

THE SOL NEWSLETTER

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Community News

The New SOL Co-chair

by René Klein Lankhorst

At the occasion of the 8th Solanaceae Genome Workshop in Delhi last November, a change in management of the International SOL Project was implemented. As of November 13th 2009 the old SOL Co-Chair has stepped down and is replaced by the new Co-Chair.

The new Co-Chair is formed by: Mathilde Causse (INRA Avignon, France), Jeanne Jacobs (Crop & Food Research, New Zealand), Glenn Bryan (SCRI, UK), Harry Klee (University of Florida, US), Sanwen Huang (BGI, China) and myself as Chairman.

First of all, let me start by thanking the former Co-Chair for all their efforts in the past years. Under their leadership, the SOL community has been united to a large extent under the "SOL umbrella" and this community appears to be in very good and healthy shape. The major scientific accomplishment has been the generation of the complete genome sequence of both potato and tomato and with that, we now are on the brink of a new phase of the SOL Project: SOL100.

It will be the pleasure of the new Co-Chair to lead the community into SOL100, in which we anticipate many more Solanaceae genomes to be sequenced and where we will study the genetic and phenotypic diversity of the Solanaceae in great depth in order to answer the central questions about adaptation and diversity posed at the very beginning of the SOL Project.

The new Co-Chair is there to serve the Solanaceae Community and we look forward to intensify our cooperation with many of you.

Happy SOL!
René

Solanum pennellii Genome to be Sequenced

Provided by Alisdair Fernie

The Max Planck Institutes of Molecular Physiology and Developmental Biology have been awarded an exceptional grant from the Max-Planck-Society for the sequencing of the *Solanum pennellii* genome. This will be carried out with additional collaborations with the VIB Ghent, Cornell University and INTA Buenos Aires.

SOL 2010

The dates for the 7th annual Solanaceae Conference, which will be held in Dundee, Scotland have been announced. The conference will take place from Sunday, September 5th (arrival and welcome civic reception) to Thursday, September 9th.

SCRI will host the event and the conference hotel will be the Apex, located on Dundee's regenerated waterfront.

The conference dinner will be held at Guthrie Castle – a uniquely Scottish venue, where you will enjoy a warm Scottish welcome and hospitality.

Please visit the website www.sol2010.org which will be live in January 2010 to register for interest and for further details of the conference hotel, the city of Dundee, and travel information.



Guthrie Castle – conference dinner venue



Dundee's waterfront with historic ship

Solanaceae Resources

Databases Available

Plant breeding research nowadays deals with a lot of data, resulting from phenotyping in field and greenhouse trials but also from high-throughput analysis of molecular markers, RNA transcripts (microarray), proteins and metabolites. Databases are becoming indispensable to manage these data and to use them for the selection of plant material and for identification of markers, metabolites and mechanisms associated with important agronomic traits.

Within Wageningen UR Plant Breeding, we are developing a relation database system, which aims to support breeding for quantitative agronomical traits. The database can be explored and queried through a web-based interface, which offers tools to present basic statistical overviews such as box-plots, histograms, but also multivariate tools like principal component analysis and cluster analysis. Graphical genotyping tools are available to show molecular marker data in relation to genetic linkage maps (Fig. 1). The same graphical genotyping visualization tool is also used to show positions of QTLs, for example, calculated on-the-fly using the kruskall-Wallis algorithm (Fig. 2). In addition, photos of each accession can be shown together with a detailed report of observations made on this accession. A biomoby web service client is incorporated within the system to allow data exchange with sequencing, metabolomics, and transcriptomics databases. The integrative analysis of different types of ~omics data is the primary focus of future developments and should allow us to postulate new hypothesis about the genetic model and genes underlying our trait of interest and will be instrumental for improving quantitative traits.

Figure 1: Genetic linkage map and graphical genotype of chromosomes 1 - 4 of the *S. pennellii* introgression line population (Eshed and Zamir 1995). Each vertical bar represents one individual of this population. The *S. pennellii* LA716 introgressions are shown in blue while the genome of the recurrent parent genome (*S. lycopersicum* M82) is shown in pink.

