צנובר Zenovar

Brix Quantitative Trait Loci for Processing Tomatoes: Case Study 2

Research goal	To identify Quantitative Trait Loci (QTL) in wild tomato species that can increase the sugar content, yield and quality of elite processing tomato varieties
Beneficiaries	Tomato growers and processors
Activities conducted in order to achieve the objectives	Identified QTL from five wild *Lycopersicon* species that improve key yield and quality associated traits of processing tomatoes. Set the stage for using the QTL in marker-assisted programs and for applying map-based cloning of the targeted QTL/genes. Field trials in California, Israel and Spain of lines intogressed with discovered QTL's. This research was the first attribution of complex genetic quantitative traits to a specific genetic locus in the plant and animal world.
Funding	BARD awards: US-1388-87, IS-1822-91C, IS US-2427-95 and IS-3009-99C; $1,045,000. Industry: $2,250,000. Other Academic Funds: $800,000.
Publications	59 journal publications, 36 of them in the top of the publications were published in the top impact factor quartile (Q1)., 8 with more than 500 citations and 2 with more than 1000 citations.
Students involved	In Israel: 3 graduate students and 2 post-doctoral researchers, all of whom currently hold positions in academia; 4 in Israel and 1 in the US.
Stakeholders' collaboration	The Introgression lines were made publicly available *via* the Tomato Genetic Resource Center at UC Davis .
Environmental impact	none
Social impact	none
Commercial engagement	Israeli, European and US Seed companies (A.B. Seeds., De Ruiter, Monsanto)
Patents	1 patent, filed by De Ruiter Seeds, currently assigned to Monsanto
Practical agricultural applications	The leading processing tomato varieties in California carry a QTL (BRIX9-2-5) originating from the *Solanum pennellii* introgression lines. This QTL improves productivity and the sugar content of the fruits.
Economic impact	Net present value of BARD's investment is $261 million, thereof $166 million already attained. The Internal rate of return is 28%. Benefit cost ratio is 74, thereof 48 already attained.

Green- Academic information; Yellow - Social and environmental information; Blue - Economic information

1 Objective: Improving Tomato Fruit Brix

The aim of the research was to discover QTL (quantitative trait loci) alleles associated with quantitative traits in wild tomato and transfer them to elite cultivars to improve fruit characteristics. Specifically, QTLs associated with fruit soluble solid content (TSS) were pursued. TSS in fruits of wild *Lycopersicon* species can reach up to 15% of the fruit's fresh weight, 3 times higher than in cultivated varieties. The studies aimed to resolve the genetic basis for this variation.

2 Research Activities

Between 1987-2002 four BARD awards (US-1388-87, IS-1822-91C, IS US-2427-95 and IS-3009-99C) were granted to Steven D. Tanksley (Cornell University) and Dani Zamir (Hebrew University). See Appendix A for full details of the awards.

In the two earlier BARD awards (1987-1994) RFLP markers in wild tomatoes tightly linked to several disease-resistance genes were identified and high-resolution maps of genes resistant to root-knot nematodes, tobacco mosaic virus, and fusarium race 2 were created.

S. Tanksley conducted pioneering work in establishing molecular linkage maps, making it possible to identify, map, and study the effects of individual loci that control a quantitatively inherited trait (QTLs). This paved the way for the next stage of collaborative work between the two researchers and adoption of the marker assisted breeding techniques of the Tanksley lab by D. Zamir at HUJI to pursue QTL for beneficial quantitative inherited traits in tomatoes.

The QTLs were discovered by inserting short chromosome segments of wild tomato species (*Lycopersicon hirsutum, S. pimpinellifolium, Solanum pennellii, L. chmiehskii and L. chesmanii)* into elite cultivars and using the advanced backcrossing breeding technique to determine which of the wild species introgressions are associated with superior performance of lines. Performance parameters included yield, total soluble solids (TSS), plant weight, fruit weight and fruit color.

