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Session Two
Monday, 22nd February 2021

DECIPHERING MECHANISMS OF NONHOST RESISTANCE AGAINST *ZYMOSEPTORIA TRITICI* IN MODEL PLANT SPECIES

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1. INTRODUCTION

Nonhost resistance (NHR) is a durable form of resistance exhibited by an entire plant species to a specific pathogen. Studies of this phenomenon often use biotrophic pathogens that penetrate plant cells and form intracellular feeding structures. In contrast, *Zymoseptoria tritici* - the causal agent of the foliar disease Septoria tritici blotch (STB) of wheat[1] - is a hemibiotroph that invades the host through stomata and remains extracellular during its entire life cycle[2]. While the molecular nature of *Z. tritici* virulence has been investigated on its natural host (wheat), NHR against *Z. tritici* remains poorly understood.

2. PREVIOUS RESEARCH FINDINGS

A previous study by Kettles *et al.* (2017)[3] at Rothamsted have identified that:

- **A number of *Z. tritici* secreted effector proteins** triggered cell-death defence response when transiently expressed in the nonhost tobacco species *Nicotiana benthamiana* (Figure 1A)
- Cell-death triggered by some of these effectors were shown to be **dependent on BAK1 and SOBIR1**, well-known co-receptors for many plant cell surface immune receptors (CSIRs) (Figure 1B)

This suggests that the **recognition** of *Z. tritici* effectors in a non-host *N. benthamiana* plant **occurs at the plasma membrane-apoplast interface** and is likely to be mediated by CSIRs.

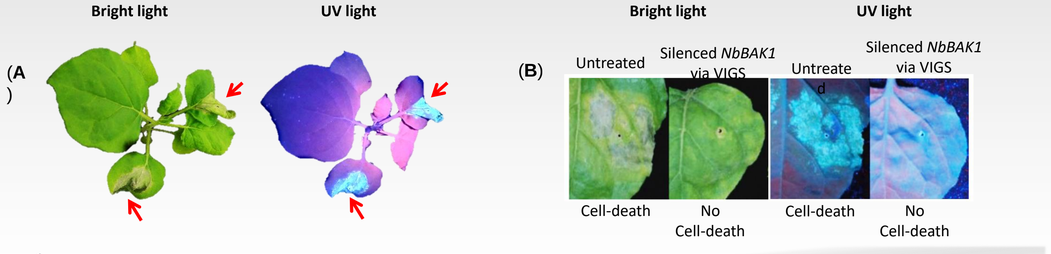

Figure 1:
(**A**) Numerous *Z. tritici* effector protein (such as Zt14 shown) trigger cell-death (red arrows) when transiently expressed in *N. benthamiana* leaves. (**B**) Cell-death triggered by expression of some *Z. tritici* effector proteins, such as Zt9 shown, in *N. benthamiana* is dependent on the co-receptor NbBAK1. Image in (**B**) is from Kettles *et al.* (2017).

3. RESEARCH PROJECT AIM AND OBJECTIVES

AIM:

Identify and characterize CSIRs facilitating recognition of *Z. tritici* effectors in *N. benthamiana*

OBJECTIVES:

- **Perform proteomic screen to identify *N. benthamiana* receptor proteins** interacting with BAK1 upon recognition of cognate *Z. tritici* effectors (Figure 2)
- **Confirm the identified interacting proteins as CSIRs** that detect *Z. tritici* effectors using Virus-Induced Gene Silencing (VIGS) (Figure 3)
- Assess whether other nonhost plants (i.e. *Arabidopsis thaliana*) could recognise the same or a different set of *Z. tritici* effectors (Figure 4)
- **Test the transferability** of effector recognition between species (Figure 5)

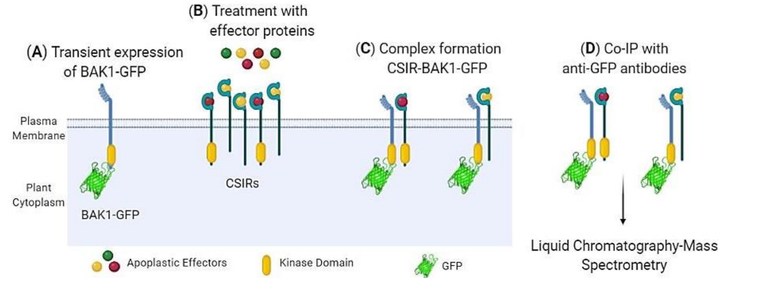

Figure 2:
In brief, *N. benthamiana* leaves transiently expressing BAK1-GFP **(A)** will be infiltrated with the purified *Z. tritici* effector proteins **(B)** leading to formation of CSIRs-BAK1-GFP complexes (**C**). These complexes will be isolated by (**D**) co-immunoprecipitation with anti-GFP antibodies. The recovered proteins will be identified by liquid chromatography-mass spectrometry (LC-MS/MS) on a Discovery Proteomics Orbitrap Fusion Platform. This approach relies on the knowledge that BAK1-CSIR complexes are formed only in the presence and upon recognition of the corresponding ligands (i.e. *Z. tritici* effectors in our case).

4. POTENTIAL IMPACT

Identification of CSIRs recognising *Z. tritici* effector proteins in *N. benthamiana* could be used to:

- Identify homologues genes in wheat plants
- Being directly introduced as transgenes into susceptible wheat plants
- Provide fundamental insights into NHR mechanism to extracellular pathogens

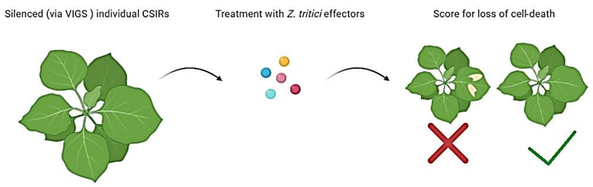

Figure 3:
Candidate CSIRs will be functionally characterised using a rapid functional genomics tool such as VIGS. Silencing of the *bona fide* receptor in *N. benthamiana* is expected to result in the loss of defence (i.e. no cell-death) in response to specific *Z. tritici* effector.

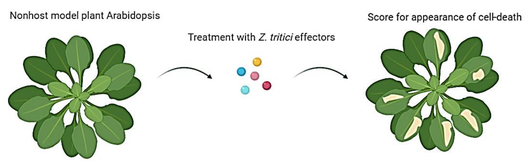

Figure 4:
A panel of selected *Z. tritici* effector proteins will be transiently expressed in a model plant *Arabidopsis thaliana* and scored for appearance of cell-death phenotype. Here, the hypothesis is that the same *Z. tritici* effectors are recognized in the different nonhost species by orthologous CSIRs.

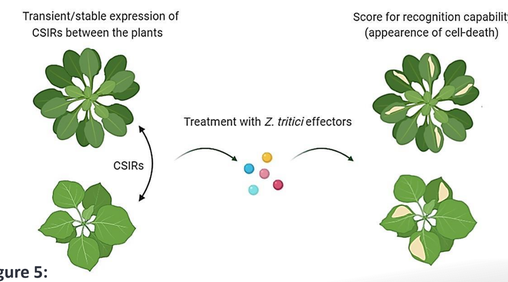

Figure 5:
CSIRs with effector-recognising capability will be transferred between the two nonhost model plant species via transient expression assays or stable genetic transformation and tested for recognition capability (i.e. appearance of cell-death)

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AKNOWLEDGEMENTS

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UKRI Biotechnology and Biological Sciences Research Council

MILLERS, SAVING YOU TIME AND MONEY! CREATING WHEAT VARIETIES WITH CONSISTENT AND HIGH BREADMAKING QUALITY

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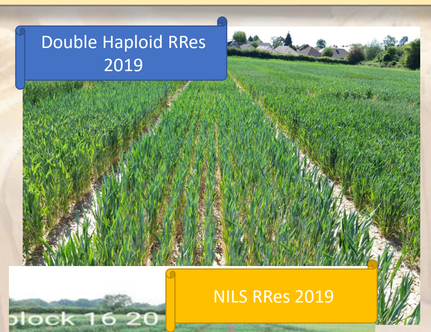
Abstract

A significant part of the variation in wheat breadmaking quality remains unexplained and intrinsic quality is also strongly affected by the environment.

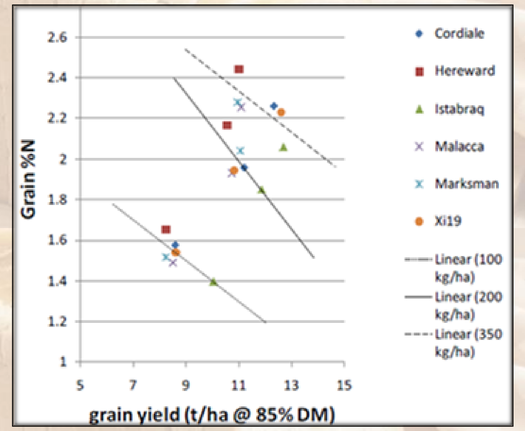
To this end, the present study uses two sets of lines from the cross Malacca x Hereward to explore the effects of quality-related QTLs and of genetic variation in grain protein deviation and stability using field trials grown in 3 environments (sites or years)

For the first set is 72 near-isogenic lines differing in 8 QTLs for 5 breadmaking traits (loaf volume, cell number and wall thickness, crumb colour and firmness). These are being grown in three environments in the UK (John Innes Centre in 2017 and Rothamsted in 2019 and 2020). Three treatments are considered: QTLs (8), alleles (2) and lines (5) which will allow us to compare allelic effect for each QTL. The lines are being randomised for each treatment with three replicates. QTLs showing effects on the phenotypic variance in the three environments will be studied further to identify candidate genes and molecular mechanisms.

The second experiment uses 104 double haploid lines. Only one treatment (line) is considered and the lines are being grown in replicated yield plots in three environments (Rothamsted 2019, Reading and Rothamsted in 2020). QTL analysis will be carried out on each field trial to control environmental effects.



Objectives



1. Maximizing cultivar response to nitrogen inputs to reduce fertiliser costs and environmental footprint

A population of 104 double haploid lines from the cross of Malacca and Hereward is being grown in three environments (Rothamsted 2019 and 2020, Reading 2020) with 150 kg/haN and a seed rate was 350 seeds/m^2.

This population will be used to carry out a genetic study for the traits grain protein deviation (GPD) and stability . **GPD** is the deviation from the linear relationship between grain yield and protein content (Left chart).

The population was selected because Hereward shows consistent positive GPD and Malacca zero or negative GPD. . The population has been mapped with high density markers so the identification of favourable alleles for GPD in Hereward will allow the development of linked markers for breeding.

Cross	Chrom	Trait	Flanking markers
(MH100 x Malacca[3])	1B	Number of cells	gwm264 and barc8
(MH58 x Hereward[3])	2B	firmness	wmc257 and wmc317
(MH9 x Malacca[3])	2D	Loaf vol	gwm102t,wmc18,gwm129t
(MH1 x Malacca[3])	4D	No cells L*	b98,gdm129t
(MH19 x Hereward[3])	4D	L*	b98,gdm129t
(MH70 x Malacca[3])	6A	No of cells	g334-b3
(MH60 x Malacca[3])	7A	Wall thick Cell diam/vol. Loaf vol	psp3001
(MH39 x Hereward[3])	7B	loaf vol	gwm537t,gwm577,barc182t

2. Identifying novel stable determinants of breadmaking quality – a genetic approach

A previous genetic study performed of breadmaking traits using the DH population of Malacca x Hereward identified favourable alleles for 5 traits related to bread characteristics: dough colour, loaf volume, crumb structure, firmness and gas cell wall thickness (Above tab).

This led to the development of a set of Near-Isogenic lines (NILs) to confirm the effects of the alleles by comparing them in the same genetic background by backcrossing with the Malacca parent. Several sister lines were developed for each allele resulting in 72 Near-Isogenic lines (NILs) which are being grown in three environments (JIC 2017, Rothamsted 2019 and 2020). Robust allelic effects (i.e. detected in at least two environments) will be identified and related to grain composition.

Determination of processing properties

Small scale analyses of dough rheology will be carried out using a Reometer. This small scale (10g) recording mixer measures dough strength and stability by recording the energy required during mixing.

Large scale analyses will be carried out on bulked sets of sister lines using industry standard methods at Heygates Ltd.

The samples will be milled on a Buhler MLU-202 to produce white flour and the water absorption determined by Farinograph. Dough elasticity and extensibility will be determined by Extensograph and bread quality by test baking.

Introduction

Bread is a staple food in the UK providing calories, vitamins, proteins and minerals to our diet. During breadmaking wheat dough is expanded by carbon dioxide produced by yeast to give gas cells which result in the porous structure of the baked loaf. This expansion is permitted by the viscoelastic properties of the dough that result largely from the structures and interactions of the gluten proteins (Shewry and Tatham, 1999). Numerous studies have shown that both total gluten protein content and variation in gluten protein composition contribute to quality. However, a significant part of the breadmaking variation remains unexplained and may result from contributions of other components such as non-gluten protein, arabinoxylan polysaccharides and lipids. For example, surface active components such as lipids stabilise the gas cells in the dough.

The cultivars Hereward and Malacca were highly successful breadmaking wheats, but in both cases the quality does not appear to be determined by gluten protein composition. Analyses of a cross between these lines show that both have QTLs for aspects of breadmaking quality while Hereward also has higher grain protein compared to yield, a phenomenon known as grain protein deviation (GPD). I am therefore using two series of lines from this cross to explore these traits: a population of 104 doubled haploid (DH) lines to map QTLs for GPD and a series of 72 near-isogenic lines (NILs) to determine the effects of allelic variation at 8 mapped QTLs for quality.

Volumeter: Measure the volume of a bakery product in cm3

Provide information about the product:

- Density of the bread crumb
- Strength of the gluten flour
- Enzyme activity

Coarse seed coat

Fine seed coat

Sieves

Break flour

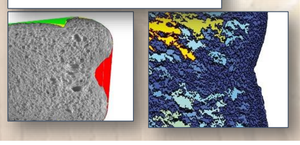
Reduction flour

Reomixer: provides practical, rapid small-scale wheat protein quality measurements derived from dough mixing characteristics.

Estimation of:
- Gluten strength
- Extensibility
- Stability

Farinograph

Estimation of:
- Gluten strength
- Extensibility
- Stability
- Water absorption



The **C Cell Colour** is an advanced digital imaging system
- Shape (important for packaging)
- Distribution of gas cells, area, volume
- Colour of Crumb and crust (consumer perception)

References

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Chart of GPD: Shewry, P. R., Wan, Y., Chope, G., Penson, S., Mosleth, E. F., & Hawkesford, M. J. (2013). Project Report No. 521 Sustainability of UK-grown wheat for breadmaking. 87.

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ROTHAMSTED RESEARCH

John Innes Centre Unlocking Nature's Diversity

Elucidating the mechanisms of aphid resistance in wheat

Student: Alexander Borg[1,2]
Supervisors: Dr. Mike Birkett[1], Dr. John Caulfield[1], Dr. Jozsef Vutz[1] and Prof. John Foulkes[2]
1: Rothamsted Research, Biointeractions and Crop Protection, 2: University of Nottingham, School of Biosciences, Division of Plant and Crop Sciences

Introduction

Aphids are a major wheat pest, inducing damage by reducing nutrient and assimilate availability via phloem feeding, by viral transmission (ex. Barley yellow Dwarf Virus) (fig.1) and by reducing photosynthesis due to aphid honeydew enabling saprophytic fungal growth on leaves.[1]

Additionally, insecticide resistance and the banning of working insecticides makes controlling aphid infestations harder.[2]

The low genetic diversity in modern wheat results in a lack of aphid resistance[3,4] (fig.3), therefore ancient and wild wheat species are being investigated to identify possible aphid resistance mechanisms which can be engineered into modern wheat.