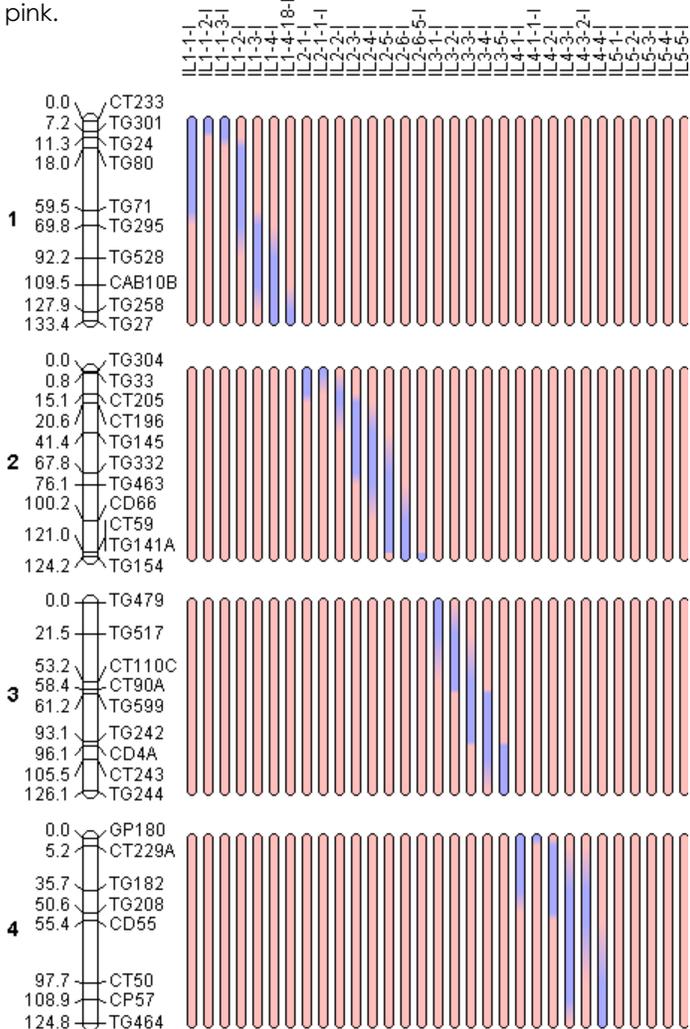
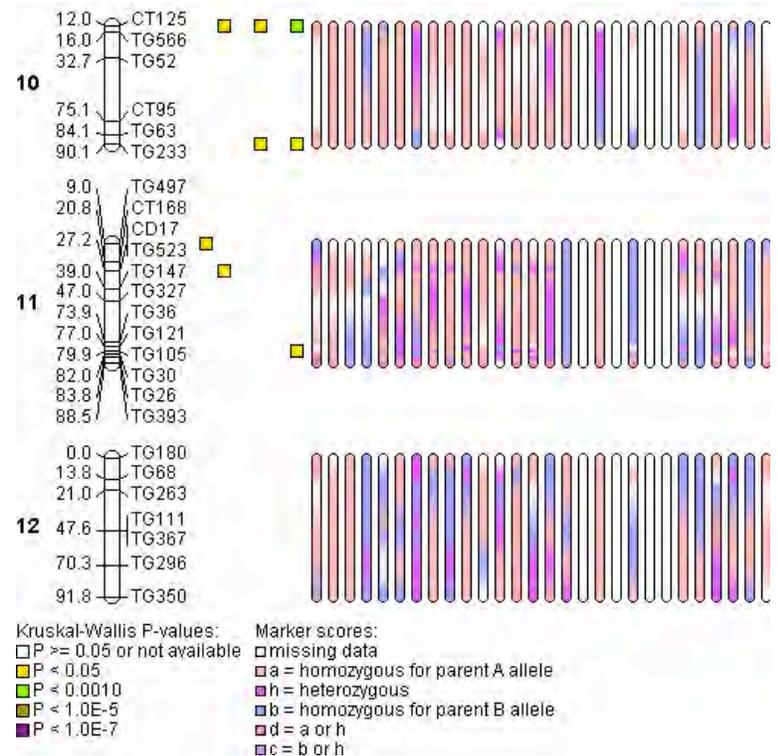


Figure 2: Chromosomes 10 - 12 of the *S. cheesmaniae* RIL population (Goldman 1995). Putative QTLs for six different traits, as determined by the Kruskal-Wallis method are shown between the genetic linkage map and the graphical genotype. Four out of six traits show a chart elements are hyperlinked to drill through to individual QTLs, chromosomes, individuals or traits.



To date, observations of 32 field trials using experimental mapping populations and ~7000 tomato landraces have been stored. The EU-SOL germplasm and phenotype database was initially developed as a tool for other scientists within the 6th framework European community project, 'High Quality Solanaceous Crops for Consumers, Processors and Producers by Exploration of Natural Biodiversity'. However, more and more of the information will also be made publicly available via the URL: <https://www.eu-sol.wur.nl>.

The database is running on a MySQL server and a new visualization layer, which will be accessible on December 9, 2009, is programmed in Java EE5. The statistical package R (<http://www.r-project.org>) is used to provide on-the-fly analysis capability to the visualization layer. Integration with external databases is achieved by implementation of a biomoby (<http://www.biomoby.org/>) compliant client. New functionality and new data are added frequently, so the usefulness of this database and its web-based interface will continue to develop in the future.

In addition to this resource, we also developed other databases. For example, a large amount of pedigree data on current and historic potato cultivars and progenitors has been collected over many years and combined into the potato pedigree database. The potato pedigree database can be queried online and allows users to create reports and dynamically created pedigree-tree images and is available at <http://www.plantbreeding.wur.nl/potatopedigree>. An ultra dense recombination map of potato, containing more than 10,000 AFLP markers, is available at <https://cbsgdbase.wur.nl/UHD/>.

