is time to fire-up the second phase of the SOL Project, which is "SOL-100". In SOL-100 we want to produce the sequences of 100 different *Solanaceae* genomes and link those to the reference tomato sequence with the ultimate aim to explore key issues of plant biodiversity, genome conservation and phenotypic diversification.

The updated version of the SOL-100 Position Paper (see page 2) summarizes our aims, our intentions and our motivations. This position paper can be freely used by all of you to leverage support and funding. In addition, the paper contains a list of *Solanaceae* that we currently envisage to sequence. This list, however, is a proposal and you all are invited to add your favorite species to this list. We also are not restricted to the number of 100 species (the current list contains 99 species!), but merely we can make this list as long as we would like ourselves.

Also, we will start adding more information to this list like some basic information about these plant species and the particular reason why we propose a certain species to be sequenced. This latter information will be crucial to raise the necessary funding to sequence those genomes. In addition, for each species we will indicate who is already sequencing what particular accessions/cultivars of a given species, or who has intentions to do so! This way we will avoid duplications and, more importantly, this will allow us to form novel consortia to work together on a particular species.

All this will be organized in a dedicated SOL-100 section on the SGN website. This part of the website probably will contain a "clickable" phylogenetic tree where all information regarding the different *Solanaceae* can be found and where project information about ongoing sequencing projects will be made available.

It is very exciting to see that SOL100 is already taking off! The first draft of the tomato genome is now available, which also holds for the first draft of the potato genome and that of *Mimulus guttatus*. In addition, *Nicotiana tabacum* is underway as are *Solanum pennellii* and *Solanum pimpinellifolium*. So at least six different species are being sequenced already, which is a good start (only 94 to go!). In addition, initiatives are on the drawing board to sequence the genomes of different accessions of, mainly, tomato and potato.

To make SOL-100 a success, it will be necessary that everyone actively start exploring the possibilities to leverage funding for parts of this project. The Position Paper will be a helpful tool to accomplish this and, in addition, the SOL Co-chair will be of assistance by supplying letters of recommendation and other additional supporting information required.

There is a forum topic called SOL-100 where you can provide information on your current sequencing efforts. The link is: [http://solgenomics.net/forum/posts.pl?topic\\_id=153](http://solgenomics.net/forum/posts.pl?topic_id=153).

On behalf of the SOL Co-chair,  
René Klein Lankhorst

## Sol 100 Position Paper

### The questions

Solanaceae include more than 3000 species with wide adaptation, shape, chemistry, and distribution. Species of the family are of great agricultural, nutritional, horticultural and medicinal importance (e.g., potato, tomato, pepper, petunia and tobacco). This enormous diversity is contrasted by high conservation of gene order and content at the macro and micro syntenic levels. Solanaceae genomes can be genetically tied to a common framework linkage map, thus facilitating the identification of genes with homologous phenotypes in the different species. These features make Solanaceae an excellent taxon with which to address a central question in biology:

**How can a common set of genes/proteins give rise to such a wide range of morphologically and ecologically distinct species?**

But also, studying the Solanaceae can generate answers to the currently highly relevant and urgent question:

**How can a deeper understanding of the genetic basis of plant biodiversity be harnessed to better meet the needs of society in an environmentally friendly and sustainable manner?**

### The aim

The SOL community will create a common Solanaceae-based genomic framework that includes sequences and phenotypes of 100 genomes encompassing the phylogenetic diversity of the group. Specific objectives are to:

- 1) Tie the available tomato, tobacco, potato and Asterid relatives coffee and *Mimulus* (monkey-flower) genome sequences to a common SOL physical and genetic map as well as other Asterid taxonomic groups, such as *Antirrhinum*, sweet potato and mint.
- 2) Select 100 Solanaceae species and Asterid outgroups (**SOL-100**) that broadly span the evolutionary tree and reflect important human uses.
- 3) Apply emerging novel genome sequencing technologies to **SOL-100** and link the sequences to the common SOL physical and genetic maps.
- 4) Genetically map simple and complex phenotypes affecting chemical, morphological, yield and fitness-related traits in the **SOL-100** species.
- 5) Construct bioinformatic vehicles to effectively access and utilize the information generated within **SOL-100**.
- 6) Foster a broadly-based international community of interacting scientists committed to exploring and conserving natural biodiversity.

### Who we are?

The International SOL Genome Project (SOL) is a 'virtual umbrella' aimed at promoting, coordinating and actively seeking additional scientists, countries and funding agencies to participate in an expedition to understand, utilize and conserve natural biodiversity (<http://sgn.cornell.edu/solanaceae-project/>). SOL includes scientists from more than 30 countries that are united and excited about the sustainable and equitable use of natural biodiversity in biological research, plant breeding and conservation of these resources for the future. The SOL community has sequenced both the tomato genome and the potato genome through grants from national funding agencies as well as international collaborative projects.

Mathilde Causse, Jeanne Jacobs, Glenn Bryan, Harry Klee, Sanwen Huang and René Klein Lankhorst

February 11<sup>th</sup> 2010

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## THE CLADES OF SOLANACEAE

Indicative list of potential target taxa for sequencing (starred taxa may prove problematic due to rarity or difficulty with cultivation); this initial list of suggestions has been chosen for ease of access to germplasm, cultivation and breadth of phylogenetic coverage.

