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Executive Summary

To explore the genetic basis of drought tolerance in tomato we employed an introgression line (IL) population of a cross between cultivated tomato (*Solanum lycopersicum*; *cv* M82) and drought-tolerant wild species (*Solanum pennellii*). The ILs in a homozygous and heterozygous condition were evaluated in irrigated and non-irrigated fields in Israel and Palestine and yield QTL were identified that act mostly in an overdominant manner and were controlled mainly by the shoot. By using genotypes carrying a pyramid of three independent QTL yield was increased by more than 50% compared to a control market leader variety both under wet and dry fields. This demonstration of the breaking of agricultural yield barriers provides the rational for implementing similar strategies for other agricultural organisms that are important for global food security. The marker generated germplasm that was developed in the project is leading to the breeding of unique sun-dried tomato varieties that are being tested as a potential gourmet food to be produced by Palestinian farmers and the local food industry. One of the greatest achievements of this project, which suffered from a number of unfortunate delays, is the establishment of a functioning molecular lab in Hebron with trained personnel. In addition this cooperation linked the Hebron group to the international tomato community in a manner that opens new opportunities for collaboration, training and scientific discoveries. Both the Hebron group and the Israeli lab take part in the EU-SOL project which focuses on tomato biology as well as a project of the DFG that aims to conduct metabolite profiling on tomatoes grown in drought conditions.

Research Objectives

Plant evolution under domestication has lead to increased productivity but at the same time it narrowed the genetic basis of crop species (Rick 1974; Tanksley and McCouch 1997; Lee 1998; Khush 2001). Wild genetic resources that are related to crop plants have been used extensively as a source for the introduction of single gene traits into modern varieties where the major focus has been on disease resistance genes; commercial tomato hybrids nowadays contain up to 15 disease-resistance genes of wild origin (Pan et al. 2000). More recently geneticists have been approaching wild species with the aim of improving crop plants with respect to complex traits such as yield, quality and stress tolerance; tomato has been a pioneering crop in the application of this method (Zamir 2001). For this purpose, different interspecific population structures have been used as a genetic framework to identify and map quantitative trait loci (QTL) that affect the desired phenotypes. One of the most efficient breeding population structures is introgression lines (ILs) that are composed of an elite variety, which carries single marker defined chromosome segments introgressed from genetically divergent germplasm. The advantage of ILs stem from the fact that the mapping analysis is performed in a leading genetic background and thus any trait identified in can be directly incorporated into variety development programs. Such an IL populations involving the wild species *S. pennellii* constitutes the biodiversity basis for the present project.

The tomato ILs provide abundant segregation for whole genome naturally selected variation affecting yield, morphological and biochemical traits (Causse et al. 2004). The use of complete genome coverage RFLP markers ILs also facilitated the identification of QTL affecting fruit sugar content and the map based cloning of the underlying nucleotide (Fridman et al. 2004). For wider applicability of the multi-species tomato IL approach the population is being anchored using a common framework of PCR based molecular markers (a few hundreds of which are available the Solanaceae Genome Network http://sgn.cornell.edu/). To date 7% of the euchromatic portion of the tomato genome is being sequences through an international SOL consortium of 12 countries (http://sgn.cornell.edu/); It is expected that by 2008 80-90% of the genome would be sequenced (Mueller et al. 2005).

The shortage of water is a central issue in the Middle East; consequently, strategies to make water use more efficient are of prime concern. Moreover, the development of drought-tolerant cultivars, capable of reaching economical yields under significantly reduced irrigation, will

strongly contribute to a regional agriculture under constant pressure to reduce water use. This study is of crucial importance in establishing a Palestinian-Israeli collaboration to tackle together the harsh problems of drought affecting the region and to develop a better understanding of the biology of drought tolerance.