The development of this resource was co-financed by the 6th framework EU project "High Quality Solanaceous crops for consumers, processors and producers by exploration of Natural Biodiversity" contract number: FOOD-CT-2006-016214, by Wageningen UR Plant Breeding and by the Centre for BioSystems Genomics (CBSG) which is part of the Netherlands Genomics Initiative / Netherlands Organization for Scientific Research.

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Tomato SBM Assembly Information is Available

We are pleased to announce the public release of the tomato SBM (selected BAC clone mixture) assembly information through the online database at <http://www.kazusa.or.jp/tomato/>.

An objective of the SBM sequencing project was to collect sequence information from gene-rich regions of the tomato genome more randomly and quickly in order to complement the ongoing international project. BAC clones that were presumably derived from euchromatic regions were pooled and randomly sequenced. This project has been coordinated with the international project by providing them with the partial sequences for acceleration of the project. The sequence information generated in this project, together with the product of the international project, would benefit researchers in the *Solanaceae* research community.

The summary of the tomato SBM assembly is as follows:

To enrich for gene-rich regions in the tomato genome, two sets of BAC clone pools, which contain 30,800 clones collectively, were generated based on the BAC end sequences and subjected to shotgun sequencing. A total of 4.2 million Sanger reads generated from two sets of BAC-pool libraries were assembled along with BAC end and fosmid end sequences that had been made publicly available on SGN. As a result, the non-redundant sequences of 540,588,968 bp consisting of 100,783 contigs were generated. A total of 40,041 complete or partial structures of protein encoding genes, apart from those of transposons and retrotransposons, were assigned to the obtained SBM contig sequences.

The online database that provides the nucleotide sequences of the SBM contigs with annotations describing the predicted genes as well as the ftp site for data download is available at <http://www.kazusa.or.jp/tomato/>.

We encourage you to use this new genome sequence resource.

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New *Coffea arabica* EST Sequences in GenBank

Scientists from the National Coffee Research Center - CENICAFE in Colombia have just published a total of 41,985 *Coffea arabica* EST sequences in GenBank. This is to date the largest deposit of sequences for this species, given that before this submission there were roughly 2,000 sequences in public databases.

The EST sequences were obtained after Sanger sequencing, quality control, contamination and vector removal from cDNA libraries of several coffee tissues. These sequences represent an essential resource for the understanding of the coffee genome and the discovery of genes of agronomic value such as those involved in production of the plant, resistance to diseases and pests, flowering, and cup quality. The information is available at:

<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucest&cmd=Search&dopt=DocSum&term=txid13443%5BOrganism%3Aanoexp%5D>.

Sequences were clustered and assembled using the TGICL tool (Pertea et al., 2003), Megablast (Zhang et al., 2000) and the CAP3 assembler (Huang and Madan, 1999). TGICL is a wrapper script, which invokes Megablast and CAP3. Sequences are initially clustered based on an all-against-all comparison using Megablast. The initial clusters are assembled to generate consensus sequences using CAP3. Assembly criteria include a 50 bp minimum match, 95% minimum identity in the overlap region and 20 bp maximum unmatched overhangs. By using this approach we obtained a total of 10,726 *C. arabica* unigenes. These unigenes and their functional annotation can be retrieved at:

<http://bioinformatics.cenicafe.org/SI/transcriptAssembly/contigSearch.php>. The EST *C. arabica* sequences and unigene assembly with SGN parameters can also be retrieved at the Solanaceae Genomics Network (<http://solgenomics.net>).

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Highlight Articles

Do potatoes and tomatoes have a single evolutionary history, and what proportion of the genome supports this history?

Written by Flor Rodríguez and David M. Spooner

Conserved Orthologous Set markers (COSII) represent a set of putative nuclear orthologs, that is, genes sharing a common ancestor by speciation, in contrast to paralogs that are duplicated copies within a genome through polyploidization or tandem duplications. Establishing orthology in divergent species is difficult in plants and identifying true orthologs is complicated by the fact that many plants are paleopolyploids and extensive gene duplication has occurred during their evolution. The discovery of such markers was initiated by Fulton et al. (2002) who screened a large tomato Expressed Sequence Tag (EST) database against the *Arabidopsis* genomic sequence and identified 1025 genes (referred to as a conserved ortholog set, or COS markers) that are single or low copy in both genomes and that have remained relatively stable in sequence since the early radiation of dicotyledons. Wu et al. (2006) improved on the discovery of more markers with better determination of orthology across wider phylogenetic distances and identified 2869 single-copy orthologs or Conserved Orthologous Set II (COSII genes), which are shared by most, if not all, euasterid plant species and *Arabidopsis thaliana*.

