

Impact case study (REF3)

Institution: University of Nottingham		
Unit of Assessment: 6; Agriculture, Veterinary and Food Science		
Title of case study: Enhancing Syngenta's tomato breeding strategy through adoption of novel UoN technology		
Period when the underpinning research was undertaken: 2006 - 2016		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Professor Graham Seymour	Professor of Plant Biotechnology	2006 - present
Period when the claimed impact occurred: 2013 - 2020		
Is this case study continued from a case study submitted in 2014? N		
1. Summary of the impact		
<p>Sustained research excellence in tomato fruit ripening at the University of Nottingham (UoN) has underpinned a change in Syngenta's tomato breeding strategy. Syngenta, a leading global agriculture company who supply one in five of all tomato seeds worldwide, has adopted UoN technology to address the challenge of extending shelf life of commercial (retail) tomatoes. UoN-Syngenta research collaborations led to the discovery of two novel genomic regions (QTLs) that control fruit softening, independent of other ripening processes that control colour and flavour. The QTLs were patent protected and deployed in Syngenta's commercial tomato breeding programme. Compared to current solutions on the market, such as the ripening inhibitor (rin) that has a detrimental effect on taste, the QTLs provide superior tomato shelf life at the same time as enhancing consumer satisfaction. [redacted].</p>		
2. Underpinning research		
<p>Tomato is the fourth most important vegetable crop, with 241,928,300t produced globally in 2017 (Food and Agriculture Organization). Thousands of varieties are grown worldwide and, since 1990, breeders have used natural slow ripening mutations to modulate the fruit ripening process and give commercial varieties an extended shelf life that is vital for fresh food supply chains. However, current state-of-the-art slow ripening mutations, such as the ripening inhibitor (rin), affect the entire ripening process and have a detrimental effect on fruit colour and flavour. Hence, a major goal of breeders such as Syngenta has been to identify novel genes that exclusively control tomato fruit softening, allowing a long shelf life, but normal colour and flavour development of the fruit. The achievement of this goal has been expedited by the availability of the tomato genome sequence and discovery of genes controlling softening by Professor Seymour's team.</p> <p>The tomato genome was sequenced by the Tomato Genome Consortium, a multi-national team of over 300 scientists from 14 countries. Professor Seymour spearheaded the UK contribution to the sequencing programme and worked closely with collaborators at Imperial College, James Hutton and the Sanger Institute to plan and deliver the BBSRC funded UK component of the programme (G1). The tomato genome sequence, published in 2012 (1), provided detailed information about the functional portions of the genome, revealing the order, orientation, type and relative position of all 35,000 genes. Specific work undertaken at UoN identified genes involved in cell wall remodelling in ripening tomato fruits that were good candidates for controlling fruit softening including 53 genes that were predominantly expressed in fruit tissue (1: supplementary information, table 12). Some genes showed a dramatic increase in expression from the onset of fruit ripening including a member of the pectate lyase (PL) gene family (Solyc03g111690) (1: supplementary information, section 5.7).</p> <p>In collaboration with Syngenta, the tomato genome sequence was used in research to identify genomic regions (quantitative trait loci or QTLs) and genes that exclusively control tomato fruit softening. The research was funded via BBSRC industry collaboration schemes (G2,G3) and leveraged Syngenta's next generation sequencing platform, other in-house</p>		

technologies and expertise. The research utilised two approaches: (i) a wild type *Solanum pennellii* tomato mapping population was used to identify genomic regions and genes associated with natural variation in fruit texture; (ii) targeted transgenic experiments were used to understand the role of candidate genes in fruit ripening.

Initial outcomes of the work identified a genomic region (QTL) on chromosome 2 associated with natural variation in fruit texture. The region contained cell wall structure genes (all encoding pectin methylesterases) and a ripening related gene (ethylene response factor) **(2)**. The discovery provided new insights in the genetic basis of ripening and led to a patent application in 2011. The patent, filed jointly by Syngenta and UoN, protected the use of specific genetic markers on chromosome 2 for use in breeding tomatoes with increased firmness **(11)**. The patent was granted in the US in 2015 and elsewhere in 2016.

Using the *Solanum pennellii* tomato mapping population, a further fruit firmness QTL was identified on chromosome 3 that was more strongly associated with natural variation in fruit texture. This research also reported the variation in fruit firmness was strongly associated with a pectate lyase (PL) gene Solyc03g111690 located within this chromosome 3 genomic region **(3)**. This PL gene was highly expressed during ripening. Gene silencing experiments showed that this gene increased fruit firmness without affecting other traits such as yield, colour, sugars, acids and other fruit metabolites. Furthermore, fruits retained their integrity following storage for 14 days at room temperature which indicated the gene had practical application in improving shelf life **(3)**. The intellectual property was protected by a joint Syngenta and UoN patent filed in 2013. The patent protected use of the genetic region on chromosome 3 containing PL and was granted in the USA (2017) and Europe (2019) **(12)**.