Clade (after Olmstead et al., 2008)	Generic diversity (approx.)	Possible target species
Schizanthus	1	<i>Schizanthus pinnatus</i>
Duckeodendron	1	* <i>Duckeodendron cestroides</i>
Goetzeoideae	4	<i>Goetzea elegans</i> ; * <i>Metternichia princes</i> ; * <i>Tsoala tubiflora</i>
Petunieae	13	<i>Petunia hybrida</i> ; <i>P. axillaris</i> ; <i>Brunfelsia uniflora</i> ; <i>B. americana</i> ; <i>Nierembergia scoparia</i> ; <i>Calibrachoa parvifolia</i> ; <i>Fabiana imbricata</i>
Schwenkieae	2	<i>Schwenkia americana</i>
Browallieae	2	<i>Browallia americana</i> ; <i>Streptosolen jamesonii</i>
Benthamielleae	3	* <i>Benthamiella</i> sp.; * <i>Combera paradoxa</i> ; * <i>Pantacantha ameghinoi</i>
Protoschwenkia	1	* <i>Protoschwenkia mandonis</i>
Cestreae	3	<i>Cestrum elegans</i> ; <i>C. nocturnum</i> ; <i>Vestia foetida</i> ; * <i>Sessea dependens</i>
Salpiglossideae	2	<i>Salpiglossis sinuata</i>
Nicotiana	1	<i>Nicotiana tabacum</i> (4x) (in progress) ; <i>N. sylvestris</i> (2x); <i>N. benthamiana</i> (4x)
Anthocercideae	7	<i>Duboisia hopwoodii</i> ; <i>Anthocercis littorea</i> ; * <i>Grammosolen dixonii</i> ; * <i>Anthotroche</i> sp.; * <i>Symonanthus aromaticus</i>
Lycieae	3	<i>Lycium barbarum</i> ; <i>Lycium carolinense</i>
Nolana	1	<i>Nolana humifusa</i> ; <i>N. galapagense</i>
Jaborosa	1	* <i>Jaborosa integrifolia</i>
Latua	1	<i>Latua pubiflora</i>
Sclerophylax	1	<i>Sclerophylax</i> sp.
Hyoscyameae	5	<i>Hyoscyamus niger</i> ; <i>Atropa belladonna</i> ; * <i>Przewalskia tangutica</i> ; <i>Scopolia carniolica</i> ; <i>Anisodus luridus</i> ; <i>Physochlaina orientalis</i>
Nicandra + Exodeconus	2	<i>Nicandra peruviana</i> ; * <i>Exodeconus maritima</i>
Datureae	2	<i>Datura stramonium</i> ; <i>D. metel</i> ; <i>Brugmansia arborea</i>
Juanulloae	7	* <i>Juanulloa mexicana</i> ; * <i>Solandra brachycalyx</i> ; * <i>Markea ulei</i> ; * <i>Schultesianthus leucandrus</i> ; * <i>Dyssochroma viridiflora</i>
Mandragora	1	* <i>Mandragora officinarum</i> ; * <i>M. caulescens</i>
Solaneae	4 (Solanum with 13 clades, 1500 spp.)	<i>Discopodium penninervium</i> ; <i>Jaltomata procumbens</i> ; <i>Solanum melongena</i> ; <i>S. aethiopicum</i> ; <i>S. nudum</i> ; <i>S. dulcamara</i> ; <i>S. laxum</i> ; <i>S. americanum</i> ; <i>S. scabrum</i> ; ; <i>S. tuberosum</i> (first draft completed); <i>S. lycopersicum</i> (first draft completed); <i>S. pennellii</i> (in progress), <i>S. peruvianum</i> , <i>S. pimpinellifolium</i> (in progress), <i>S. chmielewskii</i> , <i>S. neorickii</i> , <i>S. c heesmanii</i> , <i>S. bulbocastanum</i> , <i>S. palustre</i>
Lochrominae	5	<i>Lochroma fuchsioides</i> ; <i>L. australe</i> ; <i>Acnistus arborescens</i> ; <i>Dunalia solanacea</i> ; * <i>Saracha punctata</i>
Physalinae	6	<i>Physalis peruviana</i> ; <i>P. ixocarpa</i> ; <i>Witheringia solanacea</i>
Withaninae	10	<i>Withania somnifera</i> ; <i>W. frutescens</i> ; <i>Tubocapsicum anomalum</i> ; <i>Aureliana lucida</i> ; <i>Athenaea</i> sp.
Salpichroina	2	<i>Salpichroa organifolia</i>
Capsiceae	2	<i>Capsicum annuum</i> ; <i>Lycianthes biflora</i> ; <i>L. multiflora</i>
<b>Potential outgroup taxa from related Asterid families</b>		
Convolvulaceae		<i>Convolvulus tricolor</i> ; <i>Ipomoea batatas</i> (sweet potato)
Plantaginaceae		<i>Antirrhinum majus</i>
Phymaceae		<i>Mimulus guttatus</i> (first draft available)
Lamiaceae		<i>Ocimum basilicum</i> ; <i>Mentha piperita</i> (mint)

## Solanaceae Resources

### Solanaceae Literature Database Managed by the Radboud University Botanical and Experimental Garden, Nijmegen, The Netherlands

by Gerard M. van der Weerden

In 2000, the RESGEN PL 98-133 EGGNET project was started. The Botanical and Experimental Garden participated in this project. One of the objectives within the project to be carried out by the Botanical and Experimental Garden was to build a literature database. At the time the project started, bibliographic information was available from cards in card boxes and from online accessible literature databases. During the five years of the project, a via internet accessible Solanaceae literature database was created to disseminate bibliographical information amongst EGGNET partners, scientists, plant breeders and other interested parties. Information kept on cards was entered into the database manually and information extracted from existing literature databases was added as well. During the EGGNET project, bibliographic information regarding *Solanum melongena* and their wild relatives was entered into this database. After the project ended, the work on the Solanaceae literature database has been continued and the scope was broadened. This in spite of the fact we know that information on the internet is increasing day by day.

What makes the Solanaceae literature database different from other sources?

The database is a Solanaceae and related organisms based database. All references have been edited. This means that journal abbreviations and author names have been standardized and checked as far as possible. Irrelevant keywords have been eliminated from the list and others added to it. Abstracts are not available from this database. Besides the standardization of the references, the main advantage of this database is the organism oriented keyword search. Scientific names, synonyms and misspelled names, as well as common names, cultivar names, PI (plant introduction) numbers, clone and accession numbers, and acronyms like PVS (potato virus S) are in the keyword list.

For example, when searching for *Capsicum annuum*, *Capsicum anuum*, *Capsicum anmuum*, *Capsicum annum* or *Capsicum annumm* you always will find all publications dealing with *Capsicum annuum* which is the correct name. When searching for *Solanum tuberosum* and cv. Bintje you will get all potato references with the cultivar Bintje. To avoid that, when searching for particular information, you are overloaded with less relevant references more general keywords are added to the keyword list. These are keywords like: breeding, phytopathology, nematology, physiology, agriculture, processing, virology, ethnobotany, micropropagation, etc.

Concerning the Solanaceae, a huge number of publications is dispersed over many well-known and lesser-known journals, reports, books as well as book chapters. At this moment over 67,000 references are stored in the database and new references will be added regularly. However, a multiple from what has been entered into this database up to now is still waiting for editing and importing. To get the database published on the internet, Reference Manager® version 11 is used. The literature database is accessible via the Radboud University Botanical and Experimental Garden website <http://www.ru.nl/bgard> or directly via <http://atropa.sci.kun.nl>.



### QTL Data Submission, Analysis and Linking of QTLs to Genomes at the SGN Database

Provided by Isaak Yosief Teclé

As entire genomes are being sequenced, a challenge remains: linking phenotypic variation of complex traits to the underlying genomic variation. Quantitative trait loci (QTL) analysis is used to dissect the genetic basis underlying complex traits. At the Sol Genomics Network (SGN) (<http://solgenomics.net>), we have developed a QTL analysis software (<http://solgenomics.net/qtl/>) which allows QTL researchers to submit their raw QTL data, perform on-the-fly QTL analysis, visualize QTL map locations (Fig. 1) and do comparative analysis between QTLs and corresponding regions on genetic maps (Fig. 2) and genomes (Fig. 3) at SGN.

The QTL analysis is based on R/QTL (<http://www.rqtl.org>) and users can decide using a web interface what statistical parameters to employ for the analysis. The QTL mapping output fully integrates with other SGN analysis tools and is cross-referenced with relevant datasets at SGN and external databases. For example, using the Comparative Map Viewer (<http://solgenomics.net/cview/>), users can compare predicted QTL regions to genetic maps from the same or different *Solanaceae* species. When markers flanking QTLs match genome regions using the genome browser (<http://solgenomics.net/gbrowse/>), users can also identify the corresponding sequences. Corresponding genome regions are annotated with ESTs, unigenes, gene and protein models annotated by the International Tomato Annotation Group (ITAG) and

experimentally characterized genes. The exploration and synthesis of these data can lead to identification of candidate genes for a trait of interest.

To allow QTL comparisons across studies, traits are usually mapped to the *Solanaceae* Phenotypes Ontology (<http://solgenomics.net/tools/onto/>).

Currently, QTL data from three tomato F2 population QTL studies on fruit morphology traits (up to 46 traits per population) are available at the SGN website for viewing.

We invite the *Solanaceae* research community to upload and analyze their QTL data using the SGN QTL tool and share their research outputs with the community. Data owners can opt to make their data to remain private or be publicly accessible.

Please send your questions, remarks and suggestions to [sgn-feedback@solgenomics.net](mailto:sgn-feedback@solgenomics.net).