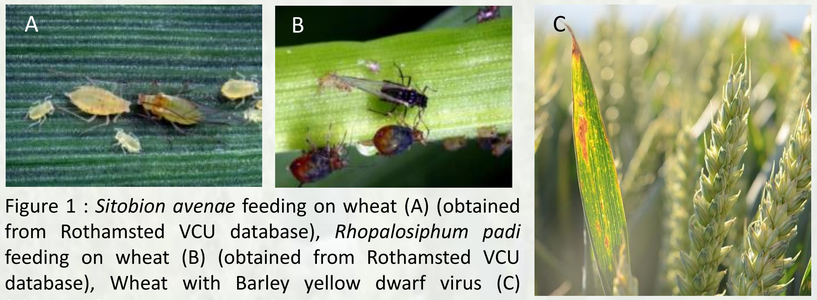
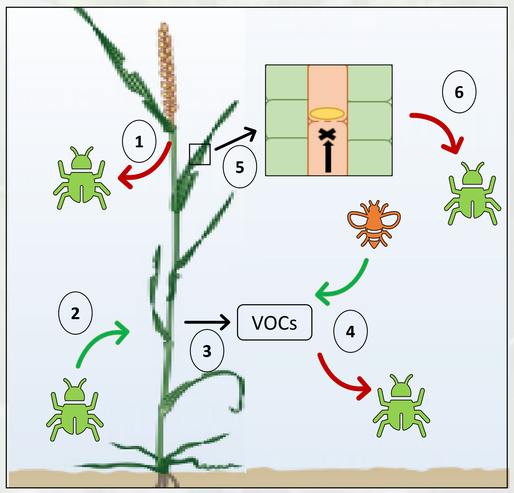


Figure 1 : *Sitobion avenae* feeding on wheat (A) (obtained from Rothamsted VCU database), *Rhopalosiphum padi* feeding on wheat (B) (obtained from Rothamsted VCU database), Wheat with Barley yellow dwarf virus (C) (obtained from Syngenta.co.uk)

Aphid resistance in ancient wheat



Screening studies show that ancient diploid wheat have the highest aphid resistance[3,4].

Resistance mechanisms include phloem occlusion and pre- and post-alighting cues (fig.2) [5,6,7].

Aphid predator attraction is also observed in *Triticum monococcum*

Figure 2: Aphid resistance in ancient wheat. 1: VOCs released by the plant prevent aphid alightment. 2: Aphids feed on the plant. 3: Aphid feeding induces VOC release. 4: Aphid induced VOCs repel aphids and attract aphid predators. 5: aphid feeding induces phloem callose deposition, blocking sap flow. 6: The blocked phloem prevents aphid feeding. Base image[13].

Figure 3: Loss of genetic diversity and resistance genes with the domestication of modern wheat. Left to right: *Triticum monococcum, Triticum dicoccum* and *Triticum aestivum*. Base image obtained from Yara.co.uk.

Aphid resistance in related grasses

- Aphid Resistant Maize adopt the same resistance mechanisms as ancient wheat[8,9].
- Mazie produce antifeedant benzoaxinoids (BXs) [9].
- The BX DIMBOA also plays a signalling role, inducing callose deposition[9] (Fig.4,5).
- *Aegilops speltoides* also produces these BXs[10].

As aphid resistant ancient wheat and some Aegilops species lack BX production[10,11], other secondary metabolites may be employed that provide aphid resistance. Of interest are *T. monococcum* MDR045 and MDR049[6] and *Aegilops longissima* 2150002[10] as they have high aphid resistance yet lack BXs.

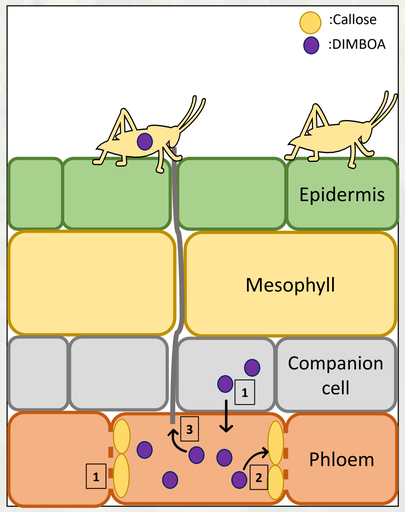


Figure 4: DIMBOA activity in maize during aphid feeding. 1: aphid feeding induces callose deposition and DIMBOA release into the phloem. 2: DIMBOA induces further callose deposition. 3: Aphid ingestion of DIMBOA acting as an antifeedant.

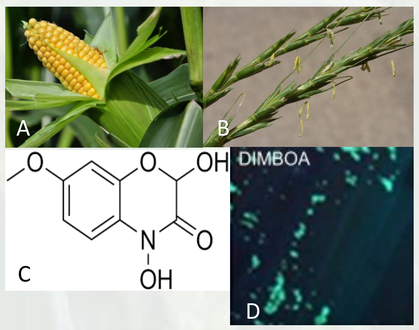


Figure 5: Maize (A) (obtained from Europa.eu), *Aegilops speltoides* (B) (obtained from kew.org), Chemical structure of DIMBOA (C), DIMBOA induced callose deposition (D)[15]

Project Aim

This project aims to further understand the aphid resistance mechanisms in *T. monococcum* MDR045 and MDR049 and *Aegilops longissima* 2150002, focusing on the secondary metabolites (SMs) used by these accessions.

This will be done by observing how SMs from these accessions, compared to aphid susceptible accessions, change with increasing aphid density infestations and across feeding time. By sampling different parts of the plant, the systematic response by the plant to aphid feeding will also be investigated.

How will abiotic factors effect aphid resistance?

Abiotic stresses characteristic of both climate and soil nutrient depletion prime aphid susceptible plants against aphid[13](Fig.6). This has not been investigated in aphid resistant wheat. This project will aim to observe whether a combination of abiotic stresses effects the aphid resistance mechanisms in ancient wheat.

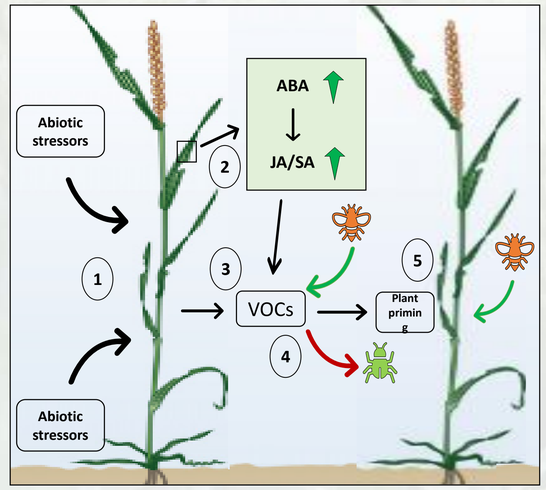


Figure 6: Abiotic stress defence priming. 1: plants are exposed to abiotic stresses. 2: ABA pathway is induced (abiotic stress defence response), in turn inducing the JA and SA pathways (pest and pathogen defence responses) priming against aphid attack. 3: ABA, JA and SA related VOCs released by the plant. 4: The VOCs repel aphids and attract aphid predators. 5: VOCs prime defence response of nearby plants, attracting aphid predators. Base image[13].

This will highlight links between the abiotic stress response and aphid resistance mechanisms and will help predict whether these defence mechanisms will be effective under certain soil conditions and changing abiotic factors due to climate change.

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NOVEL PHOSPHATE FERTILIZER FORMULATION FOR AFRICAN AGRICULTURE

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INTRODUCTION

Phosphate (P) deficiency, a major constraint for agriculture in sub-Saharan Africa, where soils are highly weathered, extremely low in available P, and fertilizer inputs are very low.

To maximise use of a small amount of P fertilizer, plant external efficiency mechanisms can be manipulated by the combination of compounds in the fertilizer and the placement of the fertilizer.

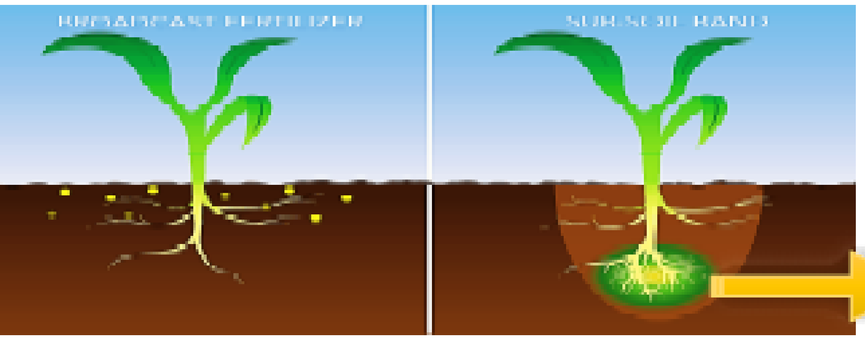

Figure 1: Properly formulated fertilizer, localized close to the developing root system increases P uptake. However, this will not happen if the fertilizer produces a very acidic or alkaline environment

Overall aims: to understand the processes controlling phosphate use efficiency in crops in highly-weathered low-P soils, and thereby to provide a basis for developing improved fertilizer formulations relevant for agriculture in sub-Saharan Africa.

Objective 1: To test a model allowing for root geometry and P solubilisation effects

Objective 2: To test the hypothesis that P efficiency in band applications can be improved by manipulating the combination and forms of P and N in fertilizer across a range of P levels

Objective 3: To assess P fertilizer response of rice genotypes

Objective 4: To identify P uptake and assimilation genes in rice indicative of P fertilizer response and P nutritional status

Objective 5: To draw conclusions for developing fertilizer formulation and management technologies

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METHODOLOGICAL APPROACHES

I- Parameterise P uptake and solubilisation model

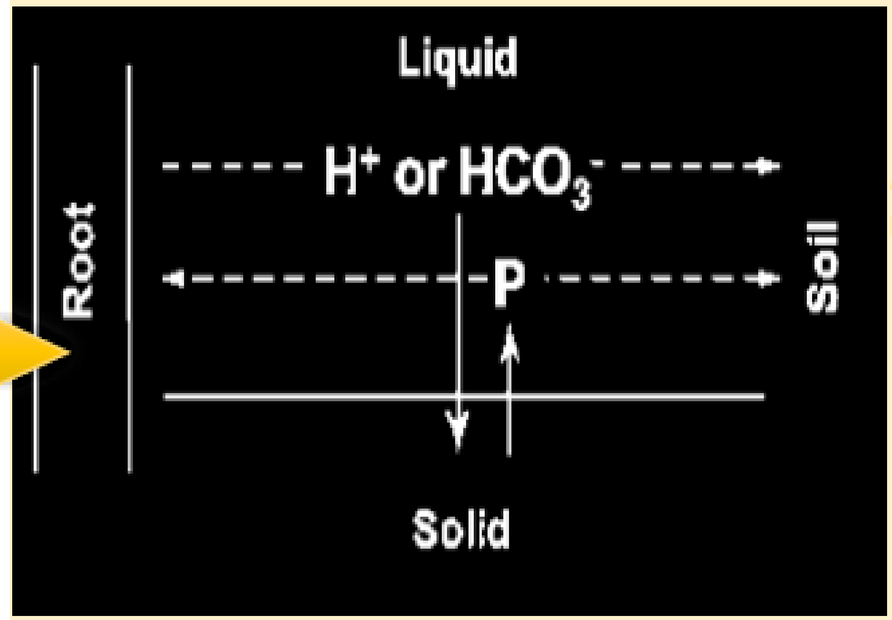

Figure 3: Model of solubilisation by pH changes
Nye (1972) J Soil Sci 23, 82–92

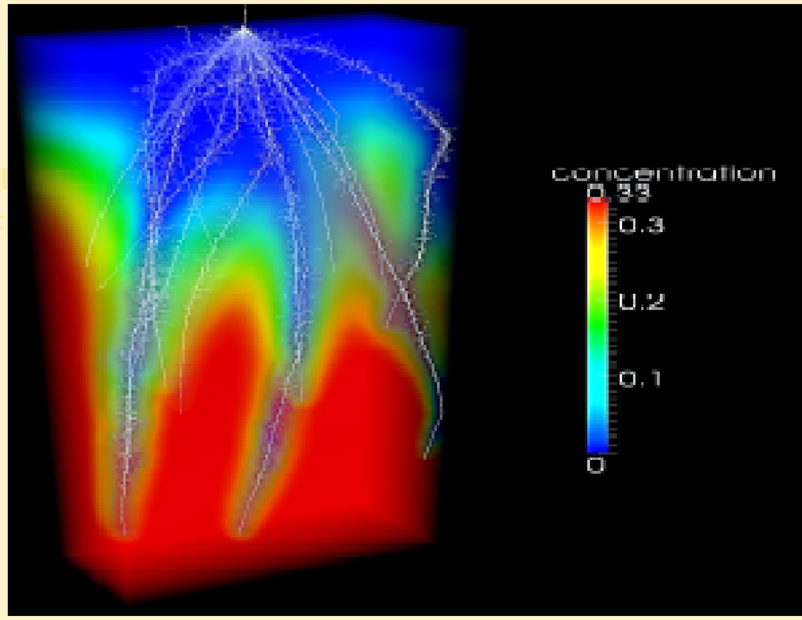

Figure 4: SIMROOT model
Postma et.al (2017) New Phytol 215: 1274–1286.

II- Test different fertilizer admixture and management

- Controlled-environment pot experiments
- Field experiment in highly weathered sub-Saharan African soils

POTENTIAL IMPACT OF THE THESIS

Improve understanding of P uptake and use efficiency in rice genotypes in highly weathered soils, and how it is affected by fertilizer formulations and management

Support the development of novel compound fertilizer formulations for African cropping systems

Identifying novel variation in Nitrogen Use Efficiency (NUE) and its physiological and genetic basis in wheat

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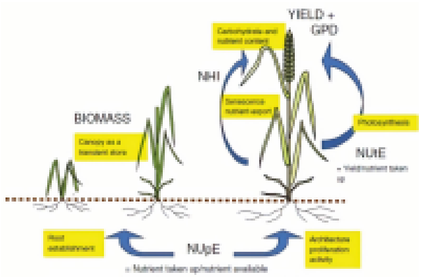
Introduction

Nitrogen (N)-use efficiency can be defined as the grain yield per unit of nitrogen supplied (from N fertilizer and soil) and is dependent on the two key components N-uptake efficiency (NUpE) and N-utilization efficiency (NUtE). The excess use of N fertilisers causes serious environmental impacts including nitrate leaching into ground water, eutrophication of water bodies, and global warming (emission N_2O; and CO_2 in manufacture of N fertilizers) (Foulkes, 2009. Therefore, a key breeding target in wheat is enhancing NUE. In bread wheat, there is relatively small genetic variation in NUpE and NutE in field investigations within adapted elite germplasm. This study therefore aims to identify novel variation for NUE by screening diverse wheat germplasm (landrace and synthetic derivative hexaploids) for NUE and associated traits and biomass and to understand its genetic basis.

Materials and Methods

Two field experiments in 2018-19 were carried out at Nottingham University and Rothamsted Research. The BBSRC Designing Future Wheat (DFW) Breeder's Diversity Toolkit of hexaploid wheat including near-isogenic lines (NILs) derived from landraces backcrossed to Paragon was screened in three replicates at each site. The NILs were developed at John Innes centre by selecting for QTLs for NUE and related traits identified in the previous BBSRC WISP project. These NILs are being used for identifying the genetic variation and its genetic basis in wheat. Second field trail was carried out at Rothamsted with 20 selected NILs. Physiological measurements were carried out described in Table 1.



Hawkesford et al., 2011

Rothamsted

Nottingham

Results

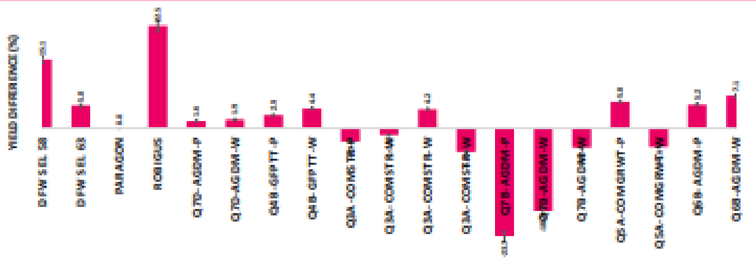


Fig. 1 Percent yield difference between paragon and NILs having NUE traits QTLs, where paragon yield is taken as reference.

