An Roinn Talmhaíochta, Bia agus Mara
Department of Agriculture, Food and the Marine

Research Stimulus Fund

Final Report

'WheatEnhance: Breeding of wheat in order to enhance yield and disease resistance – mutation of the BRI1 brassinosteroid receptor gene"

DAFM Project Reference No: 11/S/103

Start date: 01/07/2013

End Date: 31/12/2018

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Collaborating Research Institutions and Researchers: Teagasc, Ewen Mullins

Please place *one* "x" below in the appropriate area on the research continuum where you feel this project fits

Basic/Fundamental →			Applied →			Pre Commercial
1	2	3	4	5 X	6	7

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report;

Priority Area (s)	Smart and Sustainable Food Production and Processing Sustainable Living

Key words: Cereals, Breeding, Disease, Brassinosteroid

1. Rationale for Undertaking the Research

Cereals are the main source of human calories. Diseases lead to yield loss, and Fusarium head blight (FHB) disease is one of the top 10 diseases of cereals globally and a major threat to Irish cereal production. It reduces yield and contaminates wheat, barley and oat grain with mycotoxins that are harmful to human and animal health. Fungicide control of this disease is inconsistent at best. Enhancing cereal resistance to this disease is a major goal of the breeding industry in order to increase field performance of new varieties. Few genes have been identified within germplasm that have a major impact on field resistance to FHB. Previous work in the UCD lab identified that a variant of a receptor gene, BRI1, enhanced barley resistance to FHB disease under glasshouse conditions. This variant was the result of a spontaneous mutation in Japanese barley, resulting in 'uzu' variants. Herein we conducted glasshouse trials to validate the FHB resistance of 'uzu' variants of barley, test performance of this barley germplasm at Irish field level and also determine if the 'uzu' variation was associated with any deleterious effects. At the outset of this project, no such mutants were characterized in wheat. Hence, another goal of this project was to try and incorporate a similar mutation into wheat and determine the effect of this mutation on the glasshouse disease resistance and field performance. Studies were also conducted to (i) identify barley genes regulated by this 'uzu' mutation that themselves might be of benefit in enhancing disease resistance and (ii) enhance our understanding of how this 'uzu' mutation affects plants at the cellular level. Through such studies, WheatEnhance increased our potential to breed for FHB resistance in wheat and barley.

2. Research Approach

1. Use of TILLING (Targeted Induced Local Lesions IN Genomes) to generate pre-breeding material of agronomic significance.

A TILLING library of wheat mutants was screened for mutations in the *Bri1* genes. Mutants were identified and two individual lines with mutations of interest were advanced (backcrossed to reduce the level of non-desirable background mutations). These two lines have been characterised and their disease resistance profile is being analysed at field level.

2. Crossing and PCR-based identification of mutations

We developed molecular marker assays to trace mutations of interest within wheat breeding programmes. These KASPar markers will allow breeders to differentiate material on the basis of whether or not it inherited the desirable mutation contributed by the two wheat *Bri1*mutant lines. The main bottleneck with KASPar was acquiring timely results, which in turned hampered the speed of selection and backcrossing.

3. Glasshouse and field trials

Field and glasshouse trials were (and continue to be) conducted to assess the disease resistance and agronomic performance of both barley and wheat mutants of interest. Issues with germination rate and mildew in glasshouse trials impeded the trials to someextent. Thus, trials had to be repeated as necessary.

4. Gene silencing

Virus-induced gene silencing was used to validate the role of a *Bri1*-regulated gene, *LRR*, in disease resistance, thus identifying another gene to enhance FHB resistance in cereals. Additionally, two other genes encoding a mitochonidrial phosphate transporter and a methyl transferase were confirmed to contribute to FHB resistance in wheat (via virus-induced gene silencing studies). The LRR work was patented and published (Frontiers in Plant Science) and we are currently preparing a publication for the other two genes.

5. Proteomics

Proteomic analysis was used to determine how mutation of the *Bri1* gene affected other downstream signalling processes in barley, thus providing insights into brassinsteroid signalling in plants. A comprehensive barley sequence became available and this greatly facilitates the analysis of this data.