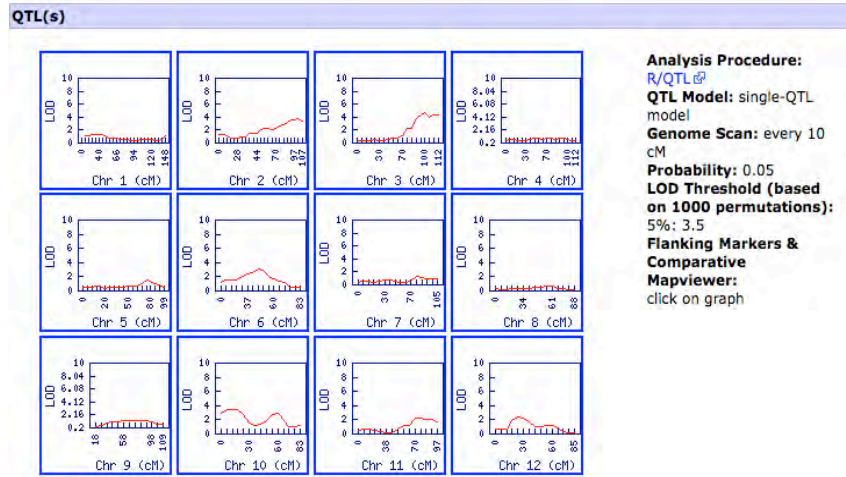


Figure 1: An example of a genome-wide QTL mapping output for a trait.

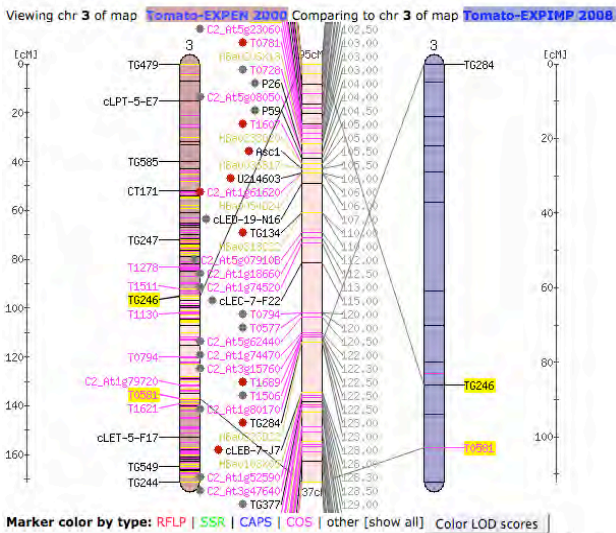


Figure 2: An example of comparative map analysis of a QTL region (right, with flanking markers TG246, T0581) and corresponding region on another genetic map (left).

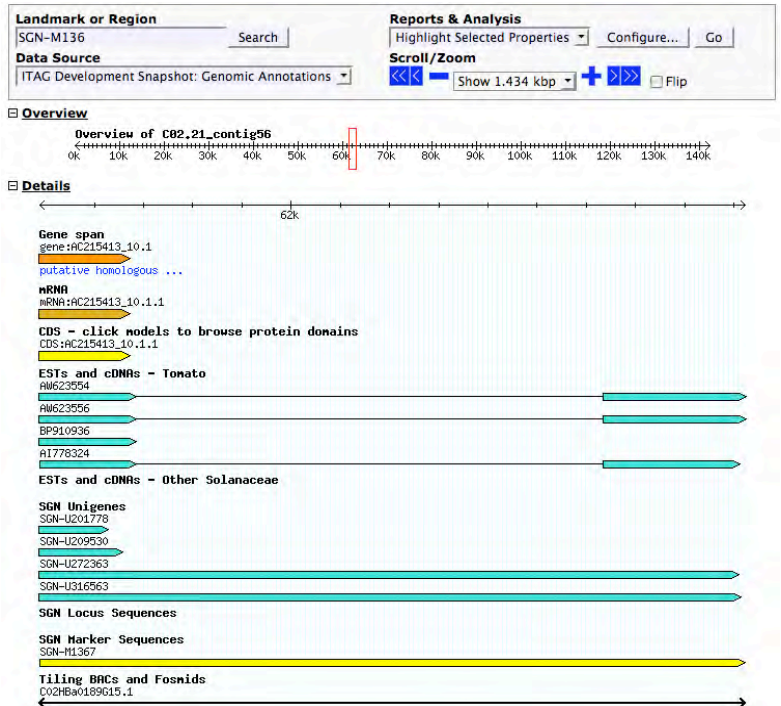


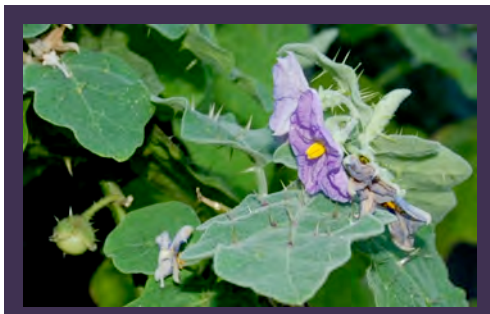
Figure 3: An example of Gbrowse search result for a QTL flanking marker showing the corresponding genomic location with annotations such as gene and protein models, ESTs and cDNAs, unigenes and in some cases experimentally characterized genes.



## Biodiversity

### Creating a Phylogeographic Platform for Studies of Domestication-related Attributes in Eggplant

Written by Rachel Meyer and Amy Litt



*Solanum. undatum* from Malaysia

#### Introduction to eggplant and its ethnobotanical uses

Eggplant (*Solanum melongena* L., subgenus *Leptostemonum*) and its close wild relatives have a two thousand year history of intense use in Asia as both food and medicine. Researchers have stated that even in Neolithic times, humans migrating from the Middle East into S.E. Asia used species that we now consider candidate progenitors of eggplant such as *Solanum incanum* as a medicine, particularly for relief of pain and infections (Daunay *et al.* 2001). Indigenous eggplant cultivars continue to play an integral role in pharmacopoeias such as traditional Chinese medicine, Ayurveda, Philippino herbal medicine, and Thai traditional medicine. Whether the fruits are used raw or cooked, eggplant that is being used medicinally is usually incorporated into meals, though topical applications are also common (Lad & Lad, 2002; Cham, 2007, Meyer unpub. data).

The ethnobotanical uses of Asian eggplant landraces vary by ethnic group and region. In different parts of southern Asia, landraces are used to treat skin cancer, hypertension (Chiej, 1984), chills and fever, bowel and hemorrhoid problems (Daunay *et al.* 2001), freckles (Wilén & Wilén, 1996), high cholesterol (Duke & Ayensu, 1985), pregnancy-related problems, boils and burns (Meyer unpub. data), and to reduce bile (Raghunatha, late 17<sup>th</sup> century).