The taxonomy of potato and tomato, and relationships to other *Solanum*, has been the subject of great controversy. Phylogenies reconstructed with only one or a few independently inherited loci may be unresolved or incongruent due to taxon and gene sampling, horizontal gene transfer, or differential selection and lineage sorting at individual loci. The availability of a wide range of COSII markers provided an ideal set of tools to help resolve such controversies. This paper screened 40 COSII markers with intron content over 60% that are mapped in different chromosomes; selected a subset of 19 of them by the presence of single band amplification of size mostly between 600 and 1200 bp; sequenced them in all 13 tomato species, in a phylogenetically divergent set of nine potato species, and outgroups, and performed phylogenetic analyses with individual and concatenated datasets. The results have produced by far the greatest taxonomic resolution to date in potato and tomato, as evidenced by support values for the main clades (Fig. 1).

Among potatoes, when total evidence (using all data in a concatenated dataset) was invoked, one single predominant history was highlighted with complete resolution within and among the three main clades. It also supported the hypothesis of the North and Central American B-genome origin of the tuber-bearing members of *Solanum* sect. *Petota* and showed a clear division between A genomes. In the case of tomato, the analyses with all sequence data completely resolved 19 of 21 clades, for the first time revealed the monophyly of five clades, and gave further support for the recent segregation of new species from the former *Solanum peruvianum*.

On the other hand, when a "prior agreement" approach (that combines the data when no significant character incongruence exists between partitions) was used other potato evolutionary histories are revealed but with less support. Baum (2007) pointed out that in cases where genes have tracked more than one underlying history, some of the differences among datasets would not be due to sampling error but to genealogical discordance. He considered the hypothetical evolutionary consequences of such discordant phylogenetic histories. If a single event of horizontal gene transfer had occurred,

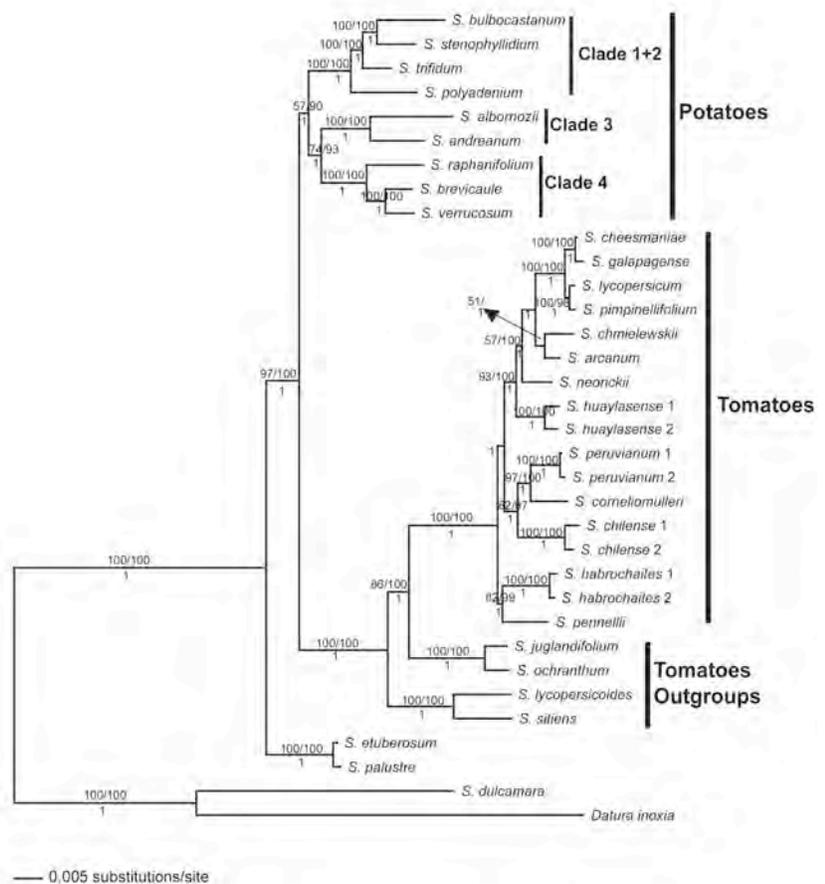


Figure 1: Bayesian phylogram based on a combined analysis in potato, tomato and outgroups. *Datura innoxia* and *S. dulcamara* were used as outgroups. Numbers after the species name indicate allelic variants. Branch lengths are drawn in proportion to the estimated number of substitutions per site and represent an average of the branch length of all trees sampled in the Markov chain that have that branch. Bootstrap values higher than 50% are indicated above branches, the first value refers to Maximum Parsimony and the second to Maximum Likelihood analyses; below branches are the posterior probability values.

one primary history and one minor history are expected. Under lineage sorting or introgression, one primary history and co-minor histories are expected. But, if hybridization occurs two co-primary histories is expected. Ané et al. (2007) developed a methodology called "concordance analysis" that estimates the distribution of such possible divergent evolutionary histories within a multi-gene data set. The concordance analysis confirmed and summarized the discordance among COSII, providing "alternative histories" conflicting with the predominant history revealed when total evidence was invoked (Fig. 2). The potential biological reason for discordance could be processes such as past hybridization, lineage sorting or fast rates of speciation.

