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WheatEnhance: Developing novel wheat lines to enhance yield and disease resistance in commercial breeding material

Key external stakeholders:

- Breeders/Researchers
- Arable crop growers
- Crop agronomists/advisors
- Policy makers

Practical implications for stakeholders:

- Fungi from the genus *Fusarium* infect cereals causing *Fusarium* head blight disease (FHB), resulting in yield losses and poor grain quality due to mycotoxin contamination.
- Mycotoxins such as deoxynivalenol (DON) exert toxic effects on human and animals.
- As European Farm-to-Fork goals strive to limit fungicide use, developing varietal disease resistance via breeding is an important tool to reduce the threat of FHB and other fungal diseases.
- BRI1 mutated lines of Japanese barley cultivars; Bowman and Akashinriki, known as Uzu lines have shown high resistance to FHB. Thus, the aim of this project was to incorporate similar mutations into wheat and prepare a population for field-based disease profiling.
- A total of 2000 wheat TILLING lines were screened, which identified nine candidates with a functional BRI1 mutation, of which two will progress to full field evaluation
- Fundamental investigations of the molecular basis of the BRI1 mutation identified a potential disease resistance gene relevant for further study. Further marker validation of the BRI1 SNP in the breeding and elite wheat lines will help breeders and researchers to identify high value candidate lines.

Main results:

- Two potential candidate lines, out of nine initially identified, have been advanced (through 4 years of glasshouse crossings) to pre-breeding seed multiplication for field-based assessments.
- Molecular investigations of the BRI1 mutation has led to the identification of a classic defence receptor profile as a potential disease resistance gene relevant for further study.

Opportunity / Benefit:

There is scope to develop improved wheat varieties to protect against diseases for both breeders and growers. There is also a need and an opportunity to advance current understanding of disease resistance mechanisms in cereals using genomic tools. Novel genes such as the one identified from this project have the field potential to reduce fungicide applications.

Collaborating Institutions:

University College Dublin

Teagasc project team: Ewen Mullins, Gerard James Hehir, Dheeraj Rathore

External collaborators: Fiona Doohan (UCD), Ganesh Thapa (UCD)

Contact Ewen Mullins Email: ewen.mullins@teagasc.ie

1. Project background:

Brassinosteroid (BR) hormones are known to be involved in many physiological processes of plant growth and development including resistance to disease and environmental stress. The characterization of BRI1 (brassinosteroid-insensitive1), a brassinosteroid (BR) membrane receptor, as a central player in the brassinosteroid signaling cascade has been assessed using various bri1 mutant lines. The mutation in the BRI1 cytoplasmic kinase domain, has enhanced yield and resistance to *Fusarium* head blight disease in Uzu barley lines, relative to its parent genotype. The Uzu phenotype in barley was originally identified as a spontaneous semi-dwarf mutation in the BRI1 gene in Japanese germplasm, and it has been widely introgressed into Asian material to improve both lodging, yield and stress resistance. More recently, barley Uzu lines carrying the BRI1 mutation have shown enhanced disease resistance against a range of fungal pathogens compared to parental barley cultivars. Molecular studies of both the wild type and mutated barley to FHB revealed the molecular mechanism of improved resistance by disruption of BRI1 in barley. Therefore, it was hypothesized that manipulation of BRI1 via chemical mutagenesis in wheat will provide genetic resources to improve FHB resistance. Following on from this, a non-GM technique known as TILLING (Targeting induced Local Lesions in Genomes) was used to screen for chemically induced BRI1 mutations in wheat germplasm with a view to developing a pre-breeding population for advanced field assessments

2. Questions addressed by the project:

- Can candidate wheat lines containing the chemically induced mutation in BRI1 be identified by screening a TILLING population of wheat?
- Is there an impact associated with the BRI1 mutation on the yield and disease resistance potentials of existing barley mutant lines under Irish field conditions?
- Can the altered response associated with a BRI1 mutation be validated under controlled conditions
- Can robust molecular markers be developed for use in breeding programmes in order to select BRI1 mutations of interest and rapidly introgress them into cereals destined for the Irish cereal market?
- Can the molecular basis by which the BRI1 mutation benefits yield and disease resistance be characterized?