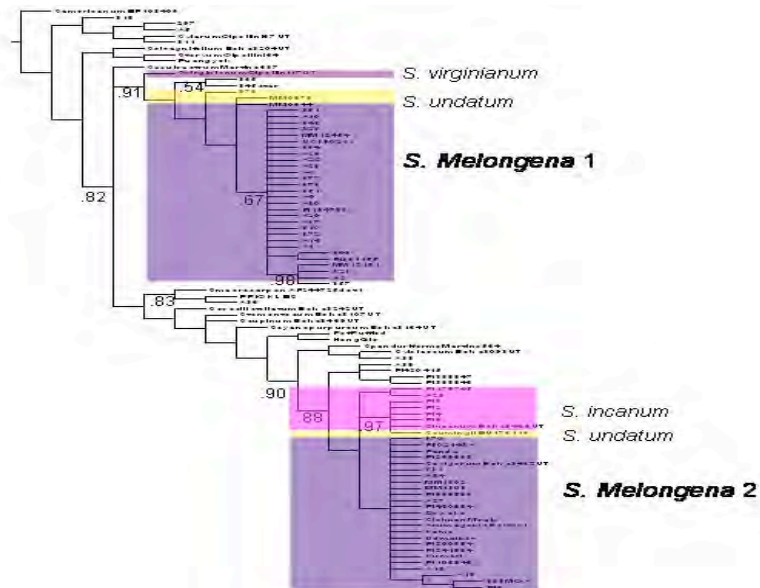
While alkaloids are the most well-known class of phytochemicals in the Solanaceae that contribute to many health-related qualities (Eich, 2008), eggplants also contain polyphenolic compounds and, in particular, hydroxycinnamic acid amide (HCAA) polyamine conjugates shown to have bioactivity that overlaps with many of the attributed health benefits (references in Whitaker and Stommel, 2003; Stommel and Whitaker, 2003). Some HCAA conjugates may also impart a bitter flavor associated with some landraces. Taste preferences for eggplant vary greatly across Asia's cultural groups, for example, bitter eggplant varieties are preferred among communities in the Himalayan foothills, while the Philippines' indigenous varieties are sweet in flavor (Meyer, unpub data). Because these compounds play a role in both flavor and medicinal potential, we hypothesize that the biosynthetic pathway for HCAA conjugates likely underwent modification as a result of selection, resulting in the accumulation of different end products in different landraces.

#### Project goals

This research project aims to explore the phytochemistry and gene regulation that underlie eggplant's medicinal and culinary attributes. In order to better understand the evolutionary trajectory of these attributes, we needed to establish a phylogeographic framework. We evaluated potential wild relatives and progenitors to try to understand the relationships among cultivars and trace the path of domestication using AFLP and ITS sequence data. Accessions are linked with ethnobotanical data, so the distribution of flavor and health-beneficial attributes can be mapped onto this phylogeographic framework. This will allow us to consider whether factors such as geographic proximity, climatic similarity, trade, migration, agroecology, and cultural similarity, may have influenced trends in the data. Based on these results, we will select genetically and ethnobotanically diverse accessions and quantify concentrations of fourteen HCA derivatives including HCAA conjugates. Accessions whose profiles are most variable will be used in Real-Time PCR analyses to elucidate how regulation of key genes in the HCAA biosynthetic pathway may influence production of certain end-products. This will allow us to identify modifications in gene regulation that may have occurred as a result of selection for domestication-related attributes.

#### Building a phylogeographic framework

Using a collection of over 200 accessions assembled from field work (China, the Philippines, India) the AVRDC, USDA ARS-GRIN, INRA, and heirloom seed companies, we are using



**Figure 1:** Phylogeny of some members of *Solanum* section *Leptostemonum* showing domesticated eggplants form two separate clades, which are highlighted in purple. Maximum parsimony strict consensus tree using ITS sequence. Maximum Likelihood strict consensus phylogeny was identical. Posterior probabilities having a value of less than one are shown.

AFLP clustering analysis and ITS-based phylogeny to create a framework for phytochemical and genomic research. Though these population genetic and systematic techniques have previously been used to study members of *Leptostemonum* including eggplant, our dataset includes more thorough sampling of landraces representing southern China (including those in the northern Mekong river valley), the Philippines, and India, which are the three hotspots of eggplant biodiversity, as well as a larger number of candidate progenitors.

The initial aim of this study was to address relatedness of accessions and dispersal/migration of landraces, however during the course of this work some critical taxonomic issues have emerged. Partly due to these taxonomic issues, both the location of domestication as well as the wild progenitor of eggplant remain in dispute. Previous analyses have been unable to fully resolve these issues because of lack of variable markers, taxonomic misidentifications, or incomplete sampling. Our analyses include candidate progenitors from southern Asia, as well as a more comprehensive collection of indigenous landraces. We are also working with Dr. Michael Nee (Solanaceae taxonomist, NYBG) to correct misidentified accessions and notify the germplasm sources.

#### Theories based on earliest evidence of domestication

The earliest written records of domesticated eggplant date back two thousand years in widely separated areas: Chengdu, China (Wang *et al.* 2008) and central India (Bhishagratna, 1961; Sharma, 2008). These equally ancient records suggest various possible scenarios: was eggplant domesticated twice? Did it undergo widespread domestication throughout southern Asia simultaneously? Or was it domesticated once and then brought from one region to the other in ancient times? Recently, 4000 year-old remains that might be eggplant were found in an archaeological site in Rajasthan, India, but the material has only been confirmed to be Solanaceae and starch granule studies have not been able to identify it further (M.D. Kajale pers. comm.; Kashyap, 2006). Thus this provocative find cannot currently be used to support initial domestication in India.

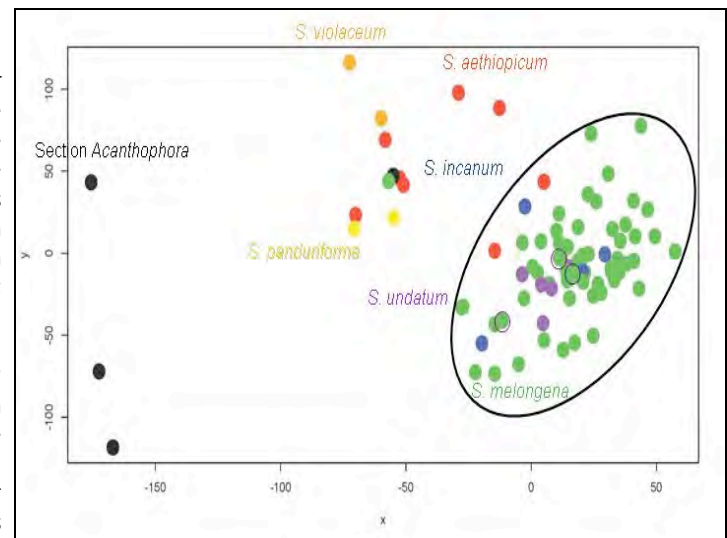
#### Theories based on taxonomy

Until the recent discovery of written evidence showing China rivaled India for the oldest history of domesticated eggplant, most researchers believed India was the site of earliest domestication. Richard Lester and his collaborators formulated the most widely known theory, that *S. melongena* (eggplant) was domesticated from *S. incanum*, an African species that Lester claimed had been spread east throughout southern Asia from Africa in Neolithic times. He proposed that in S.E. Asia *S. incanum* diversified into various wild and primitive cultivated forms, some of which were brought into India and developed into our current widespread "advanced" domesticated eggplant (Sakata and Lester, 1997; Lester & Hasan, 1991). Other hypotheses are that eggplant was domesticated in India or in East Asia directly from a wild ancestor such as *S. cumingii* or *S. undatum* (now synonymous). To further complicate matters, the relationship between *S. undatum* and *S. incanum*, and the status of *S. undatum* as a true wild species, are not resolved (Weese, 2010).