L. pirnpinellifolium was first shown to possess QTL alleles capable of enhancing TSS, important to processing tomato production. Using a set of 50 introgression lines developed from a cross between the green-fruited species *Lycopersicon pennellii* (each containing a defined *L. pennellii* chromosome segment) and the cultivated tomato, *Lycopersicon esculentum*, it was possible to map 23 QTLs that increase Brix, most of which also introduced adverse genetic traits when intogressed into the cultivated tomato. The highest increase in TSS with no negative effects on yields was due to the introgression line IL9-2-5, which harbors the *S. pennellii* allele of the LIN5 gene that encodes for an invertase, thus increasing fructose and glucose content.

Superior lines were created using map-based cloning of the Brix 9-2-5 discovered from the wild species. The performance of these lines was evaluated in California, Spain and Israel. This finding was the first attribution of complex genetic quantitative traits to a specific genetic locus in the plant and animal world in general.

3 Academic Impact

3.1 Publications

59 peer-reviewed journal publications have been published based on research from the 4 BARD awards. Of these, 36 are in the top quartile (Q1), 8 have been cited more than 500 times, and 2 have been cited more than 1000 times.

3.2 Capacity Building

In Israel: 3 graduate students and 2 post-doctoral researchers were involved in the BARD supported research for QTL research. All currently hold positions in academia; 4 in Israel (3 at Agricultural Research Organization, Volcani Center and 1 in the Weizmann Institute) and 1 in the US at U. Wisconsin.

3.3 Stakeholder's Collaboration

The *S. pennellii* introgression lines (seeds and database) generated as part of the BARD supported research, are publicly available *via* the Tomato Genetic Resource Center, at UC Davis[1].

The research provided a platform for the formation of an isogenic tomato 'mutation library' in the genetic background of the processing tomato variety M82. The mutations were characterized according to a phenotypic catalog comprised of 15 major categories and 48 sub-categories (BARD 3337-02). All data was made publicly available[2].

D. Zamir participated in a multi-national research team that provided the full genomic sequence for the stress-tolerant wild tomato species *S. pennellii*[3].

4 Commercial Engagement

AB seeds held an exclusive license to the intellectual property developed by D. Zamir and from 1992 until 2008 AB Seeds contributed around $200,000 per annum for R&D with the Zamir lab. The license had a duration of 18 years during which royalties of 2.25% on sales were shared between the Hebrew University and Cornell University.

[1] https://tgrc.ucdavis.edu/

[2] In the Solanaceae Genome Network (SGN) on a site called 'The Genes That Make Tomatoes' (http://zamir.sgn.cornell.edu/mutants/).

[3] Nature Genetics (2014); doi:10.1038/ng.3046

AB Seeds Ltd. was founded in 1994 by D. Zamir. In 2002, D. Zamir left the company's management and remained involved as a scientific consultant. AB Seeds Ltd. produces hybrid tomato seeds, and developed varieties of tomato with increased soluble sugar in the fruit, generating a sweeter fruit that is desirable to both tomato processors and fresh tomato consumers. First seed sales were in 1996. AB Seeds Ltd was sold to De-Ruiter in 1999. In 2008, the company was purchased by Monsanto and the Israeli branch became Monsanto's Vegetable Seeds Division.

4.1 Patents

Cultivated tomato plant having increased Brix value and method of producing same, Dani Zamir, Tzili Pleban, Eyal Fridman; US7235719B2; Granted: 2007-06-26, Applicant: De Ruiter Seeds, Current Assignee: Monsanto Invest BV

5 Practical Agricultural Applications

The higher the soluble solids the greater the amount of product (paste, catsup, etc.) that can be extracted from a fixed quantity of freshly harvested fruit. Introgression of Brix 9-2-5 can increase TSS by up to 20%.