This study confirms and quantifies the utility of using DNA sequences from different parts of the genome in phylogenetic studies to avoid possible bias in the sampling. It shows that 11-18 loci are enough to get the dominant history in this group of *Solanum*, but more loci would be needed to discern the distribution of gene genealogies in more depth, and thus detect which mechanism most likely shaped the discordance.

For this reason, with funding from the USDA Competitive Grant Funding, Rodríguez, Spooner, Meredith Bonierbale (International Potato Center), and Lukas Mueller (Boyce Thompson Institute) are extending these studies to a wider array of COSII markers and potato and tomato species, and screening COSII markers for conversion to PCR-based markers for genetic mapping, such as done by Wu et al. (2009) in eggplant.

The reference for the full publication can be found in the Publications section of this newsletter.

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Figure 2: Summary of the Bayesian concordance analysis in potatoes performed with 12 COSII, showing alternative evolutionary histories. Above branches are the concordance factors for $\alpha = 1, 10$ and infinite respectively. The numbers supporting the branches are concordance values indicating the proportion of the genes supporting these branches.

Capsicum pubescens: an underutilized species of hot pepper

Contributed by John Samuels

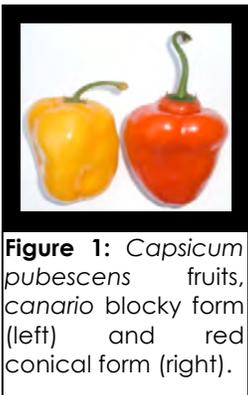


Figure 1: *Capsicum pubescens* fruits, canario blocky form (left) and red conical form (right).

Introduction

The capsicums are some of the most widely cultivated food plants on Earth. They make up a diverse genus, which originated in South America and consists of around thirty species, five of which are cultivated in various parts of the world. These are: *Capsicum annuum* L., *C. chinense* Jacq., *C. frutescens* L., *C. baccatum* L. and *C. pubescens* Ruiz & Pav. They are prized for their distinctive "pods," all of which have high culinary value.

History

C. pubescens (Fig. 1) was one of the first domesticated plants in the Americas; DeWitt & Bosland (1996) give its time of domestication as around 6000 BC. This took place in the Andean region (Pickersgill, 2007) probably in Bolivia. Many years later, post-Columbian explorers such as Garcilaso de la Vega, in the seventeenth century, noted that *C. pubescens* was the most common pepper amongst the Incas during his time (Heiser, 1987). It was undoubtedly an ingredient of food preparations used for ceremonies

involving Incan royalty and the attendant priesthood. It seems likely that its subsequent spread from South America northwards was brought about by human migrations across the land to Central America and Mexico. This probably took place as late as the twentieth century (Andrews, 1984; Smith and Heiser, 1957).

Distribution

C. pubescens is generally known as the *rocoto* in the Andes (*locoto* in Bolivia) and this name may originate from the Spanish for "rock," *roca*, as the fruits are particularly hard-fleshed. Its indigenous names include: *rócot-uchu*, *ro'quote*, *lo'koti*, or *llata* (Andrews, 1984).

The *rocoto* occurs only in cultivation (DeWitt & Bosland, 1996; Pickersgill, 2007) and is grown throughout the Andes from Columbia to Chile, and also to a lesser extent in Costa Rica, Honduras, Guatemala and in southern Mexico (Oaxaca and Chiapas). It is well adapted to cooler temperatures and is grown at an altitude of up to 3500 m, mostly in small-scale family plots. Cultivation of the *rocoto* is more or less totally unknown outside of these regions, and this may be due to its requirement for a long growth season with cool, but freeze-free conditions (Basu & De, 2003).

Characteristics

C. pubescens was first given its scientific name in the eighteenth century by the botanists Ruiz and Pavon, who discovered it in cultivation in Peru (Ruiz & Pavon, 1799). The specific epithet *pubescens* relates to the hairy or pubescent appearance of all aerial parts (Fig. 2). This characteristic, along with purple-colored flowers and black seeds (Figs. 3 & 5), sets this species apart from the other cultivated capsicums, which are non-hairy, have pale brown seeds and mostly have whitish flowers.

The *rocoto* is the largest and most robust of all the cultivated capsicums; for this reason amateur growers tend to refer to it as the "tree chilli." If allowed to grow unchecked it will easily reach over 4 m in height and as much in breadth by its second year. Its habit is initially erect, with luxuriant foliage (Fig. 4), but it may adopt a scrambling habit as the branches gain length and become heavier. Pruning is therefore necessary to ensure a compact bush that is convenient when harvesting. Leaves may reach around 18 cm in length. The root system is relatively shallow, but nevertheless extensive. After the first year's growth, lateral roots may reach 1.5 m in length. The bushes are highly productive from their second year onwards and may produce fifty or more fruits in a growing season. The *rocoto* may live up to ten years in the tropical Americas (Bosland & Votava, 2000).