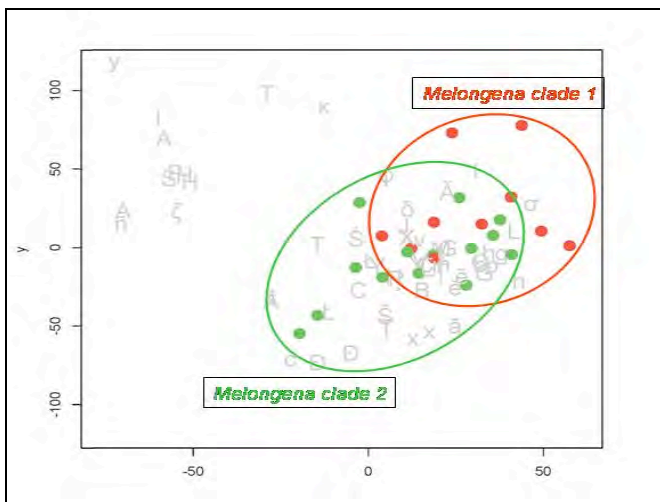
#### Preliminary results

We have screened over 15 polymorphic sequence markers for variability among eggplant and its close relatives, and few have proven useful. These included several COSII markers, microsatellite flanking regions, chloroplast regions, and several nuclear introns. The Internal Transcribed Spacer (ITS) yielded some variability and was used in phylogenetic analysis to determine relationships between domesticated eggplants and wild relatives. We performed both Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses; the MP strict consensus and the ML tree were identical. The resulting tree (Fig. 1) shows domesticated eggplants unexpectedly forming two clades (herein called 1 and 2). In the case of *S. melongena* clade 1, *S. undatum* falls in an unresolved clade with *S. incanum*. In the case of *S. melongena* clade 2, *S. undatum* forms two successive branches that are sister to *S. melongena*, suggesting that *S. undatum* is either a progenitor or part of *S. melongena*. *S. incanum* is not found anywhere near *S. melongena* clade 2. This topology suggests as one possibility two independent domestication events. However, an alternative explanation is that *S. undatum* is a plausible progenitor but that it is a hybrid, and that subsequently the cultivated eggplants underwent lineage sorting.

At the same time we performed AFLP analyses to try to address relationships below the species level. Preliminary results are shown by a Manhattan Distance plot generated in R Stat. Candidate progenitors, *S. incanum* and *S. undatum* fall within a cluster of *S. melongena* and do not form clusters distinct from each other,



**Figure 2:** AFLP Manhattan Distance plot of domesticated eggplant accessions showing species represented by different colored dots. Proximity of points to each other represents genetic relatedness. *S. incanum* and *S. undatum* accessions fall within the cluster of *S. melongena*. Hollow circles represent Lester's germplasm collections of "primitive cultivars".



**Figure 3:** AFLP Manhattan Distance plot of domesticated eggplant accessions showing the ITS *Melongena* clade 1 in red and clade 2 in green.

genetically contributed to domesticated eggplant.

For most of our accessions we have obtained geographic distribution information as well as ethnobotanical data such as taste attributes and medicinal use. Upon completion of this phylogeographic study we will be looking for correlation between phenolic profiles and ethnobotanical attributes of landraces and wild relatives. We will then compare phytochemical and ethnobotanical datasets to phylogeographic patterns and genetic regulation of parts of the phenolic pathway to see if there is further correlation. Through these efforts we hope to gain a better understanding of the key genetic regulators of the biosynthetic pathway for ethnobotanically-valuable phenolic compounds, as well as the modifications in gene regulation that occurred in conjunction with domestication and subsequent selection.

To help clarify Lester's grouping of members of *Leptostemonum* section *Melongena*, and to provide an opportunity to learn about the impressive morphological diversity of eggplants, we are working towards putting photos of collections on the SGN website. Donations of germplasm from eggplant landraces or wild relatives would be enthusiastically received.

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## Genome Updates

### Coffee

Contact: Marco.Cristancho (Marco.Cristancho@cafedecolombia.com)

**Coffee Sequencing Status as of February 2010** Approximately 50 scientists participated in the 3<sup>rd</sup> coffee genomics workshop held as part of the Plant and Animal Genome Conference in San Diego on January 10, 2010. The scientists discussed current and future sequencing efforts by the coffee research community. A group of research centers has been working on developing a cost efficient strategy to *de novo* sequence the coffee genome, as well as to secure interagency funding for the project. The strategy to sequence the coffee genome will involve the adaptation of next generation sequencing technologies (454-Roche and Solexa/Illumina), and will target high coverage *de novo* sequencing to generate reference genomes for both diploid parental species of *Coffea arabica*: *C. canephora* and *C. eugenioides*. A pilot project based on shotgun sequencing of *C. canephora* using the FLX454 Titanium technology was started in 2009 by the launching of the International Canephora Sequencing consortium including research centers in the US, France, Italy and Brazil. To date, 12 runs of 454 Titanium and one Illumina PE run have been completed. 454 sequencing was done at the University of Illinois, USA, ENEA, Italy, and Illumina PE run at Southern Cross University, Australia. The data and additional markers (SSRs) derived from the 454 *C. canephora* WGS runs are expected shortly.

Two proposals submitted to the Agence Nationale de la Recherche ANR (France) were approved for funding. The first phase of the proposal is now completed, which allowed the sequencing of BAC ends by Genoscope of the two *C. canephora* BAC libraries (*Hind* III and *Bam*HI) that were constructed in collaboration with Rod Wing at the Arizona Genomics Institute. In 2009, 73,000 *C. canephora* BAC clones were BAC end sequenced using Sanger technology at Genoscope. The second phase of the project is on going to *de novo* sequence the *C. canephora* genome using multiple sequencing platforms and high depth coverage to obtain a reference genome. CENICAFE and Cornell, on behalf of the International Coffee Genomics Network (ICGN), submitted a proposal to the InterAmerican Development Bank (FONTAGRO) in 2008. The proposal was approved in 2009 to construct a BAC library for the diploid maternal parent of *C. arabica* (*C. eugenioides*) and *de novo* sequence the *C. eugenioides* genome. The project will be started in 2010. Once completed, the high coverage reference sequences for the diploid species *C. canephora* and *C. eugenioides*, using a combination of 454 Titanium and Illumina, should serve as a solid framework for future sequencing and assembly of the genome of the allotetraploid species *C. arabica*, the main cultivated coffee species throughout the world.

### Mimulus

#### Initial Release of the *Mimulus guttatus* Genome: A comparator for the Solanaceae

Contact: Todd Vision, Dept of Biology, University of North Carolina at Chapel Hill, [tjv@bio.unc.edu](mailto:tjv@bio.unc.edu)

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The DOE Joint Genome Institute has released an initial shotgun assembly and automated annotation of the *Mimulus guttatus* genome. *Mimulus* is the first sequenced Asterid genome outside the Solanaceae; it is in the order Lamiales. Because of its evolutionary proximity, we expect the availability of this resource will be useful for the characterization of Solanaceae genomes. Preliminary studies have shown extensive conservation of synteny between *Mimulus* and the Solanaceae, and strong sequence conservation within some exons, though little conservation outside of exons (unpubl. results).

The *Mimulus* genus has long been a popular system for studies of ecological and evolutionary genetics in natural populations, particularly a number of species complexes in the western United States, one of which includes *Mimulus guttatus*; research areas have included reproductive isolation between species, cytoplasmic male sterility, meiotic drive, heavy metal tolerance, serpentine tolerance, flowering time, pollinator syndrome, and inbreeding depression [1].

The assembly and annotation of the *M. guttatus* IM62 inbred line are available from Phytozome (<http://www.phytozome.net/mimulus>) for browsing, BLAST, and download. While the total genome is estimated to be ~430Mb in size, the assembly consists of 321.7Mb arranged in 2216 scaffolds, with 6.5% gaps. For scaffolds, N50=81 and L50=1.1 Mbp. 95.7% of the genome is in scaffolds larger than 50 Kbp. The genome annotation contains 25,530 protein-coding loci. There is a separate collection of 27,504 protein-coding transcripts from EST sequencing of IM62 and other genotypes (and species). These data are also being incorporated into a forthcoming update of the [mimulusevolution.org](http://mimulusevolution.org) database, which integrates the sequence data with genetic markers, maps and other information.