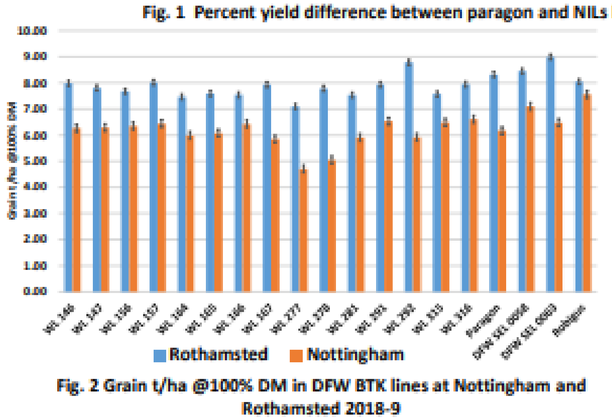


Fig. 2 Grain t/ha @100% DM in DFW BTK lines at Nottingham and Rothamsted 2018-9

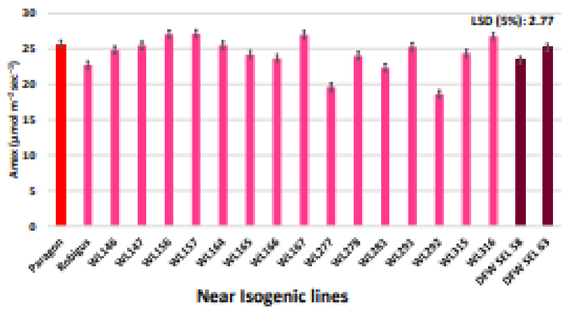


Fig. 4 Flag leaf Photosynthesis rate at Anthesis (Nottingham)

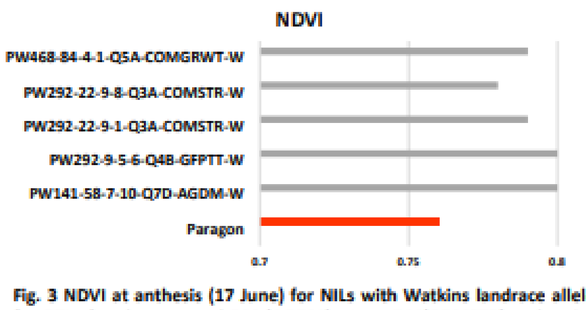


Fig. 3 NDVI at anthesis (17 June) for NILs with Watkins landrace alleles (W) for QTLs for aboveground DM (AGDM), straw DM(COMSTR) and grain filling thermal time (GFPTT) compared to paragon at Nottingham

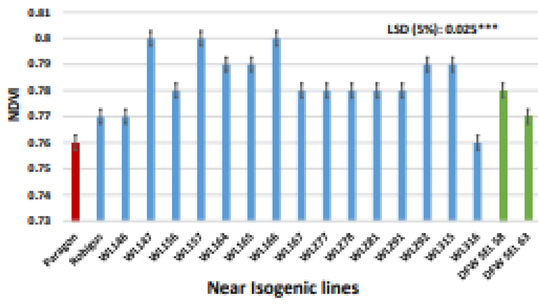


Fig. 5 NDVI for NILs Anthesis (17 June, Nottingham)

Table 1. Physiological measurements on BTK lines

Development stages	Phenological measurements	Senescence Kinetics	Root phenotyping	Growth analysis at harvest
Establishment score	Leaf photosynthesis rate (Licor 6400 Fluorpen FP100)	Normalized Difference Vegetative Index (NDVI)	Shovelomics (crown root angle, root no., root length) post-harvest	Grain yield (GY)
Growth stage scores	Hyperspectral spectro-radiometer	Flag-leaf visual score		Above ground dry matter (AGDM)
		Flag leaf relative chlorophyll content (SPAD)		Dry Matter (DM)
				N Partitioning (straw, grain)
				N remobilization efficiency

Funding Agencies

DFW · BBSRC bioscience for the future · UK Research and Innovation

Conclusions

NILs with W allele and having NUE related traits QTLs had significantly higher grain yield than paragon (Fig. 1). NILs incorporating QTLs related to NUE traits aboveground DM (AGDM), straw DM and grain filling thermal time (GFPTT) with alleles from Watkins landraces had Normalised Difference Vegetative Index (NDVI) significantly above Paragon (Fig. 3 and 5). The grain yield at Rothamsted site was higher (1-1.8 t/ha) as compared to Nottingham site (Fig. 2); and yield at the two sites was correlated among genetic lines (P = 0.01; data not shown). No significant variation for flag-leaf photosynthesis for the NILs compared to Paragon was observed (Fig. 4).

Ongoing work

A subset of NILs with NUE-related QTLs is being grown in a field experiment at Rothamsted Research in 2020-21. Transcriptomics experiments will be done on selected NILs with promising NUE QTLS to identify the candidate genes for NUE QTLs with a focus on N remobilization and senescence-related traits. In-silico gene/s identification from QTLs is being carried out by using online databases and tools.

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ROTHAMSTED RESEARCH

Session Three
Tuesday, 23rd February 2021

FUNCTIONAL CHARACTERISATION OF *FUSARIUM GRAMINEARUM* CANDIDATE EFFECTORS

Claire Kanja[1,2], Ana Machado Wood[1] Karen Irawan[3] & Kim E. Hammond-Kosack[1]

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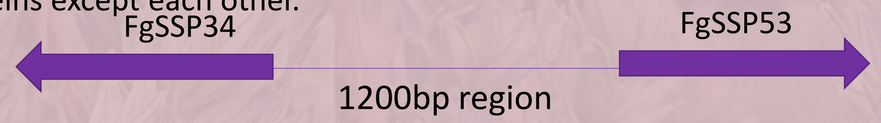
Fusarium graminearum is the causal agent of the global economically important wheat disease **Fusarium Head Blight**. FHB reduces both grain quality and yield and results in grain contaminated by harmful mycotoxins. During the fungal-floral interaction ***F. graminearum*** differentially upregulates a subset of genes encoding small secreted proteins (SSPs).

Two candidates identified by bioinformatics **FgSSP34** and **FgSSP53** are upregulated in the symptomless phase of the *F. graminearum*-wheat floral interaction[1]. These proteins are predicted to be paralogues and share no sequence homology with known proteins except each other.



CHARACTERISATION IN *N. BENTHAMIANA*

Recombinant expression of FgSSPs using Agrobacteria mediated transient expression (OD_{600} = 1). FgSSP53 but not FgSSP34 expressed from pEAQ-HT-DEST3[2] vector leads to induction of **Fig.1 (A-D)** necrosis and **E+F)** Reactive oxygen species (ROS) production.

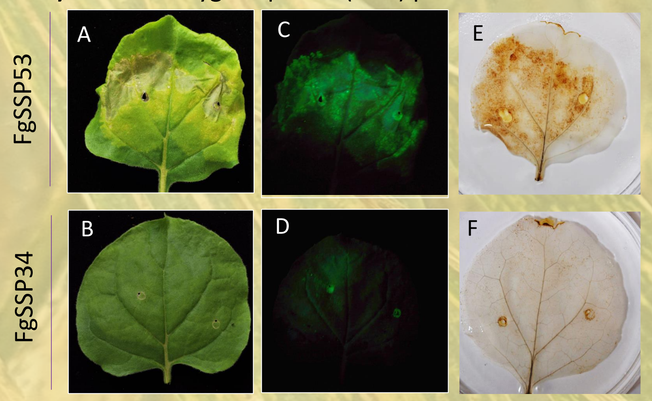


Figure 1) Photos were taken at 5dpi following agroinfiltration with vectors containing **FgSSP53** (**A,C,E**) or **FgSSP34 (B,D,E**) viewed under **A+B)** normal light and **C+D)** UV light. **E+F)** images at 4 dpi following staining with 3,3'-Diaminobenzidine (DAB) to detect ROS

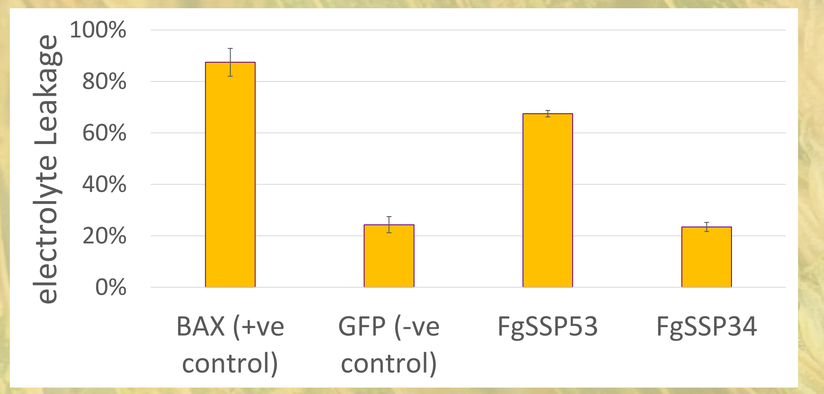


Figure 2) The necrosis induced by heterologous expressed proteins in *N. benthamiana* can be quantified by measuring the electrolyte leakage of leaf disks 4 days after agro-infiltration.

CHARACTERISATION IN WHEAT

Using the *Barley Stripe Mosaic Virus*-mediated overexpression system (BSMV-VOX)[3], overexpression of FgSSP53 but not FgSSP34 in wheat floral tissue was shown to significantly decrease *F. graminearum* disease severity.

Figure 3) Representative *F. graminearum* disease symptoms on wheat ears expressing individual FgSSPs or the iLOV control. Photographs were taken 12 days post *F. graminearum* infection. MCS- multiple cloning site

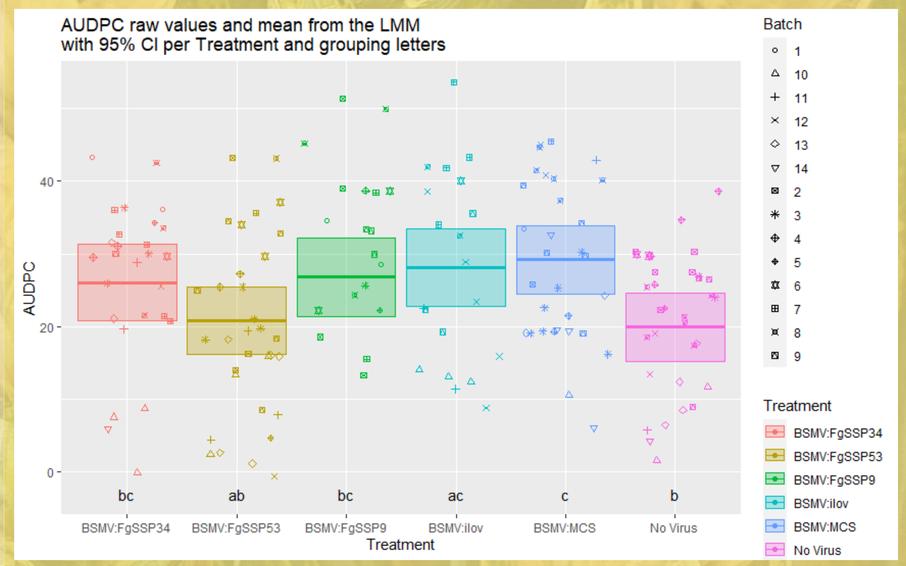


Figure 4) The estimated means and 95% confidence intervals for the area under the disease progression curves (AUDPC) values predicted using the linear mixed effectors model. For each treatment all individuals are plotted, and the symbols indicate batches. The letters correspond to significance groups calculated using Tukey's method ($p<0.05$).

CONCLUSIONS

- **FgSSP53** and **FgSSP34** have different phenotypic effects *in planta* despite being paralogues suggesting neofunctionalization.
- Although **FgSSP53** is expressed during the symptomless phase of **FHB** this effector induces defence responses in both *N. benthamiana* and wheat.

FUTURE WORK

- Explore the FgSSP induced immune response in *N. benthamiana* using RT-qPCR
- Generate *F. graminearum* knockout mutants of **FgSSP53** and **FgSSP34** and double mutants
- Explore possible interactions between **FgSSP34** and **FgSSP53** using protein-protein-interaction assays such as BiFC

ACKNOWLEDGEMENTS + REFERENCES

PhD funded by the BBSRC and Lawes Trust as part of the Doctoral Training Partnership programme between Rothamsted Research and the University of Nottingham. Studentship with KI funded by the BSPP.

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ROTHAMSTED RESEARCH
Biotechnology and Biological Sciences Research Council
University of Nottingham UK | CHINA | MALAYSIA

THE NITROGEN ECONOMY OF RICE-PASTURE-LIVESTOCK SYSTEMS IN URUGUAY.

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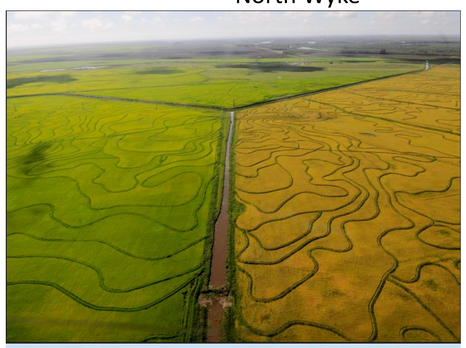
Addresses:[1] Rothamsted Research, Harpenden. [2] Cranfield University. [3] Rothamsted Research, North Wyke



INTRODUCTION

In Uruguay, rice production is considered in an integrated rotation with perennial pastures for direct cattle grazing. Country productivity has stabilised 8 Mg ha^{-1} with the application of only 75-80 kg N ha^{-1}. Because on average, about 100 kg grain per kg N added are obtained (Pittelkow et al. 2015), which at least doubles reported values for the major producer countries (Lahda et al. 2005), it is believed that this rice-pasture rotation and the animal effect has allowed the rice sector to minimise the use of N through the preservation of soil quality. The aim of the study was to quantify the country-level Full Chain NUE and the N balance (inputs-outputs) of the rice-pasture-livestock system of Uruguay, based on an N budget approach.

METHODS

. A national scale database for the last 16 year was constructed for the rice (0.165 M ha) and livestock (8.25 M ha) system.

. Records considered: rice grain and bi-products rice production and N crop use, livestock production (final food and total from bovine and ovine cattle), pasture production and pasture type composition. Local and regional literature data for atmospheric N deposition, pasture N fixation, and N losses (gasses and leaching), animal N efficiency were used. Calculous of total N animal recycling and N animal direct deposition from livestock to rice sector were conducted based on previous data.

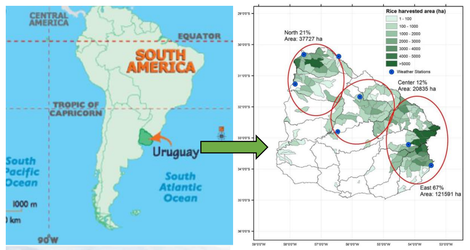
. N balance was calculated as de difference among all the inputs and the outputs of the system at a farm gate level.

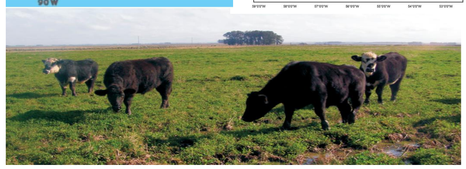
. Full chain NUE was analysed as: N in food products/ New N inputs (fertilisers, N fixation, atmospheric deposition, following EU Nitrogen Expert Panel (2015) proposal.

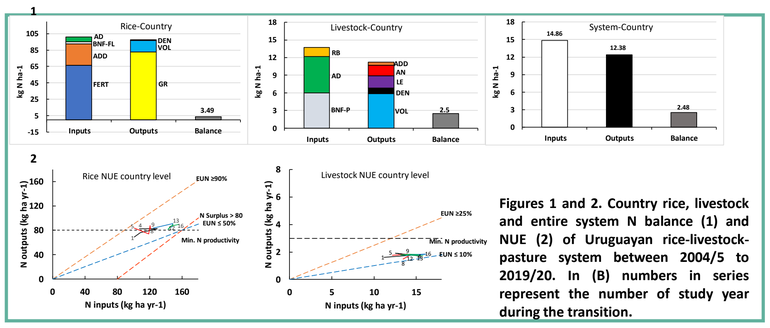
RESULTS

Average N inputs (99 kg ha^{-1}) for the rice system were fertilisers (65%) and animal direct deposition (ADD) (24%), while for the livestock sector (13 kg ha^{-1}), 41, 46 and 13% corresponded with pasture biological N fixation (BNF-P), atmospheric depositions (AD) and rice bran (RB), respectively. Main output in the rice system was the N retained in the grain (GR) (86%). Of 11.5 kg ha^{-1} as N livestock output, 78 and 14% corresponded with environmental losses (denitrification=DEN and leaching=LE) and N in animal products (AN), respectively (Figure 1). For the entire combined system a slightly positive balance was

Full Chain NUE was 74, 12 and 23% for the rice, livestock and complete system respectively for the entire period. These values were higher (rice), and similar compared with international published information but with less N addition in both systems, possibly, because a high direct total N recycling (61%) in the entire chain.