Processing tomato varieties that include *S. pennellii* introgression lines (mostly Brix 9-2-5) developed and released commercially from AB seeds are AB311, AB319, AB2, AB8, AB5, and Seminis DRI319. These varieties are dominant in California, which produces 95% of the processing tomatoes in the U.S and ~ 30% of the global processing tomatoes. The California market is divided into 5 segments; Early, (16.1%), Thin 17.3%, (AB0311, DRI-0319, N6366), Intermediate (23%), Thick (39.8%) and Pear (< 5%). AB tomatoes are for the most part (~90%) in the thin segment and the Bayer varieties AB311 and DRI-319, dominate this segment (~80% market share in 2018). It should be noted that competing companies have also introgressed the Brix 9-2-5 lines, but the overall quality of the AB tomatoes has enabled them to keep their market edge. Of the total processing tomato market in California, 12% of the total tonnage during the last 14 years has been comprised of AB varieties. See Appendix B for details.

Current breeding programs at Bayer California for new tomato varieties that focus on field traits such as yield and disease resistance all maintain the Brix 9-2-5 introgression lines.

6 Economic Impact

6.1 Investment Cost

BARD contributed $1.05 million in research funds between 1987-2002. Additional academic funds contributed $800,000. Industry investments totaled $2.25 million between 1992 – 2008[4].

6.2 The Benefits

For processors making tomato paste, a paste with higher soluble sugar level gets a higher price. To increase the level of soluble sugar in the paste, processors often dehydrate. By starting with a paste containing higher soluble sugar levels, processors save both energy costs and time. In 2016, processors in Israel paid a premium of $1.62/ton for any 0.1% increase of solid contains.[5] In Italy, the framework agreement concluded for the 2018 harvest season, determined a premium of €1.99/ton for any increase of 0.1%.[6].

A seven-year analysis of incentives in growers and processors contracts in California (1993-1999) found 14 unique incentive structures for solid content (Brix).[7] To date, there is no premium paid in California for higher Brix tomatoes, and the economic benefit is reaped by the processors[8]. An analysis of the Brix economic contribution conducted in 2004 showed that a tomato with a Brix of 5.1% vs. 5.0% was worth about $1.55/ton to the processor. Conversely, 4.9% Brix is worth less[9].

Table 1 in Appendix B details the annual fraction of the AB varieties that include *S. pennellii* introgression lines out of the total California Processing Tomatoes production and their Brix values. The dominant varieties AB311 and DRI-319 have yielded an average Brix of ~ 5.7 over the last years. Averaging over the full years of commercialization, these varieties contained extra solids (Brix) of 0.43% compared to the average of all other processing varieties. We assume a value of $1.55 per 0.1% extra Brix per ton, so on average, these varieties yielded an economic benefit of $6.7/ton. This benefit influenced the price to consumers. The wholesale and retail income in the US for processed tomatoes is 42% of the end-price. Therefore, the economic average benefit is $11.4/ton (=$6.7/0.58). The benefit is calculated for each year since 2001 and summed. As examples; in 2004 the total production of these varieties that contained 0.4% extra solids was 892,000 tons, and it summed to a $9.53 million benefit. In 2017, the benefit summed to $21.3 million (see Appendix B). After consulting with experts, we assume that in the future the demand for

---

[4] 70% of $3.2 million from AB seeds. Personal Communication – D. Zamir.
[5] See processors agreement, in Hebrew: http://www.falcha.co.il/_Uploads/dbsAttachedFiles/tomatos2016.pdf
[6] http://www.tomatonews.com/en/italy-2018-quality-incentives_2_311.html
[7] https://ageconsearch.umn.edu/record/21990/files/sp03hu13.pdf
[8] Personal communication with staff at Seminis Vegetable division; Bayer, in California.
[9] http://www.tomatoland.com/documents/182.pdf

these tomatoes will grow. However, due to uncertainty in future predictions we maintain the 2017 annual benefit also for the years 2019 – 2028.