The research **(3)** was an important breakthrough, as it reported for the first time substantial and specific control over tomato softening during the ripening process. It offered a commercial approach for extending tomato shelf life that exploited natural variation in pectate lyase within wild type tomatoes to produce new varieties by conventional, non-genetically modified (non-GM), breeding approaches. In recognition of excellence in UK government funded research, the outcomes of the research were featured on the BBSRC website. The work was also reported globally in mainstream, industry and subject specific news outlets including [The Wall Street Journal](#) (USA), [Daily Mail](#) (UK) and BBC Farming Today (UK).

3. References to the research

University of Nottingham UoA6 staff are **bold**; Syngenta co-authors are underlined.

Underpinning publications:

1. Tomato Genome Consortium (2012). The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, 485 (7400), pp. 635-641. DOI: 10.1038/nature11119. *The Tomato Genome Consortium includes over 300 collaborators from across the globe, including **GB Seymour***
2. **NH Chapman**, J Bonnet, L Grivet, J Lynn, **N Graham**, R Smith, **G Sun**, PG Walley, **M Poole**, M Causse, GJ King, C Baxter, **GB Seymour** (2012). High-resolution mapping of a fruit firmness-related quantitative trait locus in tomato reveals epistatic interactions associated with a complex combinatorial locus. *Plant Physiology* 159:1644-57. DOI: 10.1104/pp.112.200634
3. S Uluisik, **NH Chapman**, R Smith, **M Poole**, **G Adams**, RB Gillis, TMD Besong, J Sheldon, S Stiegelmeier, L Perez, N Samsulrizal, D Wang, **ID Fisk**, **N Yang**, C Baxter, D Rickett, **R Fray**, B Blanco-Ulate, ALT Powell, **SE Harding**, **J Craigon**, JKC Rose, EA Fich, L Sun, DS Domozych, PD Fraser, **GA Tucker**, **D Grierson**, **GB Seymour** (2016). Genetic improvement of tomato by targeted control of fruit softening. *Nature Biotechnology* 34, 950–952. DOI: 10.1038/nbt.3602

Intellectual Property Rights (IPR):

11. [Baxter, CJ, Chapman, NH, Puddephat, I, Seymour, GB](#) (2011). Tomato fruit having increased firmness: US2010129474 / WO2011/051120 A1
12. [Baxter, CJ, Grivet, L, Bonnet, J, Chapman, NH and Seymour, GB](#) (2013). QTL responsible for tomato fruit firmness. WO2013/153237 A1

Underpinning grants:

- G1. Sequencing the tomato genome: a reference genome for the Solanaceae. Sponsor: BBSRC, 2005-2009, BB/C509731/1. GBP226,713 PI: G Bishop, ICL (Col: **GB Seymour**, UoN)
- G2. Identification of genes that underlie a major QTL for tomato fruit texture. Sponsor: BBSRC, IPA with Syngenta, 2006-2009, BB/D00103X/1. GBP281,709. PI: **GB Seymour**, UoN
- G3. The validation, characterisation and translation of the outputs derived from network analysis and QTL mapping of tomato fruit quality traits (TomNET). Sponsor: BBSRC LINK with Syngenta, 2012-2016, BB/J015598/1. GBP277,248. PI: **GB Seymour**, UoN (PI: P Fraser, RHUL)

4. Details of the impact

Syngenta are a leading global agriculture company with a vegetable seed business of USD621,000,000 ([Syngenta Financial Report 2019](#)). **Syngenta supply one in five of all tomato seed varieties globally** making the tomato of significant value to the company [redacted]. Syngenta's new tomato variety breeding priorities have a major focus on traits that will reduce waste along the food chain and the company has sought new ways to extend post-harvest shelf life in tomato while maintaining excellent fruit quality. **Working with University of Nottingham (UoN), Syngenta have identified, patented and deployed two novel genomic regions (QTLs) within their tomato breeding programme.** Compared to current solutions on the market, such as the ripening inhibitor (rin) that has a detrimental effect on taste, these novel QTL deployment approaches provide superior tomato shelf life and flavour attributes and have **driven change in Syngenta's tomato breeding programme strategy.**

Genome sequence improves Syngenta's tomato R&D

The tomato genome sequence **(1)** has improved Syngenta's tomato research and development operations. It provides the reference genome sequence for comparison of novel germplasm, such as wild tomato species, for Syngenta's new trait discovery platform. The genome sequence has also allowed Syngenta to understand the position of all tomato genes relative to their proprietary breeding markers **(a)**. This has enabled Syngenta's breeding team to precisely understand the position of recombination events relative to genes and improve the accuracy of the tomato breeding process. Syngenta have used the genome sequence as part of their new trait discovery platform to identify and convert many elite lines for 50+ genetic loci responsible for a wide range of important agronomic characteristics **(a)**. These include genes affecting disease resistance, vigour and fruit quality characteristics such as sugar content, colour, and, working with researchers at UoN, genes involved in controlling post-harvest quality. Syngenta's Head of Global Seeds Development Traits and Regulatory stated "*the publicly available [tomato genome] sequence complements our own genomic resources and has **proved an important resource for our breeding programme***" **(a)**.