Figures 1 and 2. Country rice, livestock and entire system N balance (1) and NUE (2) of Uruguayan rice-livestock-pasture system between 2004/5 to 2019/20. In (B) numbers in series represent the number of study year during the transition.

CONCLUSIONS

This well-integrated system achieved for the entire system, and for each sector, N balances (kg N ha^{-1} year) close to the neutrality.

Cross-benefits as the animal direct deposition (ADD) from the livestock to rice sector can be identified as an important N input source to the crop. In the opposite way, the rice bran (RB) contributed in 11% of the N inputs to the livestock sector.

Compared with other producer systems, the rice sector reached a high NUE and the livestock sector, NUE´s around international reported values.

Logos, Acknowledgments & References

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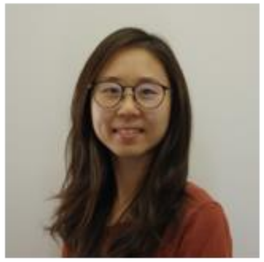
The influence of supplement source (organic vs. Inorganic) of selenium, copper, zinc, and manganese on micronutrient excretion and partitioning between urine and faeces in sheep



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ROTHAMSTED RESEARCH

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Research background

- Forage plays a key role in ruminant nutrition and the quality of animal-products. However, frequently forages lack sufficient micro-minerals for ruminant requirements.
- Direct mineral supplementation to the diet of ruminants is commonly adopted to improve ruminant nutrition.
- The excessive minerals are excreted mostly through urine and faeces, which contains higher concentrations of micronutrients than feed and is a major source of micronutrients in pasture system.
- The major factors influencing micronutrient flux in manured pasture systems are unclear.

We started from a sheep experiment

- Study factor: mineral supplement source and level
  - Forms: organic vs. inorganic of Se, Zn, Cu and Mn
  - Levels: 100% vs. 80% of level regulated by National Research Council of US (NRC)

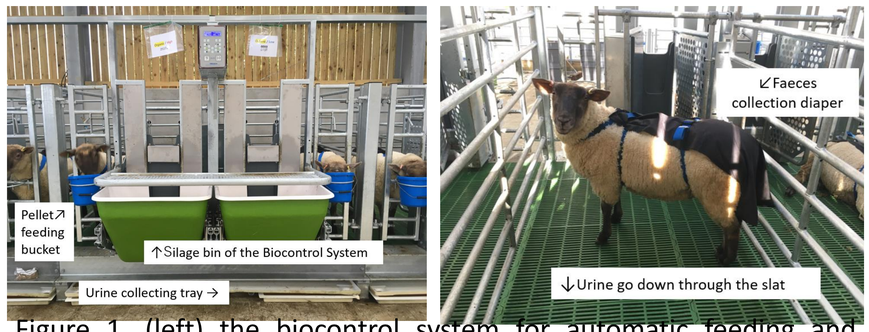

Figure 1. (left) the biocontrol system for automatic feeding and recording; (right) methods of collecting separated urine and faeces.

- Data collected:
  - ✓ Total excretion of urine and faeces
  - ✓ Macro- and micro nutrient concentrations in the feed, urine and faeces

Key findings

I. Within NRC levels, basal diet (nutrients not from supplements) is a major source of micronutrients, especially for Mn, and can dilute the effects of mineral supplements.

Table 1. Portions in total element intake of the micronutrients from different sources

Element sources	Se	Zn	Cu	Mn
Mineral supplements	53%-77%	47%-55%	42%-51%	16%-23%
Concentrate	19%-38%	28%-31%	25%-28%	17%-19%
Silage	4%-10%	17—23%	24%-31%	58%-66%

II. Both the level supplemented and form had no influence on partitioning of micronutrients between urine and faeces within the NRC supplementing level.

Table 2. Partitioning of micronutrients between urine and faeces

Treatment	Se (w/w %)		Zn (w/w %)		Cu (w/w %)		Mn (w/w %)	
	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
Inorganic-low	17.7	82.3	5.1	94.9	0.4	99.6	0.1	99.9
Inorganic-high	17.1	82.9	7.0	93.0	0.4	99.6	0.1	99.9
Organic-low	15.7	84.3	6.6	93.4	0.4	99.6	0.1	99.9
Organic-high	21.1	78.3	4.7	95.3	0.4	99.6	0.1	99.9
Form (F1) P-values	0.8279	0.2279	0.0906	0.4383	0.8279	0.2279	0.0906	0.4383
Level (F2) P-values	0.1674	0.4468	0.3579	0.7323	0.1674	0.4468	0.3579	0.7323
F1 x F2	0.2205	0.5464	0.9161	0.8558	0.2205	0.5464	0.9161	0.8558

III. The ratios of Se and Sulphur in urine and faeces were significantly different, which might influences Se availability to forages post excreta application in soil.

Table 3. Selenium and Sulphur content and ratio change in urine and faeces after supplementation

Treatment	Urine (conc./conc.)			Faeces (conc./conc.)		
	Day14/Day0		Day 14	Day14/Day0		Day 14
	Se	S	Se/S	Se	S	Se/S
Inorganic-low	1.33	3.19	1.47E-05	2.50	0.97	9.55E-05
Inorganic-high	1.77	2.72	2.48E-05	4.16	0.92	1.63E-04
Organic-low	1.14	2.91	1.55E-05	2.24	0.97	8.74E-05
Organic-high	0.96	1.61	2.05E-05	4.30	0.97	1.64E-04
F1 P-values	0.0088**	0.2095	0.3353	0.797	0.5327	0.2815
F2 P-values	0.4308	0.1154	**<0.001*** **	**<0.001*** **	0.634	**<0.001*** **
F1 x F2	0.0836	0.4508	0.1663	0.3875	0.6485	0.2413

ROTHAMSTED RESEARCH

Session Four
Tuesday, 23rd February 2021

Soil Spectroscopy and Crop Modelling for Precise Fertilizer Application in Morocco

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Addresses: [1]Cranfield University, Cranfield, UK; [2]Mohammed VI Polytechnic University, Ben Guerir, Morocco; [3]Rothamsted Research, Harpenden, UK.

METHODS

- Target crop: wheat under rainfed production,
- Auxiliary environmental variables that will be considered to explain the variations and uncertainties,
- Various machine learning algorithms and soil-crop modelling techniques will be studied once primary data are generated.

On-station NPK Experimentation at one site for two consecutive years to catch variability within a farm scale and evaluate level of precision of the new methods at field condition using crop response.

↕

Field wheat production assessment and employment of Hand-Held NIR to evaluate diverse soil-environmental condition at country level through representation of Mega- environments of wheat production.

↕

Pot experimentation for major soil types representing wheat growing areas in rainfed condition for in-depth study and precise evaluation of crop response.

↕

Developing frameworks for the best use of soil spectral library at field and Country levels.

Figure 2: Experimentation flow chart.

INTRODUCTION

Understanding the sensitivity and robustness of spectral analysis methods in the laboratory and in the field will improve applicability of spectral techniques for precise fertilizer management through soil-crop modelling. Soil spectral libraries can be developed at a scale using statistical models by optimizing the quantity, location and timing of the entries which could reduce chemical tests for calibration meanwhile improving the predictive power for new samples.

RESULTS

- Six soil types were identified for the pot experimentation which will be sampled from 5-6 sites differing in slope and agroecology.
- The spatial datasets deployed to identify these soils were Soil groups (FAO GeoNetwork Team), Infrared rainfall estimation (CHIRPS), crop coverage (African land cover viewer), and Digital elevation model of 30 m resolution (Earth Explore).
- These datasets were collated and processed using ArcGIS Pro software to get the spatial distribution and area coverage of the soil type.
- These soils include Calcic Kastanozems, Calcic Cambisols, Chromic Luvisols , Vertisols, Eutric Planosols and Luvic Phaozems which represent more than 85% of the areas where wheat is being grown in rainfed production in Morocco.

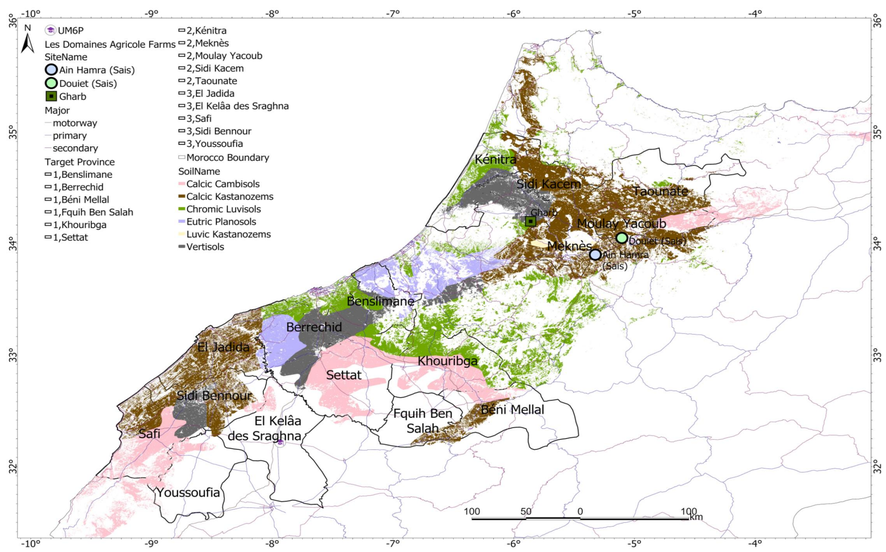

Figure 3: Spatial distribution of the major soil types.

Figure 1: Soil spectral analysis instrumentation at RRes.

CONCLUSIONS

- Soil spectral sensing techniques should be evaluated for their ultimate use in-field and with crop response.
- Developing soil spectral libraries could reduce the cost of chemical analysis for calibration and prediction of new soil samples

FUTURE WORK

Soil samples of the identified soils will be collected to carry out the pot experimentation and identify the possibility of existing and/or newly developed soil spectral libraries to predict soil properties of the samples at a scale.

Acknowledgements: The research project and studentship are financed by OCP Group via the Mohammed VI Polytechnic University, Morocco, under the overall project -'Next generation of agricultural research scientists for Africa'.

A Cereal Killer's Mask: What Triggers 3LysM Effector Expression in a Fungal Wheat Pathogen?

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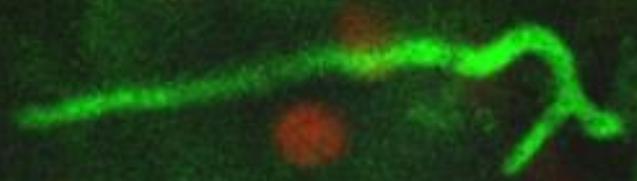
1, Rothamsted Research, Harpenden. 2, University of Nottingham



We investigated *3LysM* expression using *Z. tritici* strains expressing GFP under control of the *3LysM* promoter (3LysMP::GFP).

Expression of important *Zymoseptoria tritici* effector 3LysM may be regulated by:

1) A glycosyltransferase

- ΔGT2 and WT strains show differential GFP expression.

2) An upstream regulator

- GFP is affected by 3LysM promoter length.
- Adds evidence for an upstream regulator.

3) Nutrient availability

- GFP varies with nutrient availability/
- expression generally strongest in **plant-derived** and **weak** nutrient sources.
- Stronger expression in hyphae than spores.

4) Infection stage

- GFP upregulated as early as **24 hpi** on wheat leaves.
- 3LysM may be upregulated at specific infection stages.

Click numbered headings to find out more! -->

INTRODUCTION

Zymoseptoria tritici causes Septoria Leaf Blotch on wheat:
•Up to 50% yield loss •70% of EU fungicide usage

During initial asymptomatic phase, **3LysM** effector is secreted.

Chitin binding protein ← **3LysM** → Δ3LysM avirulent
3LysM → Prevents chitin-induced defence
3LysM → Regulated by GT2 glycosyltransferase?

Investigating 3LysM regulation and associated signalling pathways may enable inactivation of 3LysM, allowing plant chitin receptors to recognise *Z. tritici* for future control of Septoria Leaf Blotch.

FUTURE WORK:
- Investigate GFP expression during first 24 h of infection and at different fungal development stages
- Identify putative regulators of *3LysM* using Y1H and fungal mutagenesis
- Validate these results in knockout strains and explore orthologous regulators in other phytopathogens

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Kleg, K., Urban, M., Lauder, R., Hawkins, N., Evans, M., Plummer, A., Halsey, K., Lovegrove, A., Hammond-Kosack, K., Rudd, J. (2017), 'A conserved glycosyltransferase facilitates pathogenesis of plants by enabling hyphal growth on flat surfaces', PLOS Pathogens, 13 (10).
Marshall, R., Kombrink, A., Motteram, J., Loza-Reyes, E., Lucas, J., Hammond-Kosack, K., Thomma, B., Rudd, J. (2011), 'Analysis of two in planta expressed LysM effector homologs from the fungus Mycosphaerella graminicola reveals novel functional properties and varying contributions to virulence on wheat', Plant Physiology, 156 (2), pp. 756-769.

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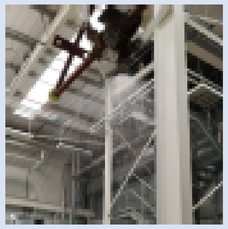
ROTHAMSTED RESEARCH
BBSRC Doctoral Training Partnerships
University of Nottingham

TECHNICAL AND PRACTICAL INNOVATIONS TO REDUCE SOIL AND WATER LOSSES BY IMPROVING SOIL PHYSICAL PROPERTIES

Authors: *Sophia Bahddou[1], Prof Wilfred Otten[1], Prof Jane Rickson[1], Dr Richard Whalley[2], Dr Ho-Chul Chin[2], Dr Mohamed El Gharous[3]*
Addresses: *[1]Cranfield University, Cranfield, UK; [2]Mohammed VI Polytechnic University, Ben Guerir, Morocco; [3]Rothamsted Research, Harpenden, UK*

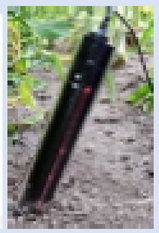
METHODS

✓ Review the different erosion processes, factors affecting soil erosion, erosion measurements and existing soil conservation techniques



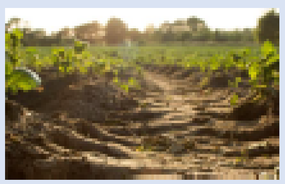
✓ Conduct laboratory-based experiments using the rainfall simulator to simulate erosion by water

✓ Develop and test an innovative system that integrates a wind tunnel and rainfall simulator to simulate the effects of soil erosion due to both wind and rainfall



✓ Study soil-water-crop interactions using tension infiltrometers and a mini-rhizotron camera to monitor changes in soil hydraulic and physical properties

✓ Test the soil-plant systems in a newly built facility to understand their impact on reducing wind and water erosion, and their resistance to extreme climate events.



✓ Conduct field trials in Morocco to test the hypotheses developed in the project under local climatic and soil conditions

INTRODUCTION

In Morocco, soil erosion is a serious agro-environmental threat considered as major constraint to plant and crop productivity. This project is directed at reducing soil and water losses in Morocco by developing novel methodological approaches and practical measures that will improve soil physical properties. This approach uses a better mechanistic understanding of the processes of erosion by water and wind, and their impacts on soil and water resources.