6.3 Economic Results

BARD invested in the initial and hence risky part of the project. According to the calculation described in the methodology section we attribute 58% of the benefits from the cultivated tomato varieties that include introgression line from the wild-type *Lycopersicon pennellii* tomato to BARD. The intogression lines are a direct research output from the two BARD awards (US-2427-95, IS-3009-99C). The earlier 2 BARD awards between 1987-1994, in which D. Zamir and S. Tanksley collaborated on disease resistance genes also contributed to the researchers understanding of the pattern of inheritance of quantitative traits in tomato. The remaining attribution is to other funds, primarily the USDA National Research Initiative Cooperative Grants Program that funded S. Tanksley in his pioneering work in establishing molecular linkage maps and development of the advanced backcross QTL analysis" strategy and in part to funds from the Israel Ministry of Science.

- Net present value of the BARD's investment is $261 million, thereof already attained $166 million
- The Internal rate of return is 28%
- Benefit cost ratio is 74, thereof already attained 48

The US economy benefit is calculated according to the production in the US.

Benefits attributed to the project that were not included in the calculation:

- An Israeli commercial company was involved in the project, and the benefit of this company to the Israeli economy is not included.

Table 1: Main Results, 2018 Million Dollar-Terms

	The Project	BARD	BARD Attained	Thereof to the US	Thereof to Israel	Other Countries
BARD's Share in the Cost	36%					
Share in the Benefit		51%				
Cost	10	4	4	1.8	1.8	
Benefit	518	264	170			
Net Present Value	508	261	166	263	-2	0
Internal Rate of Return	32%	28%	28%	33%	0%	
Benefit Cost Ratio	52	74	48	148	-1	

6.4 Sensitivity Analysis

The low and high alternative assumptions used in the sensitivity analysis were brought together to estimate results under pessimistic and optimistic scenarios. Table 2 displays the net present value sensitivity results, between the low result: $51 million, to the high result: $482 million.

Table 2: NPV - Sensitivity Analysis, 2018 Million Dollar-Terms

			BARD's Share in the Benefit		
			Low	Central	High
			41%	51%	61%
Change in Benefit	Low	50%	103	129	155
	Central	100%	209	261	313
	High	150%	315	393	471

7 Appendix A: BARD Awards

Table 3: Details of the 4 BARD awards

Project No	Full Title				
	Investigators	Institutes	Budget	Duration	Start Year
US-1388-**87**	Tagging Plant Genes with Tightly-Linked RFLP Markers				
	Tanksley, S.D Zamir, D.	U Cornell Hebrew U	$200,000	3 years	1987
IS-1822-**91C**	Cloning a Fusarium Resistance Gene in Tomato Based on Knowledge of its Map Position				
	Zamir, D. Tanksley, S.D.	Hebrew U U Cornell	$290,000	3 year	1991
US-2427-**95**	Development and Testing of a Method for the Systematic Discovery and Utilization of Novel QTL's in the Production of Improved Crop Varieties: Tomato as a Model System				
	Tanksley, S.D Zamir, D.	U Cornell Hebrew U	$300,000	3 years	1995
IS-3009-**99C**	Fine Mapping and Genetic Interactions of Nearly-Isogenic Allelic Series Representing Yield and Quality QTLs Derived from Wild Tomato Species				
	Zamir, D. Tanksley, S.D.	Hebrew U U Cornell	$255,000	3 year	1999