3. Research Achievements/Results

1. *Bri1* TILLING wheat mutants were selected and developed (back-crossed), ready to be tested for agronomic performance and of relevance to breeders.
2. KASPar assays were developed to track the mutations in these lines through breeding programmes.
3. Genes/gene variants were identified that enhance cereal resistance to Fusarium head blight disease and some of these also impact grain development.
4. We have assessed the field performance of barley *Bri1* mutants and the glasshouse performance of wheat and barley *Bri1* mutants (field assessment of the wheat lines is ongoing).
5. Proteomic analysis was used to determine how mutation of the *Bri1* gene affects the cellular signalling processes responsive to *Fusarium* infection.
6. Three scientists have been trained as part of this project, one postdoctorate and two research assistants.
7. We have published research outputs in high impact journals and presented the results at national and international conferences (including those relevant to breeders.
8. We have protected the IP: we have lodged a patent invention detailing how a leucine rich receptor gene (regulated itself by the BRI1 mutation) enhances wheat resistance to Fusarium head blight disease and now work to determine the industrial potential of such a gene.

4. Impact of the Research

Synopsis of the benefits/improvements

At the outset of this research, the potential impact of brassinsteroid signalling on disease resistance was not established in barley or wheat. This research has conclusively confirmed that modulation of the brassinsteroid signalling receptor *Bri1* in barley significantly impacts disease resistance and agronomic performance in the glasshouse and/or field, and that this effect is disease-specific. Similar analyses are being finalised for wheat, focusing on the two TILLING mutants. Thus, this study provides breeders with knowledge regarding germplasm performance and with wheat TILLING mutants. Additionally, by enhancing our understanding of how brassinsteroid signalling functions in response to disease, and identifying additional genes that affect disease resistance, WheatEnhance provides additional breeding targets and background information of relevance to the breeding industry.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

We have developed collaborative links with a wide range of scientists and industry partners during the course of this project. On the basis of the success of TILLING, we have used a similar strategy to screen mutant lines for resistance to other diseases. This formed part of a Marie Curie ETN wherein we collaborated with 21 EU partners. Additionally, we have developed collaborative links leading to a new Horizons 2020 project (INNOVAR) where we collaborate with international partners to develop new tools for germplasm assessment.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

This project validated the use of TILLING technology for introgressing traits of agronomic importance into crops.

This project identified new genes/gene variants of agronomic potential.

(iii) Outcomes with economic potential

The genes identified as conferring FHB resistance have potential economic value in terms of their impact when introgressed into other crops (e.g. maize), but more importantly, the allelic diversity of these genes should now be examined to determine if natural variation exists that impacts disease resistance. Also, some of the genes identified as part of this study also impact grain development. Future research will determine their value for augmenting grain yield parameters.

(iv) Outcomes with national/ policy/social/environmental potential

Breeders are interested in using the wheat mutants as pre-breeding material. This material, along with the results of Task 2, will be provided to breeders and they will assess its wider agronomic performance. Follow on field-based phenotyping will continue with Teagasc in support of this. The development of varieties with enhanced disease resistance is of benefit in terms of reducing environmental impact of crop production (reduced chemical inputs).

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.

- Doohan FM, Benbow H, Thapa G, Zhou B, Kahla A, Christodoulou T, Jianguang J, Perochon A,(2017). Genes for wheat resistance and susceptibility to Fusarium head blight and *Septoria tritici* of wheat. A seminar presented in Genetics and Genomics of Biotic and Abiotic Stress Resistance. In Proceedings 13th International Wheat Genetics Symposium April 23-28, 2017Tulln, Austria.
- Thapa G, Gunupuru LR, Hehir JG, Kahla A, Mullins E, Doohan FM. A pathogen-responsive leucine rich receptor like kinase contributes to Fusarium resistance in cereals. Frontiers inPlant Science, doi: 10.3389/fpls.2018.00867.
- Gunupuru, L.R., Perochon, A., Ali, S.S., Scofield, S.R. and Doohan, F.M. (2019). Virus- induced gene silencing (VIGS) for functional characterization of disease resistance genes in barley seedlings. In *Barley* (pp. 95-114). Humana Press, New York, NY.
- Two more in preparation:
    - Thapa, G., Hehir, G., Mullins, E. and Doohan, F.M. (2020). Effect of mutation on the brassinsteroid receptor *Bri1* on downstream signaling, in preparation(submission 2020).
    - Rhatore, Hehir, G., Doohan, F. M. and Mullins, E. 2020.

(ii) Popular non-scientific publications and abstracts including those presented at conferences

- A picture from this project entitled 'Inside the flower of wheat' won the Teagasc Vision of Research and Innovation competition 2017, which was published in the Teagasc Calendar for 2018.
- Poster presentation at the Society of Irish Plant Pathologists Annual Meeting 2018: 26th - 27th November 2018, Teagasc, Oak Park Carlow. Title: Field-based phenotyping of brassinosteroid (BR)-insensitive 1 (BRI1) mutant lines of barley in Ireland.

