Institution: University of Warwick		
Unit of Assessment: UOA5 - Biological Sciences		
Title of case study: Genetic improvement of vegetable crops		
Period when the underpinning research was undertaken: 1 January 2014- 31 December 2020		
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:**
John A. Walsh	Professor	01/04/2004 - present
John Clarkson	Reader	01/04/2004 - present
Guy C. Barker	Assistant Professor	01/04/2004 - present
Stephen Jackson	Assistant Professor	01/04/2004 - present
Charlotte Allender	Assistant Professor	01/04/2004 - present
Graham Teakle	Associate Professor	01/04/2004 - present
Carol E. Jenner	Associate Professor	01/04/2004 - 31/12/2010
Period when the claimed impact occurred: 1 August 2013- 31 December 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact (indicative maximum 100 words)

Warwick Crop Centre (WCC)'s research on the genetic diversity of vegetable crops, identification of resistance genes and development of marker-assisted technologies has enabled global seed companies to breed better varieties through genetic improvement. Accessions of globally important crops such as brassicas, carrots, parsnips and onions have been sent to over 40 companies. The Brassica 52k SNP Illumina beadchip is used by global agribusinesses to investigate genetic variability, and the development of pathogen screens and the adoption of marker-assisted technologies has accelerated breeding programmes at global seed companies including Limagrain, Elsoms Seeds, BASF, Hazera, and Syngenta, improving reliability, saving money and developing new markets.

2. Underpinning research (indicative maximum 500 words)

Conserving and exploiting the genetic diversity of vegetable crops

Biodiversity is key to the survival of life on Earth and in the UK alone the vegetable sector is worth GBP1,400,000,000 annually. WCC hosts the Defra-funded UK Vegetable Gene Bank (UKVGB) and our research makes this germplasm accessible [3.1]. As many vegetables are open-pollinated and heterozygous, our research provides essential trait-trapping populations as well as molecular markers and genome maps for breeders, such as a SNP-mapped fixed diversity set for lettuce [3.2]. **Barker** directs the Vegetable Genetic Improvement Network (VeGIN) which is an industry-guided national Research and Development programme established in 2009. Underpinning research, much with the international brassica genome consortium includes the pangenome of brassicas [3.3], knowledge of gene architecture, and trait maps which have supported the highly successful Illumina 52k array chip [https://emea.illumina.com/areas-of-interest/agrigenomics/consortia.html].

Genetic improvement of crop varieties

-Broad-Spectrum, potentially-durable virus resistance in brassica crops

Turnip mosaic virus (TuMV) is considered to be one of the two most important viruses affecting field vegetable crops worldwide, also affecting arable crops in certain parts of the world. **Walsh** has investigated the biological, serological and genetic variation of Turnip mosaic virus (TuMV). A BBSRC Crop Science Initiative grant mapped the recessive gene involved in broad-spectrum resistance (*retr01*) and a BBSRC CASE studentship with Syngenta, identified *retr01* as a mis-spliced version of the eukaryotic translation initiation factor, *eIF(iso)4E* enabling elucidation of the resistance mechanism [3.4], which was patented by the University of Warwick. As the resistance gene was recessive, SNP markers were developed to facilitate the rapid identification of plants carrying the resistance gene accelerating the introgression of this resistance into commercial breeding lines. **Walsh** subsequently investigated transmission and control of Turnip yellows virus and revealed prevalence in oilseed rape and vegetable brassica crops, followed by the identification of genetic plant resistance.

-Resistance to *Fusarium oxysporum* in onions *and Puccinia porri* in leeks

Fusarium Basal rot, caused by *Fusarium oxysporum f.sp. cepae* (FOC), causes significant global onion production losses with no effective control treatments. **Clarkson** developed new rapid plant bioassays involving artificial inoculation with an aggressive FOC isolate to identify novel, high level resistance within the UKVGB onion diversity set [3.5]. Following the first genome sequencing of FOC isolates, **Clarkson** identified genes related specifically to pathogenicity on onion which enabled development of a molecular diagnostic test, and RNAseq identified candidate effectors expressed in planta [3.6].

- Resistance to parsnip canker

An estimated 20% of the UK's parsnip crop each year is deemed unsuitable for sale due to root canker diseases and there are no fungicides registered for the disease. **Clarkson** has identified the three major parsnip canker pathogens, and a biomarker for resistance to the important *Mycocentrospora acerina* [3.6].