PROGRESS TO DATE

Soil preparation:

✓ Test 4 different types of soil surface roughness under the rainfall simulator to simulate water erosion

Rainfall simulator set-up:

✓Time taken for runoff generation (min)
✓Total Infiltration and runoff (ml)
✓Splashed material (g)
✓Eroded sediment (g)

Provisional results:

✓Sediment concentration is higher at the beginning of the rainfall event.
✓Random roughness generates more runoff and soil loss.
✓Oriented roughness across-slope doesn't always give better erosion control.

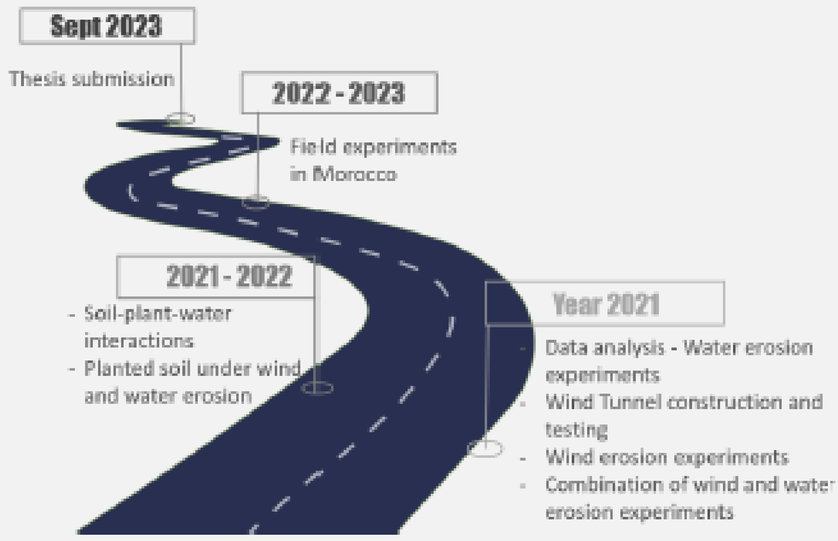
CONCLUSIONS (INTELLECTUAL CONTRIBUTION)

Give a clear vision of what needs to be developed in the experimental capabilities in soil erosion studies

Understand the erosion processes and the practical field based techniques to reduce soil and water losses

Improve environmental protection by focusing on both wind and water erosion, separately and combined

FUTURE PLAN



Acknowledgements: The research project and studentship are financed by OCP Group as part of the programme 'Next generation of agricultural research scientists for Africa'

Novel QTLs for High Dietary Fibre in the Watkins Landrace Collection

James A Brett, Noam Chayut, Simon Griffiths, Peter Shewry and Alison Lovegrove

Dietary Fibre (DF) has a number of recognized health benefits however few people eat the recommended daily minimum intake. Using the Watkins Landrace Collection this project aims to characterise novel genes and alleles for high fibre.

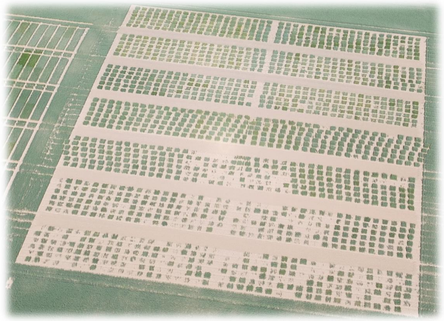
Introduction

Wheat is an important source of dietary fibre (DF) in the UK diet, with bread alone providing about 20% of the daily intake (Lockyer and Soiro, 2020). Almost all DF comes from plant sources with the majority of DF coming from plant cell walls. The main DF component of wheat grain cell walls is the pentose sugar, arabinoxylan (AX). With 70% of the UK population preferring to eat white bread we aim to increase the fibre content in wheat endosperm to deliver health benefits without changing eating habits and at no extra cost.

Paragon x Watkins mapping populations

- PxW 145
- PxW 471
- PxW 694
- PxW 007
- PxW 032

- 5 populations grown over 3 consecutive years.



2020 field trials

Phenotyping the lines

- Relative and specific viscosity of aqueous wholemeal flour preps for each line- a proxy for soluble arabinoxylan content

- **HPSEC-MALS** = intrinsic/specific viscosity
- **Capillary Viscometry**= relative viscosity.

- KU Leuven partners determine soluble-AX with colorimetric 'pentosan' assay

ROTHAMSTED RESEARCH

John Innes Centre
Unlocking Nature's Diversity

DFW

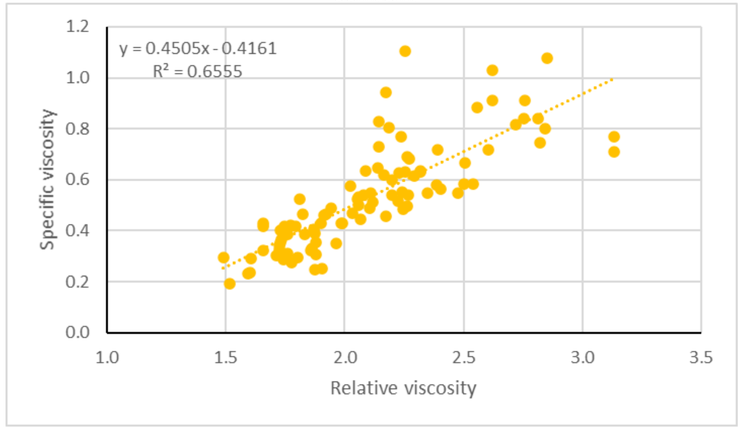
KU LEUVEN

FF FLANDERS' FOOD

UKRI Biotechnology and Biological Sciences Research Council

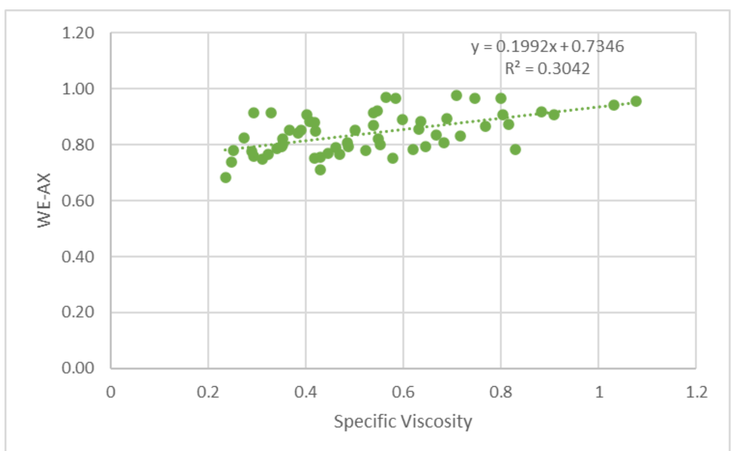
Correlating specific viscosity with relative viscosity

96 randomly selected lines were screened for soluble AX content



Specific/intrinsic viscosity (HPSEC-MALS) against relative viscosity (Capillary viscometry)

Correlating specific viscosity with WE-pentosans



WE-pentosan data from Leuven, plotted against specific viscosity

CONCLUSIONS

- Strong positive correlation between relative viscosity and specific viscosity

  -HPSEC-MALS suitable for screening large number of lines for AX.

- A weak, but positive correlation between WE-pentosan and specific viscosity.

  -These methods measure different but related parameters, which could result if different QTL

  - This could provide novel and different QTLs for the high DF trait.

FUTURE WORK

- All lines will be screened using HPSEC-MALS and pentosan methods.

- Phenotypic data input into R/qtl and will perform QTL analysis using Axiom 35k marker data for all PxW populations in this study.

- Resulting QTLs will be refined using exome capture data.

- The QTLs will be further interrogated for causal genes or alleles within these regions.

Funded by Flanders' Food as part of the FIBRAXFUN project

PLANT-BASED VACCINES FOR PREVENTION OF POULTRY VIRAL DISEASES: NEWCASTLE DISEASE

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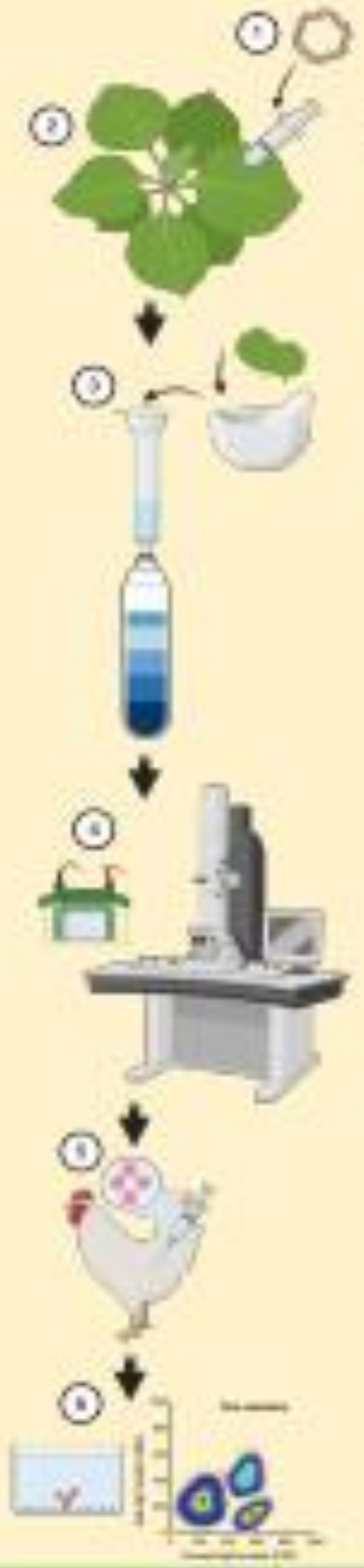
BACKGROUND AND AIMS

- The development of plant virus-based transient expression systems provides a robust tool to generate a high yield of recombinant proteins with superior speed while managing to achieve cost-efficient production.[1]
- Newcastle disease (ND) affects economic livelihoods by causing major severe losses in poultry industries and poses a risk to global food security.[2]
- The current prevention of Newcastle disease virus (NDV) infections based on live-attenuated and inactive vaccines are deemed adequate due to the constantly evolving strains of NDV .[3]
- VLPs are novel vaccine candidate that structurally resembled to whole virus vaccines but lack of genetic materials and reported to induce strong humoral as well as cellular immune responses.[4]
- This study will test a possibility to develop plant based-VLP as a vaccine candidate against NDV infections.
- NDV VLP will be assembled from F (fusion), HN (haemagglutinin-neuraminidase), and M (matrix) proteins originated from velogenic ZJ1 strain.

EXPERIMENTAL METHOD



1. pEAQ-HT carrying NDV F, HN, or M genes expression cassettes
2. Agroinfiltration
   a. Optimise the *Agrobacterium* strain for agroinfiltration
   b. Evaluate NDV protein expression in *N. benthamiana*
   c. Production of NDV VLP
3. NDV VLP purification based on sucrose gradient centrifugation and ion exchange/affinity chromatography
4. Protein characterisation and VLP visualisation
5. Chicken immunization with purified NDV VLP
6. Efficacy and immunological assay of NDV VLP

Created with BioRender.com

RESULTS

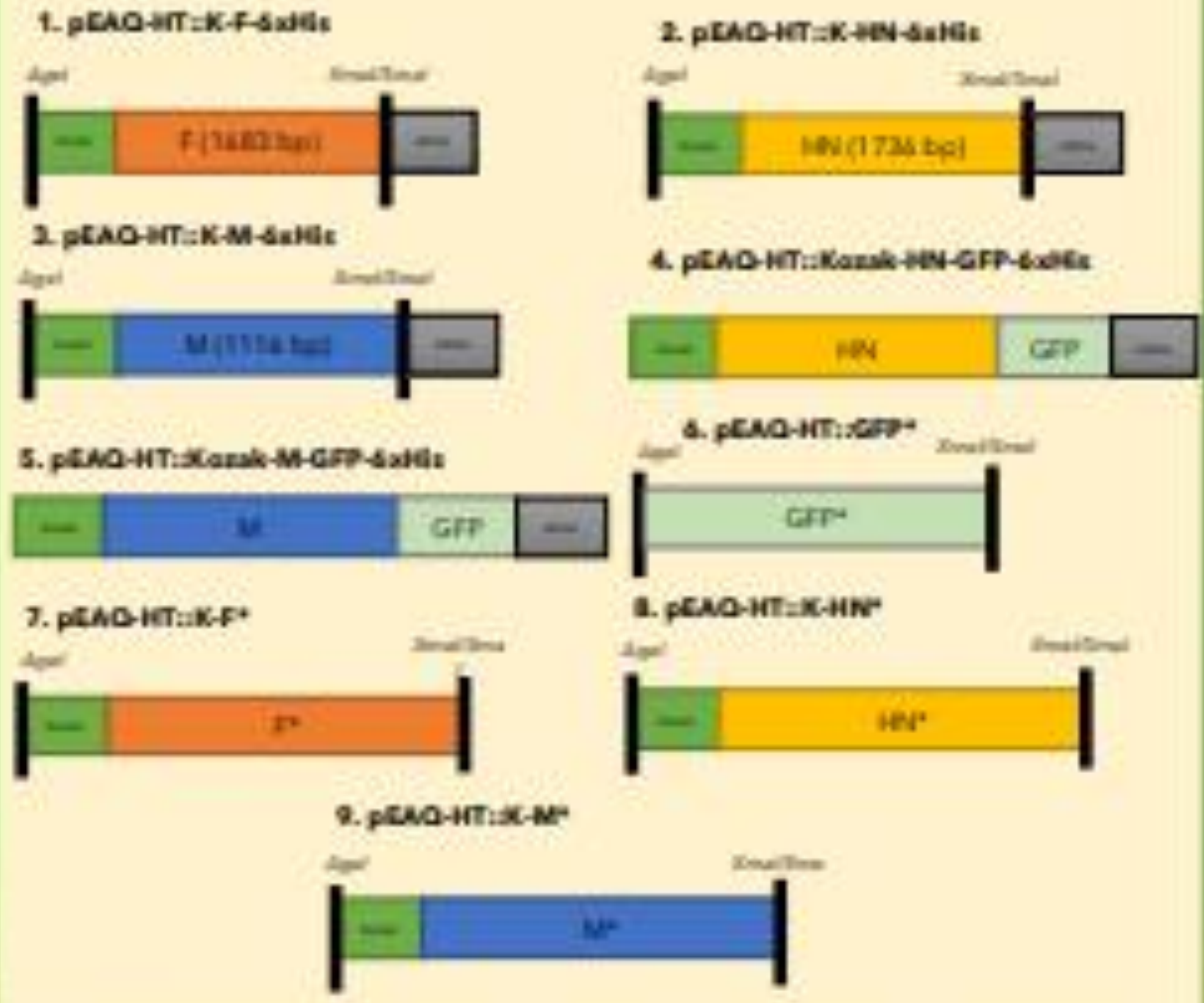


Figure 1. Schematic illustration of the cassettes for heterologous protein expression that have been successfully constructed using Gibson Assembly and restriction enzyme-mediated cloning in the pEAQ-HT vector background.
The DNA fragments are drawn not to scale.