8 Appendix B: AB Varieties Fraction of California Processed Tomatoes Between 2001 to 2017[10]

		A	B	C=A*25	D	E	F
	Variety	**Loads**	**Solids**	**Ton (000')**	**Share of AB Varieties**	**Extra Solids of AB Varieties Compared to Others**	**Premium, million $ ($1.55/0.1%/ton) + 42% for Retail-Price Terms**
2001	AB2	129	5.33	3			
	AB5	26	5.10	1			
	AB311			0			
	AB2 3155			0			
	AB Total	**155**	**5.29**	**4**	**0%**	**0.12**	**0.01**
	California Other	**338,464**	**5.17**	**8,462**	**100%**		
	California Total	338,619	5.17	8,465	**100%**		
2002	AB2	2,263	5.46	57			
	AB5	1,075	5.42	27			
	AB311			0			
	AB2 3155			0			
	AB Total	**3,338**	**5.45**	**83**	**1%**	**0.27**	**0.49**
	California Other	**425,800**	**5.18**	**10,645**	**99%**		
	California Total	429,138	5.18	10,728	**100%**		
2003	AB2	7,698	5.61	192			
	AB5	1,754	5.36	44			
	AB311			0			
	AB2 3155			0			
	AB Total	**9,452**	**5.56**	**236**	**3%**	**0.25**	**1.30**
	California Other	**356,378**	**5.31**	**8,909**	**97%**		
	California Total	365,830	5.32	9,146	**100%**		
2004	AB2	29,267	5.59	732			
	AB5	6,404	5.37	160			
	AB311			0			
	AB2 3155			0			
	AB Total	**35,671**	**5.55**	**892**	**8%**	**0.40**	**7.88**
	California Other	**412,907**	**5.15**	**10,323**	**92%**		

[10] http://www.ptab.org/history.htm

		A	B	C=A*25	D	E	F
	Variety	**Loads**	**Solids**	**Ton (000')**	**Share of AB Varieties**	**Extra Solids of AB Varieties Compared to Others**	**Premium, million $ ($1.55/0.1%/ton) + 42% for Retail-Price Terms**
	California Total	448,578	5.18	11,214	**100%**		
2005	AB2	63,841	5.77	1,596			
	AB5	2,245	5.32	56			
	AB311			0			
	AB2 3155			0			
	AB Total	**66,086**	**5.75**	**1,652**	**17%**	**0.38**	**13.83**
	California Other	**311,941**	**5.37**	**7,799**	**83%**		
	California Total	378,027	5.44	9,451	**100%**		
2006	AB2	63,530	5.69	1,588			
	AB5	1,367	5.29	34			
	AB311			0			
	AB2 3155			0			
	AB Total	**64,897**	**5.68**	**1,622**	**16%**	**0.42**	**14.89**
	California Other	**343,211**	**5.26**	**8,580**	**84%**		
	California Total	408,108	5.33	10,203	**100%**		
2007	AB2	63,882	5.58	1,597			
	AB5	898	5.10	22			
	AB311			0			
	AB2 3155			0			
	AB Total	**64,780**	**5.57**	**1,620**	**14%**	**0.39**	**13.76**
	California Other	**402,277**	**5.19**	**10,057**	**86%**		
	California Total	467,057	5.24	11,676	**100%**		
2008	AB2	69,010	5.58	1,725			
	AB5	664	5.24	17			
	AB311			0			
	AB2 3155			0			
	AB Total	**69,674**	**5.58**	**1,742**	**15%**	**0.34**	**12.94**
	California Other	**387,032**	**5.24**	**9,676**	**85%**		
	California Total	456,706	5.29	11,418	**100%**		
2009	AB2	68,806	5.58	1,720			
	AB5	445	5.27	11			
	AB311	12	5.58	0			
	AB2 3155			0			