The genome is being released under the Fort Lauderdale guidelines [2], which aim to balance the value of rapid release of genomic data with respect for the scientific interests of the generators of that data. Please feel free to use these data to advance Solanaceae biology, though note that this is a preliminary release, and undoubtedly includes various errors. Plans for publication of the *Mimulus* genome are focused on the large-scale analysis of the gene and repetitive content of the genome and its evolutionary dynamics (including synteny and chromosomal/segmental duplication, gene family evolution, gene structure evolution) relative to other angiosperms. The initial manuscript describing the assembly, annotation, and first global analysis of the *Mimulus* genome will be based on an improved, chromosome-scale assembly currently in development. If you have an interest in using these data for large-scale analysis of *Mimulus*, or contributing to a companion manuscript, please contact the authors.

## References:

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2. Sharing Data from Large-scale Biological Research Projects: A System of Tripartite Responsibility. <http://www.genome.gov/Pages/Research/WellcomeReport0303.pdf>

## Tomato

*China: Contact – Chuanyou Li (cyli@genetics.ac.cn)*

Currently, our effort is spent working on integrating the sequences produced by BAC-by-BAC as well as the whole genome shotgun approach to chr 3. Twenty-five scaffolds from the new assembly version 1.03 were mapped to chr 3 with a total length of 88.75-Mb. We are sequencing 20 BACs that will help to fill in the inter-scaffold gaps. We also identified around 3,000 intra-scaffold gaps with size ranging from 20-bp to 16-kb, which will be filled by sequencing fosmid clones and direct PCR. We are also working on a comprehensive, high quality annotation of jasmonate-related genes of tomato, which will be compared with gene families from the major sequenced plants such as *Arabidopsis*, rice, grape, *Medicago*, cucumber, maize, *Mimulus*, and soybean.

*India: Contact - Akhilesh Tyagi (akhilesh@genomeindia.org)*

At the Indian Initiative on Tomato Genome Sequencing, we have confirmed positions of 100 BACs on chr 5. Till now fifty-nine BACs have been sequenced to phase III level, twenty-five BACs are at phase II level and seven BACs are at phase I level of sequencing. The remaining nine BACs are in the early phase of sequencing or library preparation. Emphasis has been given to finish in-hand BACs and a search is on to find new extension BACs by performing overgo hybridization on the filters available for the three tomato libraries, PCR screening on the 3-D DNA pools of *HindIII* and *MboI* BAC libraries, the fosmid end sequences and SBM (selected BAC mixture) shotgun data.

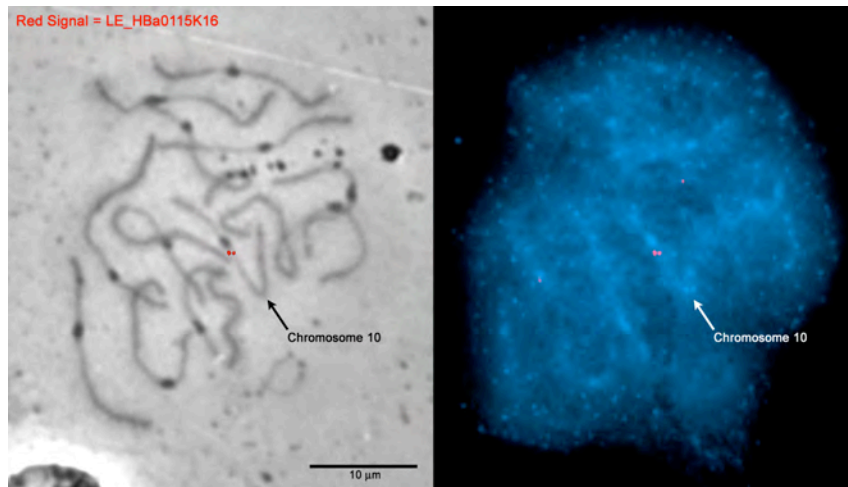
*Italy: Contact - Mara Ercolano (ercolano@unina.it)*

Italy has co-founded the Whole Genome Shotgun effort. To date, we obtained more of the 10 x coverage of the tomato genome performing both 454 WGS and pair-ends runs. Also, two slides of a SOLiD 10kb mate-pair library have been produced. Italian groups are participating in the assembly of data and in the annotation of several gene families (e.g. carotenoids, photomorphogenesis, resistance genes). The BAC-by-BAC sequencing effort has been momentarily stopped until a common strategy for filling the gaps in the shotgun sequence is agreed upon by the international consortium. The IL mapping to assign new loci and sequenced BACs to various bins is still ongoing. Deep transcriptome sequencing is underway to assist genome annotation.

*US: Contact - Joyce Van Eck (jv27@cornell.edu)*

We continue to edit the public tomato genome physical map developed in cooperation with the Arizona Genomics Institute and the group of Rod Wing. This map represents approximately 10X BAC coverage of the tomato genome and takes advantage of clones from four independent BAC libraries (*HindIII*, *EcoRI*, and *MboI* genome partials in addition to a sheared genomic library). The map can be accessed through the physical map link of SGN (<http://solgenomics.net>) and the full assembly can be retrieved from our ftp site. The map and all underlying BAC clones are available to the public without restriction. We strongly encourage those attempting to extend contigs for sequencing or other purposes to revisit the physical map as this new map uses two libraries not incorporated in the original tomato physical maps and thus will provide some new extensions. Note that SGN retains the prior map so that you can compare contigs of interest. We encourage your use of this new resource and your comments. The physical map has now been anchored to the genetic map and the 1.03 sequence assembly and provides validation of many scaffolds and also points to some inconsistencies in addition to identifying putative "bridging" BACs and BAC contigs between scaffolds. The current merging effort is being done in part to develop the necessary pipeline needed for rapid merging with the publication stage tomato genome sequence assembly once it is completed. We are currently sequencing pooled, primer tagged BACs via 454 and have sequenced 139 BACs on chromosomes 1 and 10 in the last 7 months. All of these sequences in addition to the prior 24 BACs sequenced by our team have been deposited in Genbank and are available through SGN.

A total of 214 BAC clones have now been positioned on tomato chromosomes using FISH on synaptonemal complex spreads including fourteen that have been localized and posted on the SGN since our last report. The 214 BACs are distributed among the chromosomes as follows: 1 - 41; 2 - 19; 3 - 15; 4 - 17; 5 - 13; 6 - 10; 7 - 24; 8 - 9; 9 - 18; 10 - 29; 11 - 13; 12 - 6. The recently positioned BACs include (listed by chromosome arm): 1Q, LE\_HBa0256E08; 1Q, LE\_HBa0174H02; 1Q, LE\_HBa0289N16; 1Q, LE\_HBa0302G11; 1Q, LE\_HBa0231M15; 4Q, LE\_HBa0291H22; 6Q, LE\_HBa0188N10; 7P, LE\_HBa0293I23; 7P, LE\_HBa0325D07; 7P, LE\_HBa0002D20; 8P, LE\_HBa0270A17; 8Q, LE\_HBa0213E05; 10Q, LE\_HBa0115K16 (Fig. 1); 12Q, LE\_HBa0148K11.



**Figure 1:** FISH labeling of tomato chromosome 10 with BAC LE\_HBa0115K16, located near the centromere on the long arm.