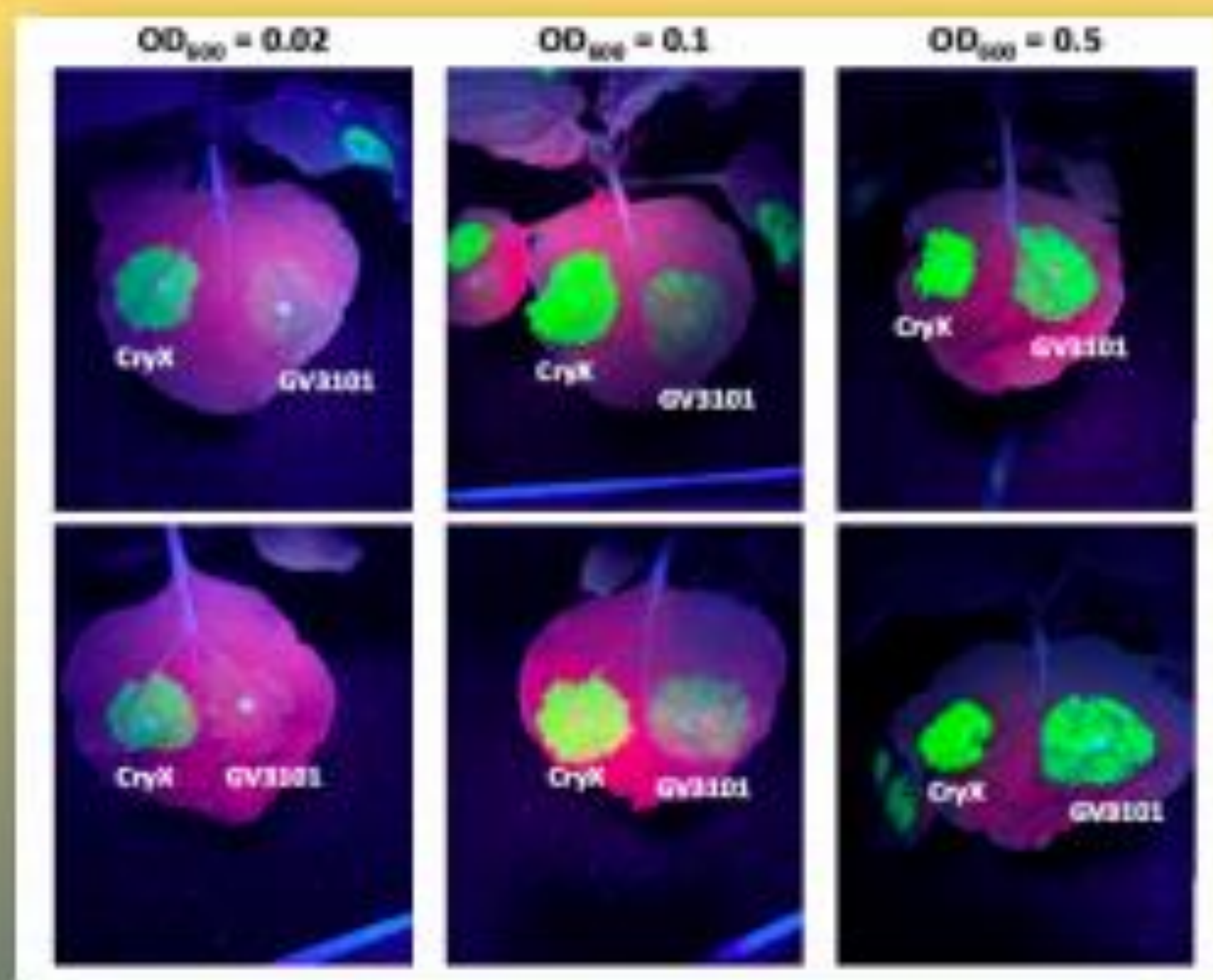


Figure 2. GFP expression after *N. benthamiana* agroinfiltration using *A. tumefaciens* NMX021 and GV3101 strains at 4 dpi. The GFP expression was observed in the two biological replicates starting from lowest OD_{600} 0.02, 0.1, and 0.5

RESULTS

- Nine vectors for the expression of NDV proteins have been successfully developed and transformed into *A. tumefaciens* NMX021 (Figure 1).
- The *A. tumefaciens* strain NMX021 is more efficient in delivering GFP to *N. benthamiana* compared with the *A. tumefaciens* strain GV3101 (pMP90) (Figure 2), and will be opted to deliver transgene in the future experiments.

ANTICIPATED OUTCOMES

- This study will be the pioneer to test a possibility of plant transient expression system for the production of VLPs as a vaccine candidate.
- This study will attempt to provide a rational design and optimisation of downstream processing to ensure high purity, recovery, and consistency of plant based-VLPs.
- Additionally, the production of cost-efficient plant based NDV VLPs will be an alternative strategy to combat the evolving strains of NDV and ameliorate the disease preparedness in developing countries.

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[2]Getabalew, M., Alemneh, T., Akeberegn, D., Getahun, D., & Zewdie, D. (2019). Epidemiology, diagnosis and prevention of Newcastle disease in poultry. *American Journal of Biomedical Science and Research*, 3(1): 50–59. https://doi.org/10.34297/AJBSR.2019.03.000632
[3]Dimitrov, K. M., Afonso, C. L., Yu, Q., & Miller, P. J. (2017). Newcastle disease vaccines—A solved problem or a continuous challenge? *Veterinary Microbiology*, 206: 126–136. https://doi.org/10.1016/j.vetmic.2016.12.019
[4]McGinnes, L. W., & Morrison, T. G. (2013). Newcastle disease virus-like particles: preparation, purification, quantification, and incorporation of foreign glycoproteins. *Current Protocols in Microbiology*, 30(1): Unit- 18.2. https://doi.org/10.1002/9780471729259.mc1802s30

ROTHAMSTED RESEARCH

Session Five
Wednesday, 24th February 2021

NOVEL BRASSINOSTEROID DWARFING GENES AS ALTERNATIVES FOR IMPROVING WHEAT GRAIN YIELDS

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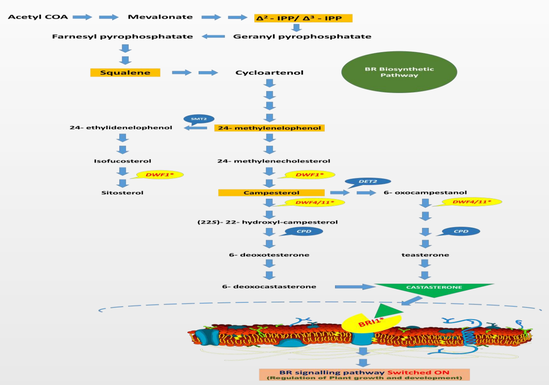
Introduction

- Yield is an outcome of the plant's capacity to capture light energy and utilize it to fix carbon dioxide into complex organic compounds.
- Smarter canopies with upright leaves on upper stem nodes, less erect on the medium and horizontal on lower nodes are more radiation use efficient (RUE).
- Leaf angle in cereals is a hormonally regulated architectural trait which is determined by the differential cell size of the collar region.
- Brassinosteroids are steroidal hormones which are extensively reported as key regulators of leaf angle in various cereal crops (Sakamoto *et al* 2006).
- Studies in rice and barley have demonstrated that lesions in the BR biosynthetic and signalling pathway result in a more erect stature that can produce improvements in grain yields (Sakamoto *et al* 2006 and Dockter *et al* 2014).
- We are trying to replicate these effects in wheat by targeting various genes in the BR pathway (as shown in Figure 1).

Figure 1: Shows the reduction of leaf angle in TaDWF1 triple mutant (plate A) as compared to Cadenza (plate B)

BR biosynthetic pathway



Castasterone is produced at the end of pathway which is the required by *BRI1* to initiate BR signalling pathway in wheat. This ultimately results in developmental changes.

Objectives

We are targeting BR pathway to develop new alleles conferring a more erect architecture that could potentially improve yields. Two strategies are being employed to develop mutants in BR biosynthetic and signalling pathway as follows:

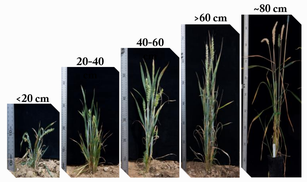
1. A field based screen to identity *TaBRI1* alleles that alter wheat architecture.
2. TILLING to identify novel BR mutants with increased leaf erectness.

A field based screen to identify novel *TaBRI1* alleles

Hexaploid wheat contains three homoeologous *BRI1* genes that are functionally redundant. We have demonstrated that *tabri1* double mutants are phenotypically comparable to Cadenza whereas the triple mutant is severely dwarfed. To identify novel *tabri1* dwarfing alleles that alter canopy architecture, we are taking advantage of the double tabri1bd mutant and conducting EMS mutagenesis, to identify mutations in the TaBRI1A gene. It is also likely that other genetic loci controlling this trait will also be identified in this screen.

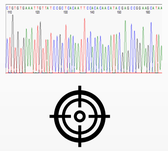
tabri1bd mutant displays a wild type phenotype and was subjected to EMS treatment.

M1 generation was sowed in the field in 12 independent plots.

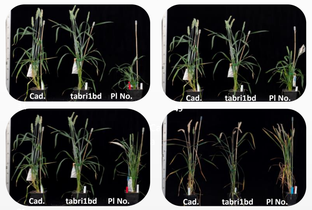


M2 individuals selected in field were classified into 4 classes based on final plant height (i.e. <20, 20-40, 40-60 and >60 cm) and were scored visually for altered leaf angle. Approximately 450 out of 600,000 M2 individuals were shortlisted from the field.

Followed by backcrossing to reduce genetic noise and characterise individuals at BC2F2 generation



Sequencing of *TaBRI1* gene needs to be done for novel SNP discovery



M3 individuals were sown in the glasshouse and the leaf angle was measured with the aid of protractor. Lines having angle less than that of Cadenza were shortlisted.

Results

- 450 M2 individuals were selected from the field having altered leaf angle (scored visually) and plant height and were grouped in 4 classes.
- Furthermore, a subset of 74 M3 individuals (from 40-60 and >60cm class) were sown in duplicates. 13 lines displaying reduced leaf angle (recorded at anthesis stage with protractor) were identified.
- These shortlisted lines were re-sowed in 2020 in glasshouse in randomised complete block design with 8 biological replicates (figure 2).

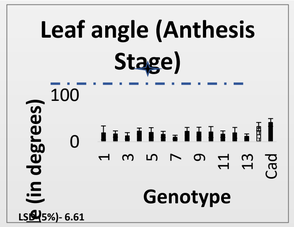


Figure 2: Describes that there are significant differences in the leaf angle in the shortlisted M4 lines w.r.t the controls.

Future work

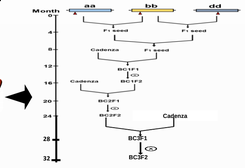
1. We will perform a genetic analysis on BC1F2 plants to establish if the mutations are recessive or dominant.
2. BR response assays will be conducted to establish if the mutations affect this signalling pathway.
3. Targeted sequencing of the *TaBRI1A* gene will be performed.
4. Other strategies, including exome capture will be used to identify mutations at other loci.

TILLING to identify novel BR mutants with increased leaf erectness

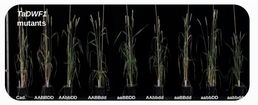
We intend to characterize *TaBRI1*, *TaDWF1* and *TaDWF4* genes. Loss-of-function mutations in these BR genes in rice and barley (Sakamoto et al 2006 and Dockter et al 2014) were used successfully to tailor crop canopy architecture. We aim to identify these in wheat, produce and characterise mutants. Cadenza TILLING lines developed by Krasileva et al 2017 will be exploited to identify deleterious mutations in these genes followed by backcrossing and phenotypic characterization.

Identification of orthologues of *BRI1* and *DWF1* genes in wheat followed by construction of phylogenetic trees to study relatedness to other species

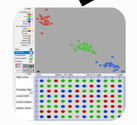
Identification of the deleterious mutations (marked in red) in the Cadenza TILLING population using Ensembl wheat database



Followed by intercrossing between identified mutant lines to stack the mutations and initiate backcossing



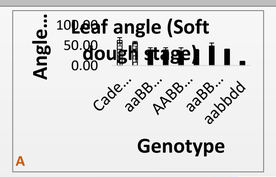
Various combinatorial mutants of TaBRI1 and TaDWF1 at BC2F2 generation. Very high level of functional redundancy is observed in these genes

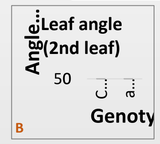


We then design KASP primers for high-thoughput genotyping of BC2F2 generation.

Results

TaDWF1





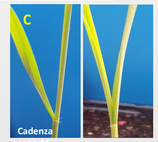


Plate A shows leaf angle (at soft dough stage) recorded in the BC2F2 generation. There is approximately 80% reduction in the leaf angle in *dwf1abd* as compared to Cadenza. In contrast at the seedling stage a 50% reduction in the leaf angle is observed in triple mutant as compared to Cadenza (B and C). This confirms the effectiveness of these genes in controlling leaf angle architecture.

TaBRI1

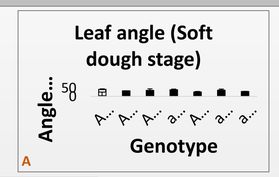


Plate A shows leaf angle (at soft dough stage) recorded at BC2F2 generation. We don't observe a substantial reduction in the leaf angle in various combinatorial mutants but the triple mutant is severely affected (as seen in plate C). Additionally we observe very high levels of Castasterone accumulates in this mutant as compared to wild-type indicating there is blockage in BR signalling (as shown in B).

Future work

1. We will quantify the anatomical differences (if any) in the dwf1abd mutant compared to Cadenza in the lamina joint (as it is primarily involved in determining leaf angle).
2. As there is reduction in leaf angle, we will conduct field trials with selected mutants at different planting densities to assess if thy can be planted at higher densities to improve yields.

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Smart Detection of Airborne Diseases During Tomato Production

ROTHAMSTED RESEARCH

INTRODUCTION

The UK produces approximately 92,000 metric tonnes of tomatoes per year.
The industry is plagued with recurring fungal infections of powdery mildew (*Pseudoidium neolycopersici*).
To prevent loss of yields, the plants are treated prophylactically, as well as when fungal infections are detected, with crop protection product. This can be costly.
The aim of this project is to be able to detect pathogens before they infect the plant. The model organism used in this study is *P. neolycopersici*.

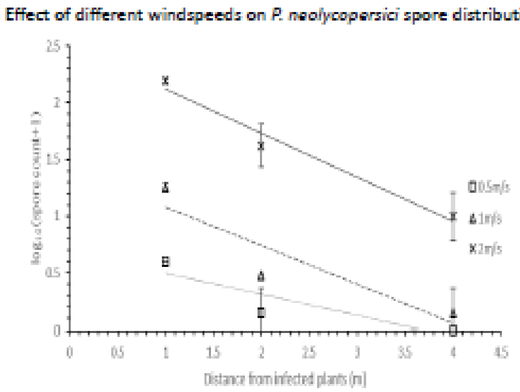
KEY FINDINGS DURING THE PHD

1. *P. neolycopersici* travels further than 4m with wind speeds of 2m/s and get dislodged at 0.5m/s wind speeds.
2. Dispersal of spores is directly related to how many spores are released.
3. Developed a loop-mediated isothermal amplification (LAMP) assay to detect *P. neolycopersici*
4. Spore traps closer to the source of inoculum detect spores faster and before visible symptoms spread to other plants
5. Spore traps at lower levels detect spore traps faster than spore traps at the top of the glasshouse

DISPERSAL OF *P. neolycopersici*

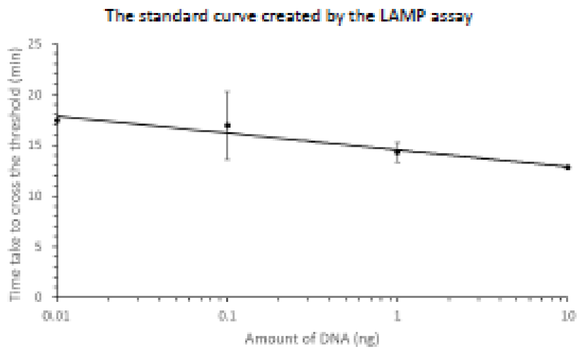
P. neolycopersici is dispersed by wind.