- Improving the quality of rocket and lettuce

Rocket and lettuce are important salad crops, however bolting (flowering) of either crop before harvesting renders the crop unsaleable. **Jackson,** an expert in the molecular mechanisms controlling flowering, identified genes in these two crops that could delay flowering, including the rocket homologue of FLOWERING LOCUS C *(DtFLC*) [3.7]. This allowed screening of UKVGC collections for late bolting lines, and expression analyses of DtFLC under varied cold treatments led to the discovery that rocket does not have a vernalization requirement.

3. References to the research (indicative maximum of six references)

[3.1] Davies, LR., **Allender, CJ.** (2017) Who is sowing our seeds? A systematic review of the use of plant genetic resources in research. Genetic Resources and Crop Evolution, 64(8), pp.1999-2008. doi:10.1007/s10722-017-0491-7

[3.2] Walley, PG., Hough, G., Moore, JD., **Carder, J., Elliott, M.,** Mead, A., **Jones, J., Teakle, GR., Barker, GC., Buchanan-Wollaston, V.**, Hand, P., Pink, D. and **Collier, R.** (2017) *Towards new sources of resistance to the currant-lettuce aphid (Nasonovia ribisnigri).* Molecular Breeding, 37 (1). 4. doi:10.1007/s11032-016-0606-4

[3.3] Golicz, AA., Bayer, PE., **Barker, GC**., Edger, PP., Kim, HR., Martinez, PA., Chan, CKK., Severn-Ellis, A., McCombie, R., Parkin, IAP., Paterson, AH., Pires, C., Sharpe, AG., Tang, H., **Teakle, GR**., Town, CD., Batley, J. and Edwards, D. (2016) *The pangenome of an agronomically important crop plant Brassica oleracea.* Nature Communications, 17. 13390. doi:10.1038/NCOMMS13390

[3.4] Nellist, CF., Qian, W., **Jenner, CE., Moore, JD.,** Zhang S., Wang, X., Briggs, WH., **Barker, GC.,** Sun, R., **Walsh JA**. (2014). Multiple copies of eukaryotic translation factors in *Brassica rapa* facilitate redundancy, enabling diversification through variation in splicing and broad-spectrum resistance. *The Plant Journal* **77**, 261-268. https://doi.org/10.1111/tpj.12389

[3.5] Taylor, A., Teakle, GR., Walley, PG., **Finch-Savage, WE., Jackson, AC., Jones, J.,** Hand, P., **Thomas, B**., Havey, MJ., Pink, DAC. and **Clarkson, JP**. (2019) *Assembly and characterisation of a unique onion diversity set identifies resistance to Fusarium basal rot and improved seedling vigour.* Theoretical and Applied Genetics, 132 (12). pp. 3245-3264. doi:10.1007/s00122-019-03422-0

[3.6] Armitage, AD., **Taylor, A.,** Sobczyk, MK., **Baxter, L**., Greenfield, BPJ., Bates, HJ., Wilson, F., **Jackson, AC**., Ott, S., Harrison, RJ. and **Clarkson, JP**. (2018) *Characterisation of pathogen-s regions and novel effector candidates in Fusarium oxysporum f. sp. cepae.* Scientific Reports, 8 (1). 13530 . doi:10.1038/s41598-018-30335-7

[3.7] Taylor, JL., Massiah, AJ., Kennedy, S., **Hong, Y. and Jackson, SD**. (2017) *FLC expression is down-regulated by cold treatment in Diplotaxis tenuifolia (wild rocket), but*

flowering time is unaffected. Journal of Plant Physiology, 214. pp. 7-15. doi:10.1016/j.jplph.2017.03.015

Grants

PI Professor John Walsh, Exploiting eIF4E-based and associated broad-spectrum recessive resistance to potyviruses in dicots and monocots, BBSRC Crop Science Initiative, 01/04/2007-31/03/2010, GBP307,074

PI Professor John Walsh, Deploying virus resistance in brassicas and understanding the interaction between the viral VPg protein and brassica eIF4E and eIF(iso)4E proteins, BBSRC CASE studentship, 01/10/2009- 30/09/2013, GBP74,410

PI Dr Guy Barker, Leafy Vegetable Genetic Improvement Network (VeGIN): Pre-breeding research to support sustainable farming of leafy vegetables and salads, DEFRA, 04/2009-01/2012, GBP2,070,824