## First Draft of the Tomato Genome Presented at PAG

by René Klein Lankhorst

During the Solanaceae workshop at the 18<sup>th</sup> Plant and Animal Genome Conference (San Diego, January 9<sup>th</sup> - 13<sup>th</sup>, 2010), the first draft of the tomato genome was officially presented to the scientific community. On behalf of the SOL International Tomato Sequencing Consortium, Roeland van Ham highlighted the results of the Tomato Next Generation Sequencing Initiative, which was initiated by the consortium at the 2008 SOL Genome Workshop in Cologne, Germany.

As part of this initiative, whole genome shotgun sequences of the tomato genome were produced using both 454 and SOLiD technology. This resulted in approximately 28 Gb of 454 data plus an estimated 60 Gb of SOLiD data. Together with the already available Selected BAC Mixture data (Kazusa), BAC-end sequences, fosmid-end sequences plus all BACs sequenced from the euchromatin of the individual tomato chromosomes, now an estimated 95 GB of sequence data is available which roughly equals a 100-fold coverage of the tomato genome.

A first assembly was undertaken using a pre-release of the Newbler assembler, version 2.3. This assembly was conducted with 55 million 454 reads, 3.8 million SBM reads, 135,000 paired BAC ends and 65,000 paired fosmid ends. The results of this first assembly were already very exciting as 800 Mb out of the total estimated 950 Mb tomato genome could be assembled into as few as 7,409 scaffolds. Even better, 95% of this assembled 800 Mb turned out to be present in a total of only 252 large scaffolds and with only as little as 9% of "Ns" in the sequences of these scaffolds. A first run of the tomato genome assembly through the ITAG annotation pipeline revealed that tomato has approximately 34,000 genes.

After the Solanaceae workshop all attending members of the sequencing consortium convened to discuss the further steps in the sequencing project, but also to celebrate the official release of the first draft of the genome! For this special occasion, Giovanni Giuliano had brought some excellent bottles of Spumante while Joyce Van Eck had arranged for some sweets and snacks. After toasting on our success, the business meeting continued with presentations from different partners. Overall, it was decided that the presented assembly (version 1.0) will be upgraded in a number of steps to version 2.0 which will be the final version for the foreseen publication of the tomato genome in spring 2010. In version 2.0, all SOLiD data will be integrated, as well as all available BAC sequences from the chromosome-specific sequencing efforts.

After publishing version 2.0, a new phase of the tomato sequencing project will start in which the genome sequence will be further polished aiming to reach the "Gold Standard" sequence we envisaged at the beginning of the tomato sequencing project. In this version 3.0, as many gaps as possible will be closed, weak parts in the assembly will be reinforced and also an attempt will be made to include as much data as possible from the currently unassembled fraction of 150 Mb of tomato DNA. Jim Giovannoni and myself will take the lead in this new phase of the sequencing project and after publication of assembly version 2.0 we will come with a proposal how exactly to proceed towards version 3.0.



Members of the tomato genome sequencing consortium celebrate the official release of the first draft of the genome.

The current version of the genome assembly is Version 1.03, which can be downloaded from the following URLs:  
<http://mips.helmholtz-muenchen.de/plant/tomato/index.jsp>  
<http://solgenomics.net/>

## Announcements

### Publications

Mallona I, Lischewski S, Weiss J, Hause B, Egea-Cortines M (2010) Validation of reference genes for quantitative real-time PCR during leaf and flower development in *Petunia hybrida*. *BMC Plant Biology* 10:4 doi:10.1186/1471-2229-10-4.

Mattoo AK, Minocha SC, Minocha R, Handa AK (2010) Polyamines and cellular metabolism in plants: Transgenic approaches reveal different responses to diamine putrescine versus higher polyamines spermidine and spermine. *Amino Acids* 38:405-413.

Shukla V, Mattoo AK (2009) Potential for engineering horticultural crops with high antioxidant capacity. *CAB Reviews*, 4: No. 066. DOI 10.1079/PAVSNNR20094066.

Singh AK, Verma SS, Bansal KC (2010) Plastid transformation in eggplant (*Solanum melongena* L.). *Transgenic Res* 19:113-119.

Yang Y, Wu Y, Pirrello J, Regad F, Bouzayen M, Deng W, Zhengguo L (2010) Silencing Sl-EBF1 and Sl-EBF2 expression causes constitutive ethylene response phenotype, accelerated plant senescence, and fruit ripening in tomato. *J Exp Bot* 61:697-708.

### Conferences

#### European Association for Potato Research and EUCARPIA "Potatoes"

June 27 – 30, 2010

Wageningen, The Netherlands

<http://www.eapr.net/sections/breeding-and-varietal-assessment>

The next conference of the EAPR section 'Breeding and varietal assessment' and the EUCARPIA section 'Potatoes' will be held in Wageningen, The Netherlands on June 27 – 30, 2010. The theme of the conference will be: Potato Breeding after completion of the DNA Sequence of the Potato Genome.

We aim for an audience ranging from practical breeders to genome scientists. The conference will offer ample opportunity for PhD students to present their research projects and offer them a highly reduced fee.

Please find the first circular, the tentative program and a registration form at the websites <http://www.eapr.net/sections/breeding-and-varietal-assessment/> and/or [www.eucarpia.org](http://www.eucarpia.org). Early registration will close on March 15, 2010. Submit your tentative title for an Oral or Poster presentation a.s.a.p. before or with your registration.

#### XXI International Congress of Sexual Plant Reproduction

August 2 - 6, 2010

University of Bristol, Bristol, UK

<http://www.sebiology.org/management/meetings/SexualPlantReproduction.html>

Registration is now open.

#### Potato Association of America

August 15 - 19, 2010

Corvallis, Oregon

<http://potatoassociation.org>

#### Capsicum and Eggplant Breeding 2010, Working Group Meeting

August 30 - September 1, 2010

Valencia, Spain

e-mail: [jprohens@btc.upv.es](mailto:jprohens@btc.upv.es)

[www.comav.upv.es/capsicumeggplant](http://www.comav.upv.es/capsicumeggplant)

#### SOL 2010 Update

SCRI and UK-SOL are pleased to announce that the 7th Annual Solanaceae Meeting will be held in Dundee, Scotland. The event will take place from Sunday, September 5th to September 9th, 2010. On Sunday, the 5th, there will be a special welcome event, including an opening lecture by Prof Sir David Baulcombe, a civic reception at Discovery Point, home to Captain Scott's Royal Research Ship Discovery, famous for its Antarctic explorations. There will be a chance to explore the ship and its visitor center. Optional tours will also be available to those wishing to see a bit more of Scotland during their visit to the UK.

The city of Dundee, the fourth largest city in Scotland, is set in a picturesque location on the shore of the River Tay, the largest and most famous river in Scotland. Dundee can be reached by train from Edinburgh, and we are intending to provide bus transport from Edinburgh airport for delegates. Dundee does have a small airport with regular flights from Birmingham and London City airports.



Photo of the venue for SOL 2010



The chosen venue for SOL2010 is the Apex Hotel, located in the heart of Dundee's regenerated waterfront district. The Apex is a short walk from the centre of Dundee, where there are several shops, bars, restaurants and other amenities. Guests staying at the Apex (only 152 rooms so book fast!) will have free access to the gym and spa facilities available at the hotel. There are other hotels for varying budgets within walking distance of the Apex.

The conference dinner will be held on Wednesday, September 8<sup>th</sup> at Guthrie Castle – a uniquely Scottish venue, where you will enjoy a warm Scottish welcome and hospitality. The dinner will also include a brief tour of some of the local countryside and one or two tourist attractions, depending on the weather!