Fruit forms

Fruits are robust, thick-fleshed and may be red, orange, yellow (*canario*) or more rarely brown; all immature fruits start off green. Occasionally purple-fruited "varieties" are described, but these are probably the result of the effect of the growing environment on the anthocyanin content of the developing fruits. Fourteen different fruit shapes have been recognized (Rick, 1950), but these approximate to four main forms. These are: spherical, blocky (similar to a sweet pepper), cylindrical and conical (Fig. 6). Fruits may measure up to 8 cm long and 7 cm across and weigh up to 100 g, or 3.5 ounces. Spherical fruits seem more popular in Central America and Mexico. At maturity, the outer pericarp may develop corky regions, similar to the *jalapeño* form of *C. annum* var *annuum*. Seeds are rugose and dark brown to black. The fruit stalk is always relatively long, robust and curved (Fig. 5).

Pickersgill (1971) determined that *C. pubescens* was genetically isolated from the four other domesticated species. The *rocoto* will, however, hybridize with its closest wild relatives *C. cardenasii* Heiser & P.G.Sm. and *C. eximium* Hunz. (the *ulupicas*) and produce edible fruit, such as the "*rocopica*" (*C. pubescens* X *C. cardenasii*).

Fruit pungency

The pericarp of the *rocoto* is moderately pungent and quite fruity in taste; however, as in other hot pepper species, the "veins" and seeds provide the heat. This is considerable and measures around 30,000-50,000 Scoville units (DeWitt & Bosland, 1996) and probably reaches as high as 300,000 units, according to growing conditions and ripeness of the fruit. *C. pubescens* possesses a distinctive range of capsaicinoids (Bosland & Zewdie, 2001; Zewdie et al, 1998), the substances responsible for the hot flavors in *Capsicum*. This may mean that the pungency of *rocotos* has a different basis to other common species, and possibly helps to explain why some people find the *rocoto* even more overpowering than the hottest *C. chinense* cultivars. *C. pubescens* is prized for its explosive flavor, and has a legendary reputation in parts of Latin America. It is known affectionately in Central America and Mexico as *chile caballo*, the horse chilli, as the fruits have a flavor that "kicks like a horse!" Its pungency is also described in Peru as *levanta muertos* ("raising the dead") and spoken of by many as *el mas picante de los picantes* ("the hottest of the hot").



Figure 2: Detail of *C. pubescens* leaf and flowering shoot, first year of growth.



Figure 3: *C. pubescens* flowers.

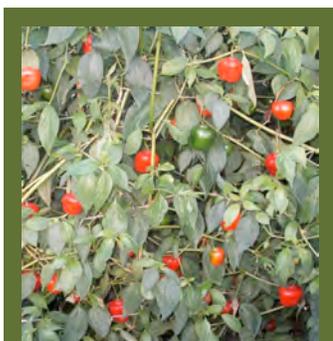


Figure 4: Fruiting bush showing luxuriant foliage, second year of growth.



Figure 5: Black seeds are a typical feature of the rocoto.

Underutilized species of the present-novel crop of the future?

The rocoto has an ancient history and has continued to be a staple source of food in upland Latin America for thousands of years. It may have been the first *Capsicum* to be domesticated but, compared with the other four domesticates, it has been the slowest to spread outside of its zone of cultivation.

Since its discovery over two hundred years ago very few studies have been performed on this species. Relatively recently, Heiser & Smith (1948) followed by Rick (1950) re-acquainted us with this lesser-known cultivated *Capsicum*. Further studies were subsequently initiated, e.g. taxonomic (Eshbaugh, 1979; Jensen et al, 1979; McLeod et al, 1979), evolutionary (McLeod et al, 1982, 1983; Pickersgill, 1971), karyology (Shopova, 1966), genetic resources and breeding (Pickersgill, 1997; Rodriguez-Burruezo et al, 2009) and domestication (Pickersgill, 2007). Our understanding of the potential of this species has continued to develop, and it is clear that the novel crop potential of

the rocoto is considerable. There have been recent attempts to establish the rocoto in highland Java; it is likely that other tropical highland regions will see similar trials in the future. It is more adaptable to its surroundings than it might first appear, and may also be suited to countries with temperate climates such as the UK (Samuels, 2008, 2009). Providing it is not exposed to prolonged, intense sunshine, and protection from freezing temperatures is possible, the rocoto will grow to be a robust shrub capable of producing a lucrative harvest for several years.

The rocoto is already a favorite with hot pepper aficionados, and its culinary potential is gradually becoming recognized on a wider scale. Over the last few years several processed rocoto products have become available. For example, one manufacturer in Miami, Florida now produces a murderously hot rocoto sauce using imported Peruvian fruits. Frozen South American rocotos are also available in specialist markets in the USA (Fig. 7). It is likely that in the foreseeable future the rocoto will be adopted for horticultural production in other parts of the world, just as its better-known relatives have been.