UoN contributes to innovation within Syngenta's pre-breeding pipeline

Through working in close collaboration with UoN across 14 years, a period that spans the current REF period, Syngenta has been able to innovate within their tomato pre-breeding R&D pipeline. Significant UoN-Syngenta collaborative research projects were funded under BBSRC IPA and LINK industry schemes **(G2,G3)** which benefitted from [redacted] investment from Syngenta **(b)**. Both BBSRC projects delivered on the planned research and

commercial objectives, providing Syngenta with two novel genomic regions (QTLs) on chromosomes 2 and 3 that could exclusively control fruit softening during the fruit ripening process (2,3). The novel genetic material was protected by joint Syngenta and UoN patents, allowing Syngenta to establish an IP proprietary position in the USA and Europe for 'Firmness Tomato 1' technology (QTL2) and in multiple countries (Australia, Canada, Europe, Israel, Korea, Morocco, Mexico and USA) for the 'Firmness Tomato 2' technology (QTL3, includes the gene pectate lyase) (11,12,a). Syngenta has invested [redacted] to maintain their IP position in the territories stated. Furthermore, **Syngenta is progressing the technologies in their commercial tomato breeding programme and is offering the technologies as licence options to competitor tomato breeding companies** on its proprietary licensing platform [Traitability](#) (a,c). Syngenta's Head of Global Seeds Development Traits and Regulatory described identification of the novel genetic material a 'major step forward as previous technologies that extended shelf life had detrimental effects on other quality characteristics' and stated 'only a small number of projects based on our university collaborations result in the discovery of gene loci that have a likely significant commercial benefit' (a).

Adoption of UoN technology within Syngenta's tomato shelf life breeding programme

Research completed under the collaborative BBSRC projects showed the expression of candidate fruit softening genes within the QTLs on chromosomes 2 and 3 was lower in *Solanum pennellii* wild type tomato compared to commercial control varieties (2,3). This provided Syngenta the opportunity to manipulate the expression of candidate fruit softening genes and develop new commercial varieties by using wild type tomatoes (donor material) containing the QTLs of interest using conventional (non-GM) breeding approaches. The process of breeding new tomato varieties through conventional breeding approaches, from gene identification to availability of commercial seed, normally takes approximately 10 years depending on complexity of the target QTLs. In 2013, Syngenta deployed the genetic material into their tomato breeding programme and have achieved excellent results to date.

Donor material containing the QTLs of interest were used to initiate backcross programs in Syngenta's nursery in Morocco (2013). For both QTL2 and QTL3, five elite breeding lines were converted over several generations across a 4 year timeline (2013-2017). The resulting converted materials were trialled during two seasons per year in a passive plastic house environment for three years in Syngenta's breeding station in Spain (2017-2020). The fruit were phenotyped for quality traits (texture, shelf life, sugar content, size and shape) across three harvests per season at Syngenta's fruit quality laboratories in Spain. Post-harvest quality traits were also measured (firmness during storage for ten days). Commenting on these traits Syngenta stated that '**Substantial improvements in fruit firmness and shelf life while maintaining excellent colour and eating quality have been obtained in various genetic backgrounds with QTL3**' (a). This outcome **confirmed the rationale for the change in Syngenta's breeding strategy** [redacted]. Syngenta stated 'The long term nature of the work is a normal factor in any crop breeding programme' (a).

Between 2013 and 2020 **Syngenta invested a total of** [redacted] **in the validation and deployment of both QTL's into their tomato breeding programme** (a). This is [redacted] of their tomato output trait programme budget, indicating sustained investment by the company into the commercialisation of UoN technology. Syngenta's Head of Global Seeds Development Traits and Regulatory stated '**adopting the UoN technology into our breeding programme has been a priority in our tomato fruit quality division and the technology is a standing item on the agenda of Syngenta Tomato Breeders Meetings**' (a).

[redacted]

Summary

Acknowledging the importance of the tomato genome sequence in development of new varieties and UoN's contribution to innovation within Syngenta's shelf-life programme, Syngenta's Head of Global Seeds Development Traits and Regulatory stated:

"Professor Seymour has been instrumental in understanding the value of the tomato genome sequence to discover novel loci controlling fruit shelf life and delivering this important resource to a global tomato breeding company. Professor Seymour's [2016 (3)] discovery has provided the first real opportunity to enhance tomato shelf life while maintaining excellent flavour. This is important because at present combining long shelf life and optimal eating characteristics have proved a significant challenge. This work is a direct result of our long-standing and fruitful partnership with the University of Nottingham, which has been sustained by Professor Seymour's collaborative approach" (a).

5. Sources to corroborate the impact

- a. Letter from Syngenta's Head of Global Seeds Development Traits and Regulatory commenting on the importance of the tomato genome sequence and detailing commercial adoption of novel UoN genetic material (September 2020) [PDF].
- b. BBSRC JeS application forms detailing initial Syngenta investment in collaborative research to identify novel tomato genes controlling fruit ripening [PDF].
- c. Screenshot of Syngenta's Traitability webpage offering 'Firmness Tomato 1' and 'Firmness Tomato 2' technology as licence options (accessed 15th September 2020) [PDF]