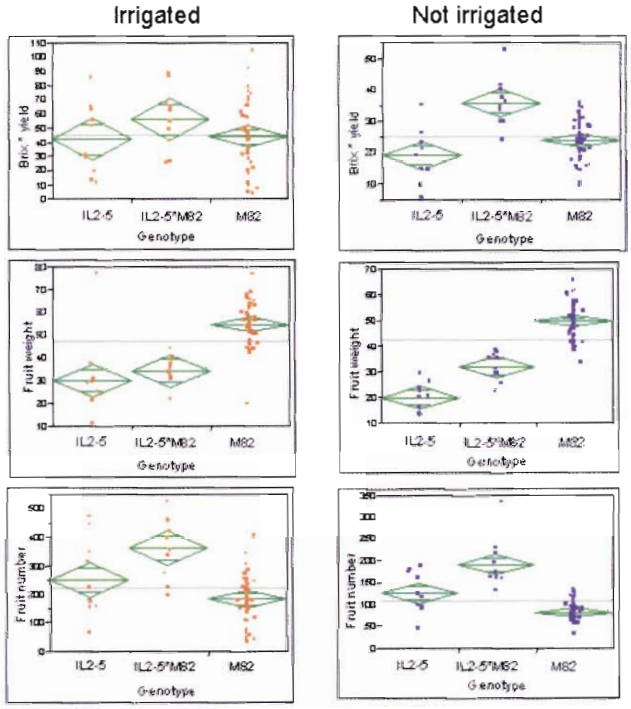
Research Accomplishments

Drought tolerance in tomato - We employed only the IL population involving the drought-tolerant wild species (*Solanum pennellii*). The ILs in a homozygous and heterozygous condition were evaluated for six yield components in irrigated (wet) and non-irrigated (dry) fields and a bin-mapping approach was used to characterize the response to drought stress with respect to the expression of yield-related quantitative trait loci (QTL) (Figure 1; Gur et al. umpublished). We detected transgressive segregation for all the yield-associated traits in both environments. Most of the yield related QTL were expressed in both environments (77%), while only 16% were wet specific and 7% were specific to dry conditions. A large proportion of the yield improving QTL were overdominant in both environments (45% in the wet and 43% in the dry). To characterize the mode of action of the QTL, we used a reciprocal grafting approach, which indicated that most of them were controlled by the shoot, while only two ILs showed effective root action. This approach also allowed the identification of hidden QTL that could not be detected in non-grafted plants. The results suggest that improvement of yield under drought conditions in tomato is achieved mainly by the action of overdominant shoot QTL, which confers yield stability. This study demonstrated the potential of QTL bin-mapping of exotic variation to dissect genetic and physiological components of a complex trait such as yield in extremely different environments. The study illustrates the value of the *S. pennellii* ILs for identifying a QTL that increases yield of tomatoes both under irrigated and non-irrigated field condition (Gur and Zamir 2004). Hybrids heterozygous for three independent yield-promoting introgressions had 50% higher production than that of a control market leader variety under both wet and dry field conditions (that received 10% of the irrigation water). Although this research led to the identification of QTL that dramatically improve yield under non-irrigated conditions, the production of fresh tomatoes in this stress environment was still not enough to support an economically viable tomato industry in Palestine.

Sun dried tomatoes - Sun dried tomatoes are gourmet products that fetch higher prices than fresh tomatoes - particularly when the sun-dried fruits are organically grown. The Israeli-Palestenian teams have initiated the breeding of unique sun dried varieties characterized by the following attributes (Figure 1): 1) The tomatoes are small due to the introgression of IL2-5 which harbor QTL for reduced fruit weight (Frary et al. 2000) and yield heterosis; thus these fruits dry quicker and maintain their tomato like shape (as compared to large fruits that tend to disintegrate). 2) The varieties are suited for cultivation in fields with low irrigation due to introgression of wild species origin. 3) The metabolic quality of the less irrigated fruits is improved and thus the sun dried taste better. 4) Tomato lines were bred with different colors and shape and thus unique sun dried products could be developed.

Based on IL experiments and the breeding work we can define the objectives and the basic germplasm for the sun dried project. Sun dried tomatoes fetch a higher price than regular industry tomatoes, particularly if they are marketed as high quality processed products with olive oil and spices (Figure 1). The quality of sun-dried tomatoes is superior when grown in non-irrigated fields or under reduced water input; this is mainly reflected by higher sugar content, which is responsible for the improved taste. In addition, due to the reduced irrigation the tomato fruit are of smaller size and thus the rate of drying is faster and the fruit maintain their integrity in the olive oil. However, under reduced water input yield is reduced compared to normally culture fields. To overcome the yield penalty from the reduced watering of the plants and still benefit from the high quality of the final product we screened the *S. pennellii* introgression lines for genomic regions that improve yield under non-irrigated conditions. One of the *S. pennellii* introgressions (IL2-5 see http://www.sgn.cornell.edu/) was characterized by a smaller fruit size coupled with strong yield heterosis reflected in elevated sugar yield (BY; Figure 2). Whereas in the non irrigated treatment the yield of the M82 was 2.5 Kg per m^2 and the yield of the homozygous IL2-5 was 2.2 Kg per m^2, the hybrid between the two lines (heterozygous for the wild species introgression) produced significantly higher yield (50%) than both parents (3.6 Kg per m^2). The unique morphology and yield heterosis advantage of this hybrid facilitated the production of high quality sun-dried products from these tomatoes. This introgression will be combined with additional *S. pennellii* genomic segments that are associated with yield heterosis (Gur and Zamir 2004) to generate a unique sun-dried product that will be produced by Palestinian farmers. To further enhance the uniqueness and

Figure 2: Yield associated traits associated with IL2-5 that reduces fruit size and increases yield both under irrigated and non-irrigated condition.



applicability of the bred sun-dried varieties we are combining the breeding material with two types of diversity sources: 1) The recessive mutation *jointless-2* (*j2*) which completely suppresses the formation of pedicel abscission zone (Figure 1) thus facilitating the harvest of fruit completely devoid of the sepal part. 2) Various fruit color mutations that modify carotenoid content of the fruit providing the opportunity to produce a product that will be attractive (Figure 1). Furthermore, due to the health benefits of the different carotenoids that are present in our initial breeding material the resulting product would have higher health benefits and perhaps could be marketed as such.