3. The experimental studies:

The experimental work in this project was divided across several objectives:

- Complete a molecular marker screen of chemically-mutagenized wheat populations to identify lines with specific mutations in BRI1 and complete up to 4 rounds of backcrossing to remove unwanted mutations from selected high value wheat lines
- Determine the effect of the BRI1 mutation on protein phosphorylation and the regulation of specific genes via 2D protein phosphorylation studies and gene expression profiling
- Field trials to determine the yield potential of BRI1 mutant barley (relative to wild type) under Irish conditions.

4. Main results:

- Nine novel wheat lines were identified as containing the BRI1 mutation from the TILLING population screened. These 9 lines were successfully back crossed with Cadenza (original parental type) for four generations to obtain BC4 seed. Two (line 1371 and line 233) have been advanced for seed multiplication and have been KASP validated as carrying a specific BRI1 mutation that will disrupt brassinosteroid signalling as required. The seed multiplication process is on-going to support disease screening against STB/FHB pathogens and parallel phenotyping with Cadenza.
- Field trials were conducted on selected BRI1 barley material UZU lines Akashinriki Uzu and Bowman Uzu compared to their parental lines Akashinriki and Bowman, to determine the performance of the BRI1 mutation under Irish conditions with a specific focus on disease performance and phenotypic variation (e.g. plant height, number of tillers, spike length, number of seeds per spike, lodging, yield, harvest index). The plant height and spike length of Akashinriki Uzu was significantly lower than its parental type, however there was no difference between Bowman and its Uzu line. Moreover, there was not yield difference between the Uzu lines and their parental types. There was severe powdery mildew infestation observed in both parents and mutant lines due to moist climatic conditions; however no *Fusarium* head blight occurred.

- Glasshouse generation of BRI1 mutants was completed with molecular marker (KASP) verification included to ensure purity of the mutant line in the backcrossing steps. The BC4 generation of mutant lines BRI1^{233} and BRI1^{1371} are currently undergoing initial disease assessments. The BC4 lines of the other 6 mutant lines were backcrossed, KASP validated and seeds bulked for storage to screen against disease resistance in field. A database of disease and phenotyping data from both field and glasshouse trials has been generated and is available to both UCD and Teagasc users as reference for any future expansion on BRI1 mutant wheat/barley time for disease resistance against diseases such as Septoria for wheat and Ramularia for barley.
- An additional objective was to determine the genes and proteins affected, due to the BRI1 mutation in cereals. Whole genome expression data identified primary defence genes and an LRR classic defence receptor as a target for further study, i.e. potential disease resistance genes. A detailed proteomic analysis confirmed that the BRI1 mutation led to phosphorylation differences in associated proteins. These differential phosphorylated proteins are now being analysed to identify the crucial regulatory proteins that will be targeted for further validation. Gene silencing studies have confirmed that an LRR gene, which was differentially re-regulated due to the BRI1 mutation, directly affects FHB resistance.

5. Opportunity/Benefit:

- The end users of the outputs from this project are breeders and famers. Specifically the BC4 material that will be field assessed in 2021 is an important pre-breeding resource that will be made available to breeders pending Oak Park trials. In light of Farm-to-Fork goals, identifying novel sources of disease resistance to reduce chemical inputs is both a legislative and societal objective.

6. Dissemination:

1. 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, 2017 Tulln, Austria.
2. Thapa G, Gunupuru LR, Hehir JG, Kahla A, Mullins E, Doohan FM (2018). A pathogen-responsive Leucine Rich Receptor (LRR) like kinase contributes to *Fusarium* resistance in cereals. Frontiers in Plant Science, 9:867.
3. 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”.
4. 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.

Compiled by: Dheeraj Rathore and Ewen Mullins