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This article was adapted from: "The Prolific Pod: Capsicums Through Time, Space And Diversity" - available in 2010.



Figure 6: Variety of rocoto forms and colors, Antigua market, Central Highlands, Guatemala. Photo: © Doris Shepherd Wiese.

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Figure 7: Frozen rocoto retail product, California, 2009
Photo: Child of Night.

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Genome Updates

Pepper

Contact: Sunghwan Jo (shjo@kribb.re.kr)

As the assemblies of the tomato and potato genomes have been announced, Korea Sol extended our efforts to pepper genome sequencing. Pepper is the most important vegetable for the fresh market and processed food additives in Korea. The pepper genome (3000 Mb) is about three times larger than that of tomato (950 Mb), but the pepper genome is known as highly syntenic with the tomato and potato genomes according to previous comparative analysis based on genetic markers and sequence. Our plan is to produce a whole genome sequence coverage of *Capsicum annuum* cv. CM334, the same resource used for the BAC library. To decipher the genome sequence, we will combine two platforms, Genome analyzer II (former Solexa) for whole genome shotgun sequencing (~50x genome coverage) and 454 FLX Ti for pools of selected BACs for enriching the genic region. After the assembly of the Genome analyzer II data, the selected BAC mixture sequencing data will also be included and then tied together with the tomato genome. Depending upon the Korean progress, the whole genome assembly may be performed in collaboration with the BGI plant genomics group (Dr. Sanwen Huang). Our goal is to complete the first draft of the pepper genome by the end of next year.

Potato

Contact: Christian Bachem (Christian.Bachem@wur.nl)

The PGSC is continuing to update the Doubled Monoploid (DM) genome sequence with additional fosmid-end sequences, BAC-end sequences, 454 reads, and Illumina reads. We anticipate making a new release of the genome assembly in early 2010. Additional sequencing efforts on the heterozygous RH clone advanced and analysis of the RH and DM genomes are underway. The consortium is also working on annotation of genes within the DM as well as further analysis of the genome for a manuscript describing the potato genome.

Here is a brief list of resources for the PGSC that are available to the community:

1. Potatogenome.net provides information on the PGSC at: <http://www.potatogenome.net/>
2. Pre-publication access to the DM potato genome sequence is available at: <http://www.potatogenome.net/index.php/Data>
3. Blast searches against the DM potato genome sequence as well as other potato/Solanaceae sequences is available at: <http://potatogenomics.plantbiology.msu.edu/index.php?p=blast>

As we are entering the post-genome phase for potato research, the PGSC will be organizing a meeting at the Plant and Animal Genome meeting in January 2010 to begin initial discussions on directions, common interests, and collaborations in post-genomic potato research that are of general interest to the potato community.

The meeting will be held at 1:30 pm in the Stratford room on January 12th, 2010 at PAGXVIII. For people with interests in post-genomics efforts in potato but cannot attend the meeting, please email your comments directly to potato-genome-i@googlegroups.com. Additional information on the meeting can be obtained from Christian Bachem (Christian.Bachem@wur.nl).

Tomato

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

We are pleased to announce the creation and full public release of a new physical map of tomato, which should help in identification of additional extension BACs. 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 shared 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. We have added a small set of additional anchor BACs and are proceeding with manual editing to improve the map and we will then begin selecting possible minimal tiling paths. This updated physical map will be placed on SGN shortly. 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. With respect to tomato genome sequencing we have completed approximately 6X coverage of the tomato genome via 454 and continue toward our project goal of 10X. We are also in the process of sequencing pooled, primer tagged BACs via 454 and preliminary analysis of the de-convoluted data looks very promising with 20 of the approximately 60 BACs sequenced to date assembling to single contigs without any finishing effort.

A total of 200 BAC clones have now been positioned on tomato chromosomes using FISH on synaptonemal complex spreads including twelve that have been localized and posted on the SGN since our last report. The 200 BACs are distributed among the chromosomes as follows: 1 - 36; 2 - 19; 3 - 15; 4 - 16; 5 - 13; 6 - 9; 7 - 21; 8 - 7; 9 - 18; 10 - 28; 11 - 13; 12 - 5. The recently positioned BACs include (listed by chromosome arm): 2P, LE_HBa0090J13; 2Q, LE_HBa0176A14; 2Q, LE_HBa0303I24; 3Q, LE_HBa0101C24; 5P, LE_HBa0282A06; 5P, LE_HBa0149J17; 7Q, SL_Mbol0141H03; 7Q, SL_EcoRI0101G19; 7Q, SL_EcoRI0095F20; 8P, LE_HBa0025I17; 8Q, LE_HBa0027D09; 10Q, LE_HBa0334K22.