For the sun-drying process the group in Hebron developed an initial protocol: The tomatoes are harvested at the peak of their maturity and go through several washings before they are cut and laid out to allow full exposure to the sun. Over a period of 2-3 days the rays of the sun dry the tomatoes. The slow gentle drying process imparts that special flavor distinctive of sun-dried tomatoes. The tomatoes are placed in large bins, lined with poly bags, and cooled to maintain

color and flavor. Sun Dried Tomatoes must be treated with care and should be considered semi-perishable (like many fine gourmet foods). These Tomatoes have a sweet, intense tomato flavor, brilliant color, and are a good choice for use in cooking, sauce and salad preparations. Halved sun dried tomatoes were jar packed in olive oil as a prototype for a high value gourmet product (Figure 1).

PCR Marker Framework - To link the ILs of *Solanum pennellii*, on a common map we are using orthologous COSII markers, which are PCR-based markers developed from a set of single-copy conserved orthologous genes (COSII genes) in Asterid species (Wu et. al. unpublished http://sgn.cornell.edu/markers/cosii_markers.pl). Each COSII gene (representing a group of Asterid unigenes) matches only one single-copy Arabidopsis gene and phylogenic analysis supports the classification as orthologs. "Universal Primers" were designed to amplify orthologous counterparts (exons and/or introns) from other related solanaceous species including those that lack of sequence data. SGN includes sequence information of each of 300 COSII gene as well as the mapping information of those mapped COSII sequences. This COS framework is being used by the Solanaceae community to link divergent Solanaceae genomes on a single comparative map that will be tied together to the sequence map (Mueller et al 2005).

Scientific Impact of Collaboration

Good personal contacts have already been established with the Israeli partner Prof. Dani Zamir and Dr. Ayed Mohammad. Dr. Bilal Ghareeb has spent sometime during 2004 in Rehovot in Israel with Prof Zamir and his team to set up molecular techniques. Dr. Ghareeb spent also sometime during 2000 at experimental fields in Akko with Prof Zamir and his team to set up crop harvest treatments. Planning meetings were held in Jerusalem (IMCA) to discuss research issues for a MERC-USAID project. In addition to the international collaboration inherent in the project the work is well embedded in the international Solanaceous genome initiative. Partners have met at the 2nd International Solanaceous genome conference where they were important participants. The proposed project would dovetail with these ongoing collaborations and furthermore all results obtained from the work are being deposited onto the website of the Solanaceous Gene Network a database created by Dr. Lukas Müller. Cornell University, New York, USA.

Description of Project Impact

The project has been successful in developing the following areas: 1) Methods for utilization of wild germplasm in breeding. 2) Enhanced understanding of heterosis. 3) Detailed evaluation of the effect of water stress on development of tomato plants and the influence that genetics and heterosis has on this phenomena. 4) Establishment of new sun dried tomato varities and agro - and post harvest techniques required to exploit the novel crops. 5) The results of this research could create a viable water efficient agro-industry in the Palestinian Authority. 6) This project which led to further education of young scientists in molecular biology assisted plant breeding and helped in linking the labs in Rehovot and Hebron to the international scientific community.

Strengthening of Developing-Country institutions

The lab equipment and chemicals was purchased with the help of our Israeli partners. The lab in Hebron University is now set up and conducting molecular and PCR-based analysis (Figure 1). Mr. Anas Ahmad Arafat Abu Zeineh is a graduate student working together with Zamir and Mohammad. He came to Rehovot with little background in practical molecular biology and genetics and during a period of six months worked closely with Dr. Zachary Lippman (a post-doc in Zamir lab) and received detailed hands on training in all aspects of the work. Presently he is back in Hebron and is running the molecular biology laboratory lab with frequent visits to Rehovot to analyze the data together with Dr. Lippman.

This project facilitated the participation of the Palestinian partners in four international activities in a manner that will integrate us with the world scientific community: 1) The International Solanaceae Genome Project (SOL) - The long-term goal of the SOL program is to create a network of map and sequence based resources and information to address key questions in plant adaptation and diversification. Presently more than 30 countries (including Palestine and Israel) are united under the virtual umbrella of SOL (see http://sgn.cornell.edu). 2) EU – The Palestine and Israeli partners participate in a project just approved by the EU (Framework 6) entitled: High Quality Solanaceous Crops for Consumers, Processors and Producers by Exploration of Natural Biodiversity. 3) The partners have received a grant from the DFG Trilateral Cooperation (in collaboration with Dr. Alisdair Fernie, Department of Lothar Willmitzer Max Planck Institute for Molecular Plant Physiology, Golm, Germany) on metabolic content of

drought grown tomatoes. 4) Presentation of our results in a poster at the Second Solanaceae Genome Meeting in Italy (September 2005)<http://www.solanaceae2005.org/>.

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Figure 1
Top Left: A non-irrigated tomato field in the Hebron area.
Top Right: A non-irrigated tomato field in Akko.
Middle Left: The molecular laboratory being set up in Hebron (2005).
Middle Right: Sun dried tomatoes in olive oil – a Hebron Product.
Bottom Left: Assortment of colored, small-fruited tomatoes bred for sun-drying.
Bottom Right: Jointless (left) and jointed (right) tomatoes.