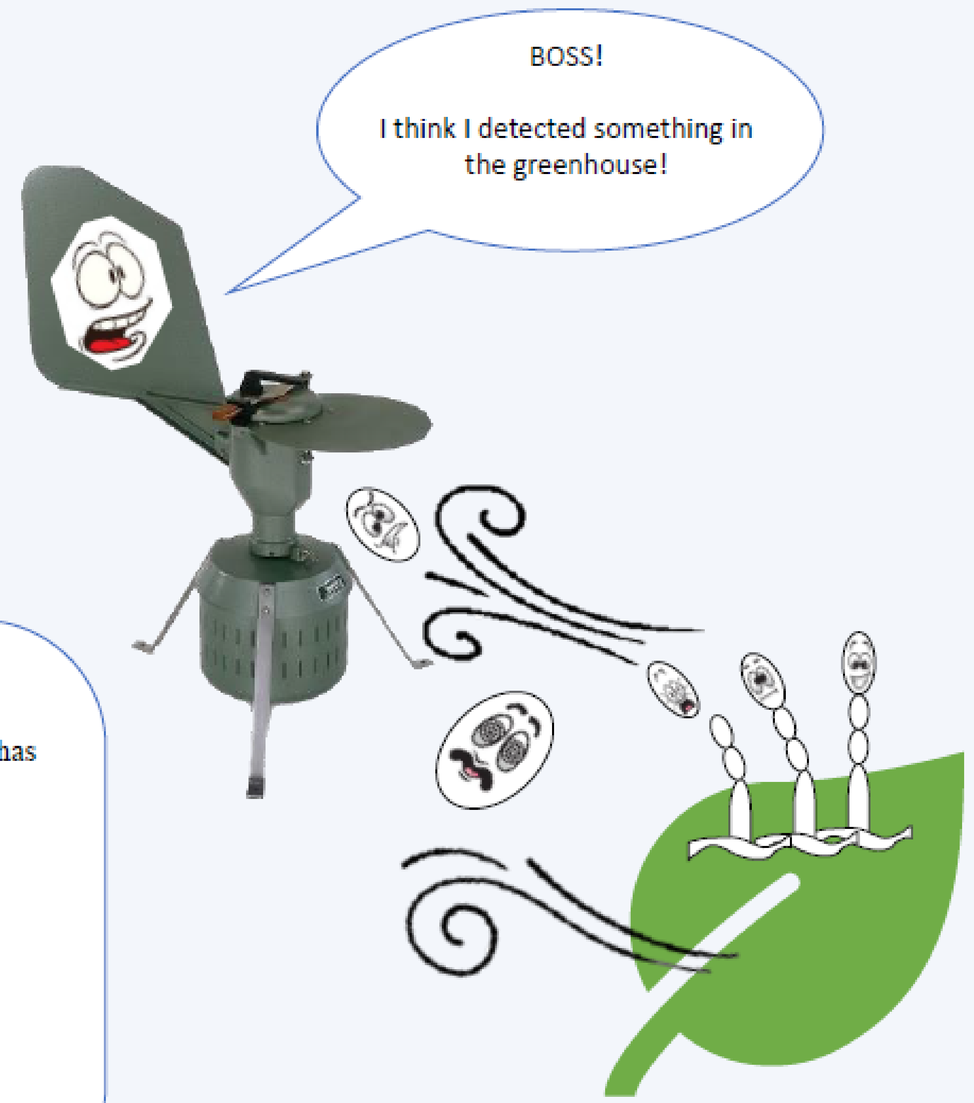
An experiment in the wind tunnel was conducted too see what windspeeds are required to dislodge *P. neolycopersici* from infected tomato plants.



MOLECULAR DETECTION METHOD

A LAMP (Loop-mediated isothermal amplification) assay has been developed that targets actin.

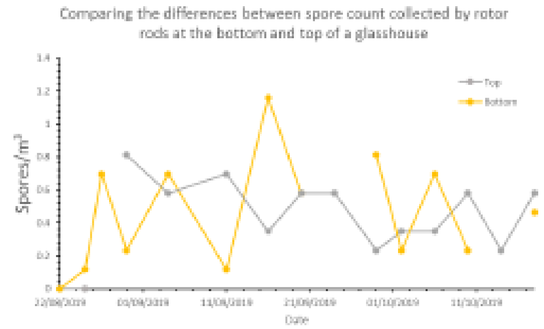


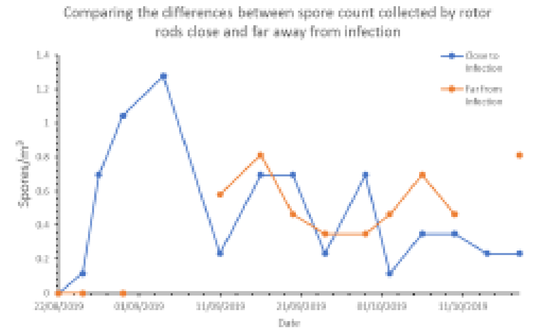


GREENHOUSE EXPERIMENTS

The spore traps sample the airborne particles onto sticky surfaces over a short/long period of time or over a long period of time.

Conducted a pilot study to understand whether spore trap placement will have an effect on the speed at which we detect *P. neolycopersici* in a glasshouse.





ACKNOWLEDGMENTS

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TRANSCRIPTOMIC DATA AND PHYTOHORMONE ANALYSIS PROVIDE INSIGHTS INTO WHEAT TILLERING CONTROL IN RESPONSE TO NITROGEN SUPPLY

PETROS P. SIGALAS[1], PETER BUCHNER[1], STEPHEN G. THOMAS[1], MALCOLM J. BENNETT[2], FRANK JAMOIS[3], MUSTAPHA ARKOUN[3], JEAN-CLAUDE YVIN[3] AND MALCOLM J. HAWKESFORD[1]

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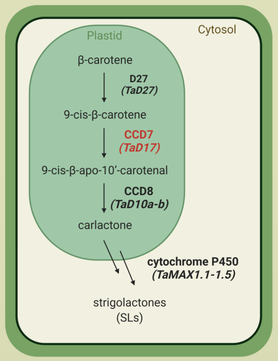
Introduction

- The main responses of plants to nutrient-deficient conditions include changes in root and shoot architecture.
- Above ground plant architecture is highly influenced by changing tillering patterns.
- Tillering is known to be regulated by the interaction between 3 classes of phytohormones, auxin, cytokinin (CK) and strigolactones (SLs).
- It is well established that low P triggers SL production leading to tillering suppression. Similarly, SLs are required for tiller suppression under low N.
- Even though N is the main limiting nutrient that affects cereal growth and productivity, there is limited information about the molecular mechanism of tillering control by N levels.

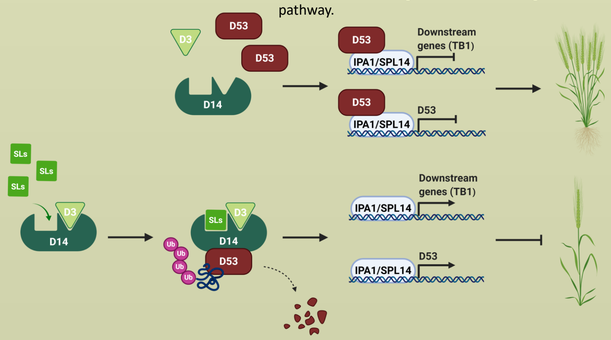


SL biosynthesis (Left)

SLs are carotenoid cleavage products. Firstly, DWARF27 (D27) catalyses the conversion of all-trans-β-carotene into 9-cis-β-carotene. Subsequently, CCD7/D17 and CCD8/D10 are working in concert to produce carlactone, the precursor of bio-active SLs. The conversion of carlactone to bio-active SLs is catalysed by a cytochrome P450 encoded by *MAX1*. Monocots have multiple *MAX1* paralogues involved in different steps of carlactone conversion to orobanchol.

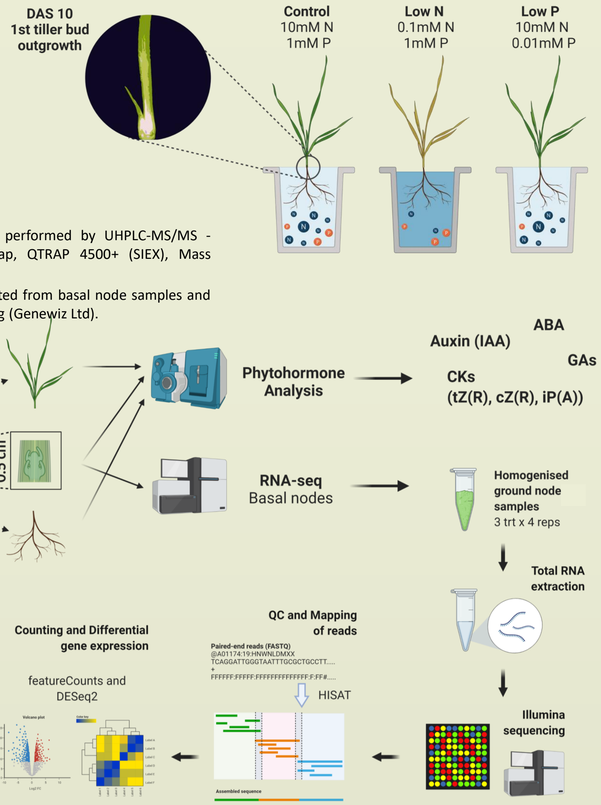
SL signalling (Below)

In presence of SLs, D14 receptor binds SLs triggering a conformational change which allows D14 to form a complex with D3. D14/D3 complex leads to the ubiquitination and degradation of D53. D53 is the main repressor of SL signalling pathway which could physically interact with SPL14 and suppresses the transcription of downstream genes. TB1 has been shown to be among the downstream genes of this pathway.



Material and Methods

- 10 days old hydroponically grown *T.aestivum* var Cadenza plants were subjected to N or P limitation for 8 days.
- For nutrient limitation, plants were supplied with 1% of the control conc. of N or P.
- 8 days after nutrient limitation, root, shoot and basal nodes were collected for phytohormonal and transcriptomic analysis (n=4).
- The phytohormonal analysis was performed by UHPLC-MS/MS - Triple Quadrupole Linear Ion Trap, QTRAP 4500+ (SIEX), Mass Spectrometer.
- For RNA-seq, total RNA was extracted from basal node samples and sequenced with Illumina sequencing (Genewiz Ltd).

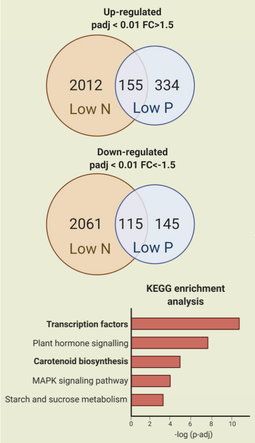


RNA-seq data analysis

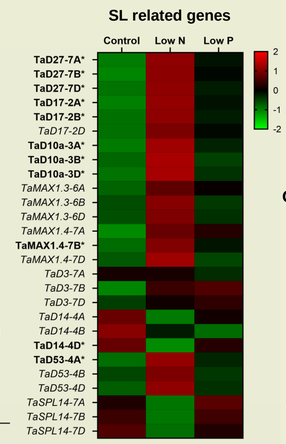
- On average each sample had 39M paired-end reads. **HISAT** tool was used for mapping reads to the RefSeqv1.0 with overall alignment rate of 85%.
- Mapped reads were assigned to genes with **featureCounts** (avg. 23.2M) based on RefSeqv1.1. Pseudo-alignment was also performed with **kallisto 0.46.0.4** for calculating the number of transcripts per million reads (TPM).
- Differential expression analysis was performed with **DESeq2** tool.
- **g.Profiler** (https://biit.cs.ut.ee/gprofiler/gost) was used for enrichment analysis.

Illustrations were created with BioRender.com

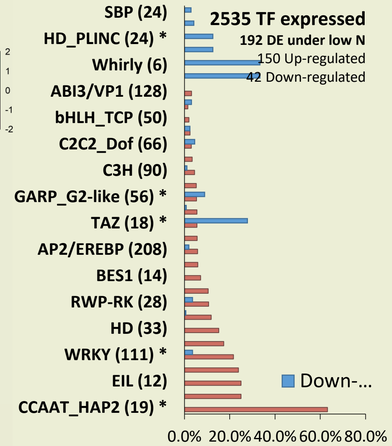
Results



Top 5 significantly enriched **up-regulated** KEGG in N deficient nodes. Enrichment analysis was performed using a custom build KEGG reference containing more than 46000 genes.



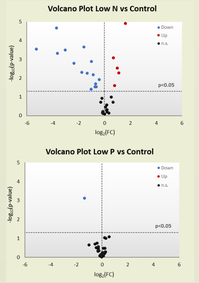
Heatmap comparison of SL-related gene expression in basal nodes under Low N and Low P. Data are mean Z-scores of TPM from 4 replicates. Significantly DE genes are in bold and with asterisks.

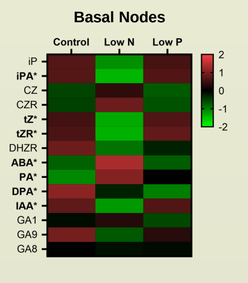


Percentage of expressed TF that were significantly DE in N deficient nodes per TF family. The number of TF expressed (TPM>0.5) per family is in brackets. Only families with significantly DE members are included. Significantly enriched families are indicated with asterisks.

Discussion

- N limitation had a stronger effect on both transcriptomic and metabolic changes. This also supported by the developmental changes recorded under low N (data not shown).
- Down-regulated genes under low N are related to housekeeping functions and metabolic processes, whereas upregulated genes are involved in signalling pathways and transcription regulation.
- The phytohormonal analysis suggested that most of the changes occurred in low N nodes. Decreased CKs and IAA content, whereas ABA conc. found significantly higher.
- As highlighted by RNA-seq, (apo)-carotenoid biosynthesis (SLs, ABA) is among the top upregulated KEEG and GO enriched terms (GO:0016106).
- The identification of TF families significantly affected by low N and their relation with TB1 provide candidate genes for the molecular regulation of tillering.
- **Future work** - The *d17* triple knock-out mutant generated using TILLING mutant lines as part of the project will be an important tool for further functional studies.





(Left) Volcano plot Low N vs Control and Low P vs Control of UHPLC-MS/MS targeted phytohormones in all tissue with p-value threshold (y) 0.05. **(Right)** Heatmap comparison of phytohormone levels in basal nodes under Low N and Low P. Data are mean Z-scores of the absolute conc. from 4 replicates.

Abbreviations: *isopentenyl adenine (iP), isopentenyl adenosine (iPA), trans-zeatin (tZ), cis-zeatin (cZ), dihydro-zeatin (DHZ) and their riboside (-R), indole-3-acetic acid (IAA), abscisic acid (ABA), phaseic acid (PA), dihydrophaseic acid (DPA) and gibberellin (GA).*

ROTHAMSTED RESEARCH

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The University of Nottingham

CHARACTERISATION OF MAJOR GENES MEDIATING RESISTANCE TO SEPTORIA TRITICI BLOTCH DISEASE IN WHEAT

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Zymoseptoria tritici is a highly destructive wheat pathogen that can cause crop losses of up to 50%. Traditionally *Z. tritici* has been controlled with resistance (*Stb*) genes and fungicides, but the high selection pressure results in these protections being broken. New diverse sources of resistance are needed.

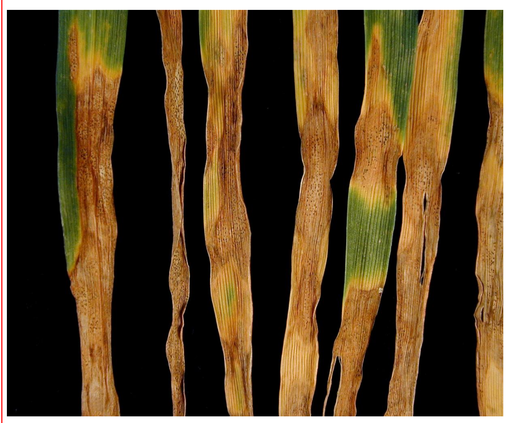

Figure 1: Symptoms of severe Septoria tritici blotch infection. Necrosis and black pycnidia (enabling fungal reproduction) are visible.

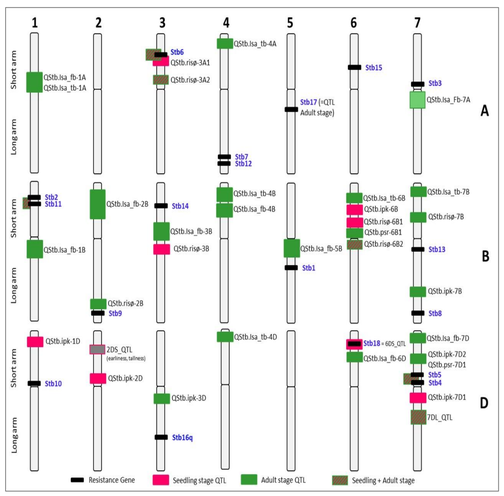

Figure 2: Chromosomal locations of *Stb* genes and resistance QTLs. More than 20 *Stb* genes are known.