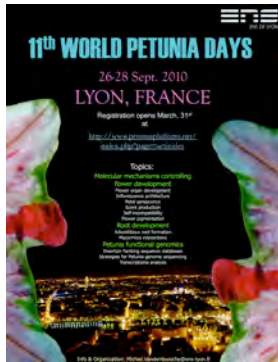
Please visit the website [www.sol2010.org](http://www.sol2010.org) for further information. The website also shows further details of the conference hotel (<http://www.apexhotels.co.uk/hotels/dundee-city-quay/>), other hotels close by, the city of Dundee, tourism (<http://www.angusanddundee.co.uk/>) and travel information.

The conference program will include sessions on: SOL Biodiversity and Evolution, Plant Growth and Development, Biotic Stress, Abiotic Stress, Informatics and computational Biology, Tools and emerging technologies, The SOL Genomes, Translational Genomics and Molecular Breeding, Metabolomics & Proteomics, as well as the usual crop specific workshops. When submitting abstracts, authors should indicate whether they want to be considered for a talk or poster. The Apex Hotel has several meeting rooms available for smaller meetings (10 – 40 people) so if you have ideas for additional sessions or small meetings you would like to hold at SOL2010, please get in touch with the organizers as soon as possible.

Please register now by going to the registration page accessed via the main SOL2010 site, to ensure that you get the 'early bird' rate, which ends on May 31st. We encourage you to book the conference hotel at your earliest convenience - the rooms and other facilities at the Apex are of excellent quality, and the hotel has provided a highly competitive rate.

We look forward to seeing you in Dundee!

Glenn Bryan, Gerard Bishop, Graham Seymour, Karen McLean, Anne Rendall.



## 11th World Petunia Days

Lyon, France

September 26 - 28, 2010

Dear Petunia scientists and – SOL lovers,

The 11th World Petunia Days will be organized at the ENS of Lyon (Ecole normale supérieure de Lyon)/RDP lab (Reproduction and Development of Plants Laboratory) from September 26 - 28 in the beautiful city of Lyon, France.

Traditionally, the Petunia Days have an informal and friendly character, so that people can exchange ideas, materials, techniques, and unpublished data without any inhibition. Together with the Petunia Platform, <http://www.petuniaplatform.net>, this meeting therefore aims to promote collaboration among Petunia scientists. Topics of the seminars traditionally cover molecular mechanisms controlling flower development (flower organ development, inflorescence architecture, petal senescence, scent production, self-incompatibility, flower pigmentation), root development (adventitious root formation, mycorrhiza interactions) and Petunia functional genomics (Insertion flanking sequence databases, transcriptome analyses, strategies for Petunia genome sequencing). New topics on Petunia research are also welcome. Registration and abstract submission will open March 31, 2010, at <http://www.petuniaplatform.net/index.php?page=activities>. For additional info and organization send an email to: [Michiel.Vandenbussche@ens-lyon.fr](mailto:Michiel.Vandenbussche@ens-lyon.fr).

Lyon has its own airport (LYS) with direct flights to most European countries, and a direct flight connection to New York. Lyon can also easily be reached by high-speed train (TGV) from the Paris airport in around 2 hours.



## Solanaceae Recipes

### Indian Nightshades on the Table

Provided by Rachel Meyer

#### *S. aethiopicum*

The fruits of the scarlet eggplant can be eaten green or red, though not many have acquired the taste for the bitter mature fruits. In the Himalayan foothills of India people call varieties of *S. aethiopicum*, "B". Some people there with diabetes eat these frequently. Wash the fruits, slice in quarters and lightly fry with ginger, tomato, and chilli. Add salt to taste.

*S. macrocarpon*

The fruits of the gboma eggplant are more similar in taste to *S. aethiopicum* than *S. melongena*. In Africa, people have cultivated this crop for edible leaves, though in India it seems that only fruits are used and sold in the marketplace. Widely but not commonly used in the plains around the Himalayas, fruits are frequently associated with having a high iron content. Cook them as you would *S. aethiopicum*.

*S. melongena*

Every household in India will have their own version of "barta", a very common eggplant dish. If you have a gas range stove, wash eggplants (leaving calyx on) and place them one at a time directly on the burner. Cook on a medium flame and rotate often until the skin is blackened and the eggplant seems to be deflated. Medium-sized round varieties are easiest to prepare this way. Remove from the fire and let the fruits cool. Peel off the blackened skin and calyx, rinse, and then mash the eggplants. Heat some oil in a skillet. This could be mustard oil, gingely oil (sesame oil), coconut oil, or ghee, depending on which part of India you choose to represent with your barta dish. Sauté one medium onion, garlic cloves, and a tomato (optional), and add the mashed eggplant. Cook for two minutes, then remove, add salt, lime, and lots of fresh cilantro.

*S. torvum*

Though this species is from the New World, it has grown to be very popular in Indian cuisine. Fruits are sometimes sold in a dried form after having been soaked in salty buttermilk. I have also seen *S. americanum* sold this way. A little bit is eaten at dinner to stimulate the appetite as its bitterness is thought to complement the flavor of main dishes. Some people use this as a vegetable for curry or simply fry the fruits. Eating lots of this fruit fried is said to kill intestinal worms. The fruits can also be boiled in water to make a tea, which is said to relieve symptoms of cold and cough.

*S. pseudocapsicum* and *S. sisymbriifolium*

Many people think these fruits are poisonous, and so the plants are usually treated as ornamentals if it is cultivated. But in the Nilgiri hills it isn't uncommon to see people eating the sweet, tangy fruits and spitting out the seeds. Proceed with caution, but don't be afraid to at least sample. Some people say there are anti-cancer benefits to ingesting these fruits.

*S. virginianum*

All parts of this plant are used. Juice of the fruit can be taken as a blood purifier. You could consider this the Indian version of going to Jamba Juice for a shot of wheat grass.

### Braised Snapper with Roasted Tomato, Pepper, Olives & Capers

<http://www.cdckitchen.com/recipes>

#### Ingredients

4 tablespoons olive oil	1 1/4 cup dry white wine
1 onion, roughly diced	6 vine-ripened tomatoes, roughly diced
1 garlic clove, crushed	1/4 cup pitted kalamata olives
1 red bell pepper, roughly diced	1/4 cup drained extra fine capers
3 sprigs fresh basil, roughly chopped	2 large whole red snappers, scaled, gutted
1 sprig fresh oregano, finely chopped	12 ounces fresh baby spinach leaves
1 red jalapeno chili pepper, finely chopped	

#### Directions

Heat 3 tablespoons of the oil in a heavy large frying pan over medium heat. Add the onions and garlic and sauté until soft, about 8 minutes. Add the bell peppers, basil, oregano and chili pepper and sauté until the peppers are crisp-tender, about 5 minutes.

Deglaze the pan with the wine and simmer the liquid to reduce it by half, about 10 minutes. Add the tomatoes and simmer gently until the tomatoes are very tender and juices form, about 35 to 50 minutes. You may need to top sauce up with a little water as it is cooking.

Remove the sauce from the heat and add the olives and capers. Season the sauce lightly to taste with salt and pepper. Be sure not to add too much salt at this point, since the olives and capers will add a salty flavor to the sauce as it continues to cook.

Using a large sharp knife, score 4 slits over each side of each whole fish. Place the whole fish over the tomato sauce. Cover with a tight fitting lid or foil. Return to medium heat until the fish easily comes away from the bone, about 30 minutes. Meanwhile, heat the remaining 1 tablespoon of oil in a heavy large sauté pan over medium-high heat.

Add the spinach and season with salt and pepper. Sauté the spinach just until it wilts, about 2 minutes. Spoon the spinach onto a platter. Serve the fish alongside the spinach. Spoon the tomato and pepper sauce over and around the fish, and serve.