PI Katherine Denby, Co-I John Clarkson, A Systems Approach to Disease Resistance Against Necrotrophic Fungal Pathogens, BBSRC, 01/05/15- 30/06/16, GBP471,160

PI John Clarkson, Exploiting next generation sequencing technologies to understand pathogenicity and resistance in Fusarium oxysporum, BBSRC, 01/12/13 – 31/03/17, GBP507,382

PI Steve Jackson, Manipulation of bolting time for improved quality and greater sustainability in lettuce production, BBSRC, 01/04/2009- 30/09/2014, GBP754,527

4. Details of the impact (indicative maximum 750 words)

The Warwick Crop Centre provides global seed companies with germplasm, training and resources to improve the sustainability of vegetable crops through genetic improvement. Warwick research has also provided access to quicker and more reliable trait evaluation assays and genetic markers, accelerating breeding programmes and saving money. As a result, companies have invested significantly, adopted screening technologies and developed new varieties with improved resistance to diseases and environmental challenges.

Conserving and exploiting the genetic diversity of vegetable crops

Crop breeding for elite varieties has constrained available genepools. Beneficial traits reside in natural genetic diversity and Warwick's UKVGB curates >14,000 accessions of globally important crops such as brassicas, carrots, parsnips and onions. Since 2014, UKVGB has distributed 5770 accessions to 203 groups worldwide, 37% being to commercial companies [5.1], including prominent global agribusinesses such as Elsoms Seeds, BASF Hazera, Limagrain, Syngenta and Tozer. Warwick also develops and makes available mapping populations for breeding programmes. Elsoms Seeds [Text removed for publication] has used over 200 lines, leading to significant commercial releases including canker-resistant parsnips and bolting resistance in rocket: "*gene bank accessions and plant diversity sets provide us with valuable knowledge and resources to inform and support investment in our breeding programmes and develop more sustainable crops*." [5.2].

Brassicas are globally important crops. Their morphological diversity is reflected in their genome complexity. Warwick's brassica genome sequence, transcriptome data and Barker's expertise were fundamental in the development of Illumina's Brassica 52k SNP beadchip. The Multinational Brassica Genome Project, seed companies, academic and commercial research organisations produced the first Brassica array with Illumina in 2012. This world-first reference sequence continues to be used by global agribusinesses to characterise natural genetic variation, confirm variety novelty and for compliance with GMO legislation, providing Illumina with a recurring revenue stream [5.3].

Establishment of marker-assisted technologies in breeding programmes

Rapid advances in vegetable improvement demand expertise in modern molecular breeding technologies. Warwick has provided hands-on support to upskill breeding programmes by Elsoms Seeds, BASF and Hazera.

Barker’s, Clarkson’s and Teakle’s work with Elsoms Seeds “*shaped the company’s thinking around our own research and approach to breeding*” [5.2] through the adoption of marker-assisted breeding. Since 2015, Elsoms Seeds has invested [text removed for publication] and trained four breeders establishing their genetic marker screening laboratory and benefited by “*speeding up traditional plant breeding methods by being able to follow commercially important traits in our breeding lines, reducing trialling and phenotyping costs”* [5.2].

Parsnip production is valued at GBP29,000,000 per annum in the UK, but another estimated 20% is deemed unsuitable for sale due to parsnip canker disease. Elsoms Seeds are the global leader in parsnip breeding and Clarkson’s research improved understanding of the pathogen and provided a screening assay to identify resistant varieties [5.2]

Global production of onions is valued at GBP9,500,000,000. Fusarium basal rot, caused by *Fusarium oxysporum f.sp. cepae,* causes significant production losses with no effective control treatments. Alliums have complex genomes and research into breeding to improve plant resistance is resource-limited. Clarkson’s pathogen virulence gene screen enabled BASF APV to identify appropriate Fusarium pathogen isolates and his onion seedling test has been implemented in BASF ABV’s global breeding programme, underpinning their Fusarium basal rot screening platform [5.4]. Hazera, another global vegetable breeder, has also adopted Clarkson’s plant disease assays enabling them to identify “*new onion germplasm lines with a relatively high level of basal rot resistance*” [5.5, 5.6].