(iii) National Report

N/A

(iv) Workshops/seminars at which results were presented

- Poster presentation at the Society of Irish Plant Pathologists Annual Meeting 2018: 26th - 27th November 2018, Teagasc, Oak Park Carlow. Title: Field-based phenotyping of brassinosteroid (BR)-insensitive 1 (BRI1) mutant lines of barley in Ireland.
- Keynote talk at the European Fusarium Seminar Conference in Tulln, Austria in 2018.
- Seminar at Irish Plant Science Association Meeting 2014 – "*The hunt for disease resistance genes*".
- Poster at US SCAB meeting 2013 advertising the project objectives – "*WHEATENHANCE- The hunt for disease resistance genes*".
- A seminar presented at the FESPB conference in 2014- "*Orphan genes and diseaseresistance*".
- A seminar presented the Plant Genomics Congress London 2015 – "*Plant DiseaseResistance genes*".
- A seminar presented at Limagrain France in 2015 – "*Irish crop science research - genetictargets for disease resistance breeding*".
- A seminar presented at The European Fusarium conference 2015 – "*A novel wheat geneenhances wheat resistance to FHB and to DON*".
- A seminar presented at 5th International Symposium on Fusarium Head Blight 2016 (2nd International Workshop on Wheat Blast) - FHB RESISTANCE FROM RECEPTORS TO DOWNSTREAM SIGNALLING.
- Poster presentation at 2017 Plant and Animal Genome Meeting (PAG XXV) – "Disease assessment of brassinosteroid (BR)-insensitive 1 (BRI1) mutant lines of barley under Irish agronomic conditions and phenotyping of BRI1 wheat TILLING lines".

(v) Intellectual Property applications/licences/patents

A Patent has been filed at UCD NOVA with INVENTION NAME: A RECEPTOR KINASE THAT ENHANCES CEREAL RESISTANCE TO FUSARIUM HEAD BLIGHT.

(vi) Other

The results were showcased at various international and industry visits to UCD and Teagasc.

5. Scientists trained by Project

Total Number of PhD theses: 0*

*Although none were directly funded, this project provided background information for a PhD completed as part of the EU Marie Curie ETN CerealPath. Keshav Malla, UCD, Graduated September 2019.

Total Number of Masters theses: 0

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project	Total Time contribution (person years)
UCD	Fiona Doohan	1
Teagasc	Ewen Mullins	0.43
Total		

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers	3	8.64
PhD students		
Masters students		
Temporary researchers		
Other		
Total		**8.64 years**

8. Involvement in Agri Food Graduate Development Programme

N/A

9. Project Expenditure

Total expenditure of the project: €727081.48

Total Award by DAFM: € 770,202

Other sources of funding including benefit in kind and/or cash contribution(specify): € 9455.60 (Teagasc)

Breakdown of Total Expenditure

Category	UCD	Teagasc	Total
Contract staff	0.00	156,427.24	156427.24
Temporary staff	0.00	0.00	0
Post doctorates	231,345.10	0.00	231345.1
Post graduates	0.00	0.00	0
Consumables	100,465.52	29,462.08	129927.6
Travel and subsistence	17,316.10	7,108.86	24424.96
Sub total	**349,126.72**	**192,998.18**	542124.9
Durable equipment	22,319.11	0.00	22319.11
Other	0.00	0.00	0
Overheads	104,738.02	57,899.45	162637.47
Total	**476,183.85**	**250,897.63**	727081.48

10. Leveraging

N/A

National Funding:
Science Foundation Ireland: We secured a 1.7 million euro grant, WheatSecurity, which built on the funding within WheatEnhance.

EU funding:
CerealPath Marie Curie ETN: We secured 1 million euro (4 million euro budget) to train 15 doctoral students in cereal disease control (led by UCD).
Horizons 2020 Project INNOVAR: We developed a project which aims to deliver new tools for plant variety testing (Prof. Doohan is the Scientific coordinator; 8 million euro budget).

11. Future Strategies

1. We will publish the field results and work with breeders to exploit the wheat germplasm developed during this project; this will be included in Horizons 2020 project INNOVAR and in a new EU project under development.

2. We are currently exploring a licensing agreement industry to determine the potential of the LRR gene.
3. We will publish the proteomics research and thereafter, exploit these findings to better characterise brassinosteroid signalling in plants of relevance to breeders.