		A	B	C=A*25	D	E	F
	Variety	**Loads**	**Solids**	**Ton (000')**	**Share of AB Varieties**	**Extra Solids of AB Varieties Compared to Others**	**Premium, million $ ($1.55/0.1%/ton) + 42% for Retail-Price Terms**
	AB Total	**69,263**	**5.58**	**1,732**	**13%**	**0.24**	**9.14**
	California Other	**444,161**	**5.34**	**11,104**	**87%**		
	California Total	513,424	5.37	12,836	**100%**		
2010	AB2	53,677	5.74	1,342			
	AB5			0			
	AB311	28	5.31	1			
	AB2 3155	197	5.59	5			
	AB Total	**53,902**	**5.74**	**1,348**	**11%**	**0.45**	**13.32**
	California Other	**422,168**	**5.29**	**10,554**	**89%**		
	California Total	476,070	5.34	11,902	**100%**		
2011	AB2	37,402	5.67	935			
	AB5	1	5.20	0			
	AB311	1,302	5.75	33			
	DRI 319	108	5.71	3			
	AB Total	**38,813**	**5.67**	**970**	**8%**	**0.45**	**9.59**
	California Other	**424,517**	**5.22**	**10,613**	**92%**		
	California Total	463,330	5.26	11,583	**100%**		
2012	AB2	26,122	5.50	653			
	AB5			0			
	AB311	2,010	5.79	50			
	DRI 319	786	5.90	20			
	AB Total	**28,918**	**5.53**	**723**	**6%**	**0.39**	**6.26**
	California Other	**460,699**	**5.14**	**11,517**	**94%**		
	California Total	489,617	5.16	12,240	**100%**		
2013	AB2	9,824	5.40	246			
	AB5			0			
	AB311	7,192	5.58	180			
	DRI 319	12,443	5.84	311			
	AB Total	**29,459**	**5.63**	**736**	**6%**	**0.44**	**7.05**
	California Other	**452,832**	**5.19**	**11,321**	**94%**		
	California Total	482,291	5.22	12,057	**100%**		
2014	AB2	5,133	5.35	128			

		A	B	C=A*25	D	E	F
	Variety	**Loads**	**Solids**	**Ton (000')**	**Share of AB Varieties**	**Extra Solids of AB Varieties Compared to Others**	**Premium, million $ ($1.55/0.1%/ton) + 42% for Retail-Price Terms**
	AB5			0			
	AB311	18,935	5.72	473			
	DRI 319	27,921	5.66	698			
	AB Total	**51,989**	**5.65**	**1,300**	**9%**	**0.55**	**15.78**
	California Other	**501,439**	**5.10**	**12,536**	**91%**		
	California Total	553,428	5.15	13,836	**100%**		
2015	AB2	1,607	5.60	40			
	AB5			0			
	AB311	32,325	5.72	808			
	DRI 319	37,638	5.80	941			
	AB Total	**71,570**	**5.76**	**1,789**	**13%**	**0.58**	**22.93**
	California Other	**489,675**	**5.18**	**12,242**	**87%**		
	California Total	561,245	5.25	14,031	**100%**		
2016	AB2	1	5.80	0			
	AB5			0			
	AB311	40,496	5.81	1,012			
	DRI 319	33,478	5.70	837			
	AB Total	**73,975**	**5.76**	**1,849**	**15%**	**0.46**	**18.61**
	California Other	**422,496**	**5.30**	**10,562**	**85%**		
	California Total	496,471	5.37	12,412	**100%**		
2017	AB2	1	6.10	0			
	AB5			0			
	AB311	27,379	5.77	684			
	DRI 319	31,039	5.70	776			
	AB Total	**58,419**	**5.73**	**1,460**	**14%**	**0.55**	**17.64**
	California Other	**356,939**	**5.18**	**8,923**	**86%**		
	California Total	415,358	5.26	10,384	**100%**		

9 Appendix C: Information providers: Personal communication

- D. Zamir - Co-PI for BARD grants, Plant Sciences and Genetics, Hebrew University
- Shaul Graph – The main extension expert for processing tomatoes in Israel
- Arnon Osri – Processing and cherry tomato breeder at A B. Seeds, Bayer – Crop Science, Israel
- Chad Kramer - Tomato Breeder, Bayer Vegetable Seeds, CA.
- Grace Warner – Technical Sales Representative, Seminis Vegetable Seeds, CA, Bayer – U.S. Crop Science
- Prasad Yadavali - Technology Development, Seminis Vegetable Seeds, CA, Bayer – U.S. Crop Science