UK: Contact - Gerard Bishop and R. Lopez-Cobollo (g.bishop@imperial.ac.uk, r.lopez-cobollo@imperial.ac.uk)

Based on the bioinformatic analysis of the BAC/fosmid end sequences using our Gbrowse viewed Golden Path (AGP based) and by screening the 3D BAC superpools using chr4 markers (27) we identified 23 BACs and 3 fosmids on chr4. All of them have been confirmed to be on chr4 by IL mapping. We have sequenced to phase II 10 BACs and 1 fosmid: SLMbol0090M22, LE_HBa0091M11, LE_HBa0054E15, SL_Mbol0048I03, SL_EcoRI0091C05, SL_Mbol0069J23, SL_Mbol0105G02, LE_HBa0051E04, LE_HBa0102P20, LE_HBa0028J23, LE_HBa0025G05 and a fosmid: SL_FOS0162E05. With this analysis and the received sequences, we have created 3 new contigs, filling 1 gap and enlarged 6 contigs. Moreover, we are reordering 6 contigs along the chromosome and changing the relative orientation of 1 contig on chr4.

Other BAC/Fosmids: SL_EcoRI0041M09, LE_HBa0012C21, SL_Mbol0028K07, LE_HBa0114K12, SL_EcoRI0049J22, LE_HBa0052C01, SL_Mbol0137H03, SL_Mbol0003A06, LE_HBa0035L11, SL_Mbol0142E15, LE_HBa0215J17, LE_HBa0024B06, SL_FOS0010B17, SL_FOS0279J18 are ready to be sequenced depending the recently released WGS assembly.

We have also been focusing on ensuring that the components of the ITAG (International Tomato Annotation Group) pipeline hosted at Imperial College will annotate successfully Next Gen data and hence the new assemblies being prepared. This includes handling properly custom accession codes, empty protein fasta files, annotation instances not encountered before, and in general dealing with a large amount of data in an efficient manner. Moreover, we are participating in the new initiative to compare and validate Next Gen data assemblies from the Dutch (Roeland van Ham) and the French (Mondher Bouzayen) groups. Specifically, we are currently mapping SOLiD data produced at the Imperial College based on a 7-8 kb mate-pair library, against the available assemblies to assess levels of coverage. Preliminary results show that ~13% of these SOLiD reads can be mapped to the Dutch assembly giving rise to ~2x coverage. We are also exploring the methodology to be used for the mapping of 454 transcriptome data, the results of which will be integrated in the genome annotation in the future.

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

At the Indian Initiative on Tomato Genome Sequencing, we have confirmed positions of 100 BACs on chr5. Till now, fifty-eight BACs have been sequenced to phase III level, twenty-one are at phase II and fifteen are at the phase I level of sequencing. The remaining six BACs are in the early phase of sequencing or library preparation. A search is on to find new extension BACs by performing overgo hybridizations on the filters available for the three tomato libraries, PCR screening on the 3-D DNA pools of HindIII and Mbol BAC libraries, the fosmid end sequences and SBM (selected BAC mixture) shotgun data.

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

We produced more than 2Gbs of the tomato genome performing both 454 WGS and paired-ends runs. Moreover, 2 slides of a SOLiD 10kb mate-paired library have been obtained, giving around 630,000,000 reads for both R3 and F3 primer. The data have been promptly uploaded in the general repository of the whole genome shotgun approach. Assembling efforts combining different strategies (BACs, fosmid ends and EST mapping) are in progress. Now we are facing a challenging task, the development of an algorithm able to find out, starting from a genome assembly and SOLiD mate-pair reads, the most probable path joining all the contigs. This algorithm will be used for validating and comparing the two tomato genome assemblies from the Dutch/French assembly team. The final aim is to determine the best assembly, and it will be publicly released and in the same time annotated through the ITAG pipeline. A specific prediction tool for specific gene families (R genes) is being tested on the partial genome that is now available. Moreover, we are proceeding to re-map seeds from BACs in various chromosomes in order to provide anchor points to NextGen data for assembly.

Announcements

Publications

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Conferences

Plant and Animal Genome Conference XVIII

January 9 - 13, 2010
San Diego, CA
www.intl-pag.org

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
www.comav.upv.es/capsicumeggplant



Solanaceae Recipes

Rocotos Rellenos (stuffed rocoto peppers)

Provided by John Samuel

See Rocotos article on page 5 of this newsletter.

Ingredients

500g ground beef (or vegetarian alternative)
 12 fresh, ripe rocotos (with stalks)
 2 white onions, finely chopped
 3 cloves garlic, finely chopped
 2 hard-boiled eggs, chopped
 1/3 cup raisins

medium strength chilli powder - add according to heat desired
 1 teaspoon chopped *quirquina* leaves (or European coriander,
 if not available)
 2 tablespoons fresh peanuts
 12 slices strong cheese
 3 tablespoons vegetable oil

Method

1. Fry the beef, onion and garlic until well cooked.
2. Remove from heat and add other ingredients, stirring in well.
3. Cut the top off each rocoto, remove seeds and "veins" (according to pungency required); fill each with the mixture.
4. Place a slice of cheese on the top of each and replace tops.
5. Bake in a hot oven for around 20 minutes.
6. Rocotos should be cooked through but still firm-fleshed.