Genetic improvement of crop varieties

Turnip mosaic virus is considered to be one of the most significant viruses affecting field vegetable crops worldwide and also infects oilseed rape. It can reduce weight yields by up to 64% and effects on quality can render crops unmarketable, affecting supply and livelihoods. Walsh, in collaboration with Syngenta, mapped the recessive gene involved in broad-spectrum resistance (*retr01*) and developed SNP markers, markedly accelerating crop improvement. Syngenta purchased the patent [5.7] rights in 2012 and has introgressed the gene into [text removed for publication] elite parent breeding lines. Development of the within-gene marker accelerated the international resistance breeding programme “*by five years*” and “*halved the time necessary for the backcrossing programme and introgression of the resistance gene into commercial plant genotypes and saved a considerable amount of money*” [5.8]. In 2020, Syngenta has “[text removed for publication] *Autumn/Storage type Chinese cabbage varieties for the European market and [text removed for publication] Spring varieties for the European and Chinese markets in later stage testing that have the resistance*” [5.8]. A second patent on recessive broad-spectrum resistance to potyviruses in plants is also being pursued by Syngenta, worldwide [5.7]

Walsh has also worked closely with Limagrain, Tozer Seeds, Sakata, Rijk Zwaan and Enza Zaden regarding Turnip yellow virus which affects oilseed rape with yield reductions of up to 30% resulting in losses of GBP67,000,000 – 180,000,000 per annum in the UK and greater losses in mainland Europe and also significantly reduces yield of vegetable brassicas. Walsh demonstrated almost 100% incidence in some crops, improving Limagrain’s understanding of the severity of the virus, informing their breeding programmes and raising their customers’ awareness [5.9].

Sclerotinia sclerotiorum is a major disease of lettuce globally. Clarkson’s research on the genetics and biology of *S. sclerotiorum* enabled Tozer Seeds to efficiently screen their lettuce populations for resistance which resulted in rapid identification of resistant lines and acceleration of their breeding programme. [5.10]

Salad Rocket is unsaleable once it flowers. Jackson developed molecular markers for flowering time genes and screened UKVGB rocket accessions for late bolting varieties with Elsoms. Following a Knowledge Transfer Partnership in 2011, a new variety of rocket called ‘Sweet Intensity’ was developed for UK, Europe and USA markets with a value estimated to be GBP12,000,000 [5.2]. Parallel work with lettuce is underway with Rijk Zwaan.

5. Sources to corroborate the impact (indicative maximum of 10 references)

[5.1] **Davies, LR.**, **Allender, CJ.** (2017) Who is sowing our seeds? A systematic review of the use of plant genetic resources in research. Genetic Resources and Crop Evolution, 64(8), pp.1999-2008. doi:10.1007/s10722-017-0491-7

[5.2] Statement, Vegetable Breeding Manager, Elsoms, verifying impact on informing which breeding programmes to invest in and develop more sustainable crops

[5.3] Statement, Associate Director, Illumina, demonstrating that Warwick's research and Barker's expertise were fundamental in the development of Illumina's Brassica 52k SNP beadchip

[5.4] Statement, Global Crop Coordinator Phytopathology, BASF, verifying impact on improving their diagnostics and pathogen impact monitoring,sped up assessments of the onion germplasms level of resistance, then implemented in their global breeding programme

[5.5] Statement, Traits and Technology Team Lead, Hazera, verifying impact on their breeding programmes.

[5.6] Hazera Press release https://www.hazera.com/2018/01/no-tears-future-onions/#more-7662

[5.7] Patent relating to virus resistant plants (2013) Walsh, J. A. ,US 2013/0117879 A1 'PLANT EUKARYOTIC TRANSLATION INITATION FACTOR4E'
https:/patents.google.com/patent/US20130117879/en
Patent on recessive broad-spectrum resistance to potyviruses in plants: Walsh J.A., Nellist C.F., Barker G.C. and Jenner C.E. (2010). Plant eukaryotic translation initiation factor 4e. Patent pursued by University of Warwick in the European Community, Japan, Korea, Australia and the USA. Numbers: US20130117879, WO2011161466A1. Syngenta purchased rights to the patent and are pursuing it worldwide: Number: EP 2585478 A1.
https://www.freepatentsonline.com/y2013/0117879.html

5.8] Statement, Global Breeding Lead, Syngenta, verifying impact of significantly accelerating the resistance breeding programme "*by five years*" and "*halved the time necessary for the backcrossing programme… and saved a considerable amount of money*".

[5.9] Statement, OSR Maritime Breeder, Limagrain, Warwick research informed breeding programmes and helped Limagrain raise awareness with customers.

[5.10] Statement, Director of Research and Development, Tozer Seeds, verifying impact on their breeding programmes.