RESISTANCE SCREENING

- Most tested wheat genotypes show greater resistance than known susceptible cultivars Riband, Taichung 29 and KWS Cashel.
- Synthetic lines and lines with multiple resistance genes have high resistance levels, suggesting that pyramiding *Stb* genes (particularly those derived from wild relatives) could be an effective crop protection strategy.

Wheat Line	Known Resistance Genes	Number of Isolates Tested	Average Days from Inoculation to Chlorosis/Necrosis Development	Average Days from Inoculation to Pycnidia Development	Average Leaf Coverage of Chlorosis/Necrosis (%)	Average Leaf Coverage of Pycnidia (%)	Average Spores Produced (X106 per leaf)
Taichung 29	None known	78	12.9	16.5	97.2	18.0	2.16
Riband	*Stb15*	90	14.2	17.8	78.8	21.4	2.88
KWS Cashel	None known	89	14.8	17.5	82.4	32.5	3.50
Estanzuella Federal	*Stb7*	69	14.1	19.1	87.0	8.7	1.39
Israel 493	*Stb3, Stb6*	77	13.5	22.7	62.9	0.8	0.46
TE9111	*Stb6, Stb7, Stb11*	89	17.8	20.7	37.8	0.7	0.34
Synthetic 6X	*Stb5*	68	15.3	25.3	50.7	0.9	0.22
Synthetic M3	*Stb16q, Stb17*	40	15.4	N/a	38.4	0.0	0.14
Kavkaz-K4500	*Stb6, Stb7, Stb10, Stb12*	61	17.6	N/a	21.3	0.3	0.25
Tadinia	*Stb4, Stb6*	69	17.1	19.5	54.8	9.0	0.69
Balance	*Stb6, Stb18*	44	16.5	22.8	61.2	2.5	0.30
Synthetic M6	*Stb8*	37	16.6	23.0	51.4	8.8	0.56
Bulgaria 88	*Stb1, Stb6*	36	16.8	23.5	47.3	2.2	0.30
Veranopolis	*Stb2, Stb6*	35	16.2	24.4	42.9	4.2	0.54
Tonic	*Stb9*	27	15.2	21.6	76.6	12.6	1.04
Salamouni	*Stb6, Stb13, Stb14*	31	17.3	22.8	49.9	3.5	0.45
Lorikeet	*(Stb6), Stb19*	31	16.8	N/a	27.4	0.0	0.31

Table 1: The symptoms caused by *Z. tritici* isolates on wheat seedlings containing different *Stb* genes. Green cells indicate a more resistant phenotype, red cells indicate a more susceptible phenotype.

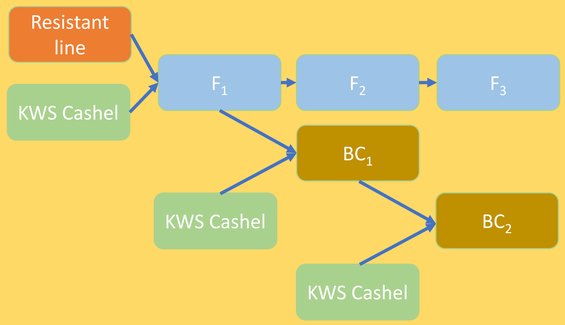

Figure 3: The breeding scheme used to produce mapping populations.

KASP MARKER DEVELOPMENT

- Backcrossed resistant lines with KWS Cashel.
- F_3 mapping populations phenotyped and genotyped to analyse KASP marker reliability.
- In the Estanzuela Federal × KWS Cashel F_3 population, one of eight markers tested was significantly associated with the time taken for initial pycnidia development.
- Lack of linkage for other markers due to difficulty differentiating between parental phenotypes.

Phenotype Measured	P for Correlation with Allele Reported by AX-95175098	P for Correlation with Allele Reported by AX-94780124	P for Correlation with Allele Reported by AX-94918531	P for Correlation with Allele Reported by AX-94475129	P for Correlation with Allele Reported by AX-94980296
Days to Chlorosis/ Necrosis development	0.476	0.581	0.309	0.105	0.854
Final % Chlorosis/ Necrosis coverage	0.971	0.810	0.704	0.998	0.999
Days to Pycnidia development	0.048	0.351	0.743	0.095	1.000
Final % Pycnidia coverage	0.192	0.148	0.567	0.104	0.463
Spores per leaf	0.791	0.807	0.717	0.882	0.999

Table 2: The probability of each functioning marker being correlated with each measured symptom in the Estanzuela Federal × KWS Cashel F_3 population

WIDER RESEARCH APPLICATIONS

- KASP markers will be directly useful in breeding for STB resistances.
- Knowledge of field effectiveness of each *Stb* resistance gene will help target breeding efforts to the most useful.
- Will develop a list of *Stb* resistance genes effective against UK *Z. tritici* isolates. The most effective so far are *Stb3*, *Stb5*, *Sth16q/17*, and *Stb19*.
- Using VIGS to test candidates for the *Stb19* and *Snn3* resistance genes. Identifying these could help develop our understanding of resistance mechanisms.

Acknowledgments.

Thanks to Bart Fraaije for providing the Septoria isolates utilised in the resistance screens, and to RAGT Seeds for providing mapping populations and genotyping data.

References

Saintenac, C., Lee, W.-S., Cambon, F., Rudd, J. J., King, R. C., Marande, W., … Kanyuka, K. (2018). Wheat receptor-kinase-like protein Stb6 controls gene-for-gene resistance to fungal pathogen *Zymoseptoria tritici*. Nature Genetics, 50: 368–374.

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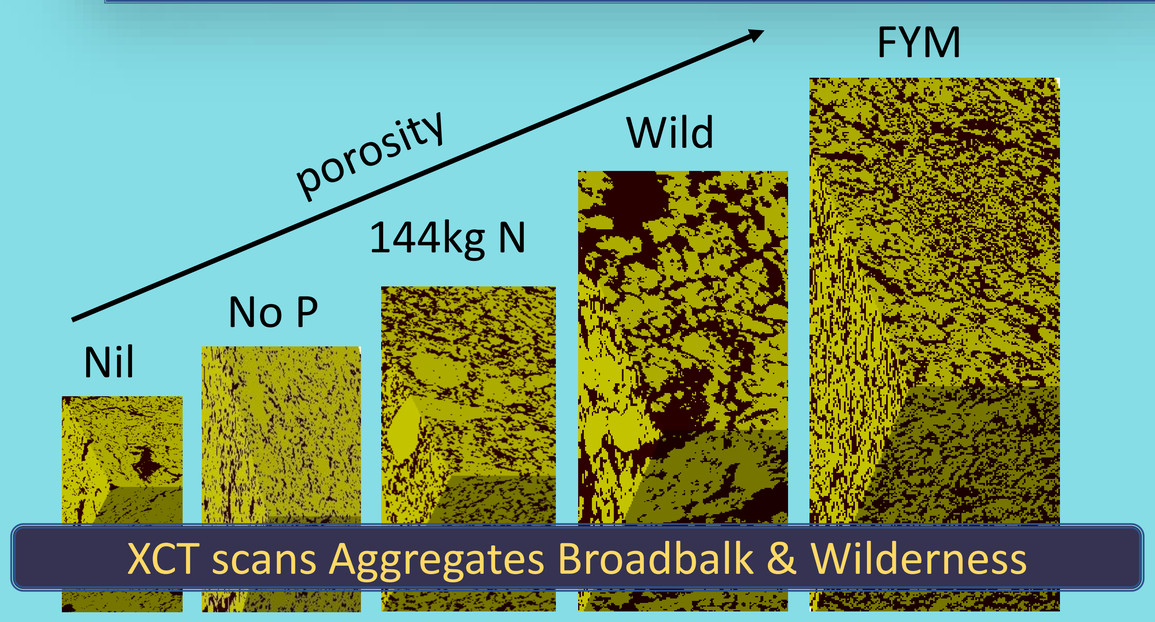
Session Six Wednesday, 24th February 2021

William Rickard

The Why of Soil Measurement

What or Why

There are many parameters of soil that can be measured. Soil health requires a measurement that is based on theory that works for all soil types and can track temporal changes. Soil is a complex system of hierarchal processes, the interactions between microbial activity, the abiotic and land management are important in the creation of soil structure. This structure directly relates to soil functions associated with soil health.



XCT scans Aggregates Broadbalk & Wilderness

Connected Porosity

X-ray computed tomography shows that soils with better connected pore networks have a greater capacity to transport air, water, nutrients and genes throughout the soil system.

Can tension infiltration, soil moisture probes and soil water release characteristics be used to directly measure or infer connected porosity at the aggregate scale?

Soil Hydraulic Properties

Practical ways of measuring connected porosity?

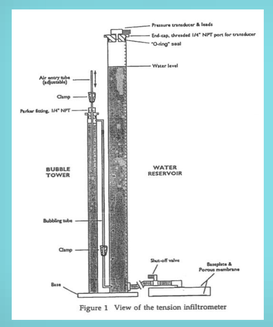


Figure 1 View of the tension infiltrometer

Soil Health Metric for Sustainable Agricultural Systems.

Munisath Khandoker, Stephan Haefele, Andy Gregory & Nick Ostle; Rothamsted Research & Lancaster University.

Soil is the foundation of all agricultural systems. In addition to agricultural production, soils provide other important functions often referred to as ecosystem services. The ability of the soil to carry out these functions is commonly referred to as '**soil health**'. (Figure 1).

Ecosystem Services:

- Medium for roots to grow
- Buffer pollutants and contaminants
- Support biodiversity
- Store and transmit water
- Store carbon

Soil Health = Ability to carry out these functions.

Figure 1: List of ecosystem services; ability to carry out these functions known as soil health.

Why are Soil Health Metrics Needed?

Achieve '**sustainable intensification**' - increasing production whilst reducing environmental footprints.

UN Sustainable Development Goal 2: 'End hunger... and **promote sustainable agriculture**'.

To achieve this, metrics for soil health are urgently needed. This is especially important in light of food security and climate change.

Project Aim:

Develop soil metrics that can quantify and monitor soil health, as related to agricultural production and ecosystem services.

Measuring Bulk Density:

Drive 5 x 5 cm corer into soil using hand sledge. **1.**
Carefully remove ring and excess soil. **2.**
Place whole sample in labelled water-tight bag. **3.**
Weigh and record sample. **4.**
Dry sample overnight in the oven at 105˚C. **5.**
Weigh dry weight of soil. **6.**
Account for stones - sieve dry sample in 2mm sieve. **7.**
Weight sample collected in 2mm seive - minus this **8.** measurement from wet and dry soil weights.

Figure 4: Accounting for stones when measuring bulk density: Sieve dry sample using a 2mm sieve and weigh sample collected in the 2mm sieve – minus this measurement from wet and dry soil weights.

Lancaster University | ROTHAMSTED RESEARCH

Acknowledgements: I would like to thank my supervisors: Andy Gregory, Stephan Haefele (Rothamsted Research) and Nick Ostle (Lancaster University).

Methods:

The aim of fieldwork is to define soil health and determine the suitability of existing protocols for assessing soil health, and where necessary develop and improve techniques.

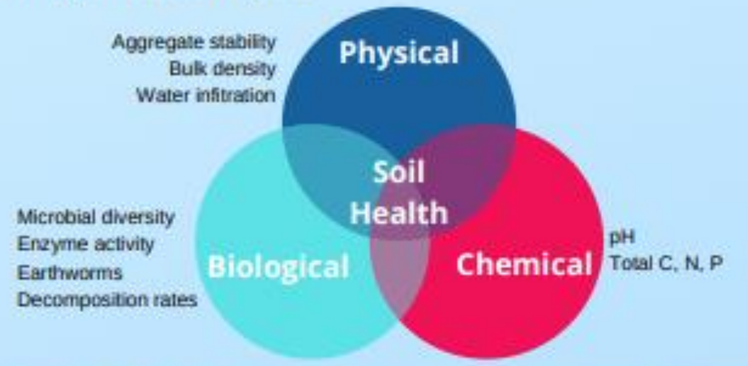


Figure 2: List of biological, chemical and physical soil health indicators selected based on existing literature.

Tests will be carried out on the Highfield LTE (Figure 3). 10 samples will be taken from 13 plots in a "W" shape. We will also use different testing methods when measuring each soil health indicator: i.e. aggregate stability - comparing effectiveness of low-cost tests (Slakes app) versus a lab-based SOP.

Figure 3: Highfield Reversion Experiment, Harpenden (since 2009): Plots formerly under long-term grass, arable and bare fallow (since 1949) divided into 3 and these three treatments superimposed.

Hypothesis:

- Soils ploughed out of permanent grass would be less 'healthy' compared to soils under permanent grass.
- Aggregate stability tests using standard lab protocols would be more informative than the Slakes app.

We aim to test this by measuring bulk density, infiltration and soil moisture. Also, measuring aggregate stability using SOP versus Slakes app.

AGRICULTURAL BIOPRODUCTS FROM MEDICINAL AND AROMATIC PLANTS

Musa Kisiriko[1,2,3], Jane L. Ward[1], Michael H. Beale[1], Maria Anastasiadi[2], Leon A. Terry[2], Abdelaziz Yasri[3]

[1]Rothamsted Research, [2]Cranfield University, [3]Mohammed VI Polytechnic University

INTRODUCTION

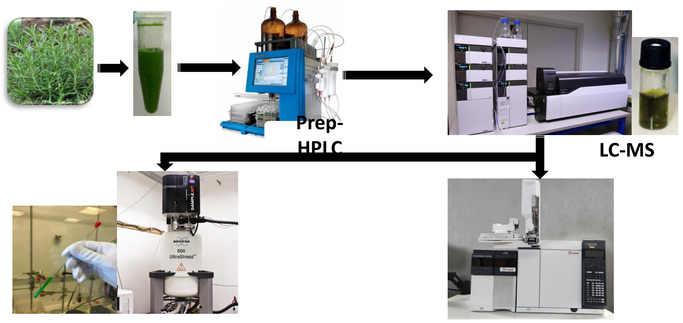
Biostimulants and Bioprotectants (Bioproducts) have over the past few decades gained appreciable recognition as the ecologically friendly and sustainable alternatives to synthetic fertilisers and pesticides [1, 2].

Secondary metabolites from plants are major sources of these bioproducts, and medicinal and aromatic plants (MAPs) contain a diverse array of such compounds. MAPs are abundant in arid areas of Morocco but have largely not been assessed for their biostimulant or bioprotectant potential.

This project seeks to develop novel biostimulant and bioprotectant compounds and formulations from these MAPs growing in arid areas of Morocco for use in African agriculture.

METHODS

Isolation and characterisation of extracts and novel compounds

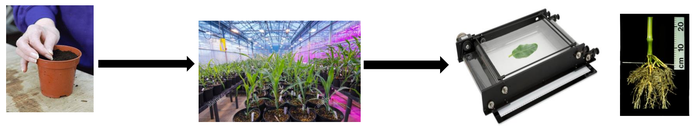


NMR (Polar Compounds)

GC-MS (Non-polar Compounds)

Bioactivity tests

Biostimulant activity



Seeds or plants to be treated with extracts, purified compounds or formulations in greenhouse studies

Physiological parameters (plant height, root length, leaf area, shoot and root fresh/dry weight etc.) will be measured.

Bioprotectant activity

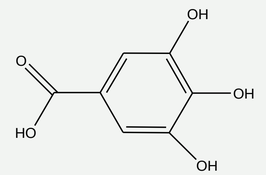
Antifungal tests on fungal strains relevant to Moroccan/African crops

Bioprotectant tests against cochineal beetles on cactus plants

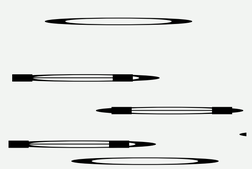
ROTHAMSTED RESEARCH

PRELIMINARY RESULTS

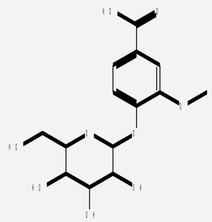
- Several metabolites belonging to different compound classes have been identified from 15 selected MAPs sourced from Morocco.
- The metabolome of the medicinal plant, *Acacia gummifera* has been characterised for the first time in any study.



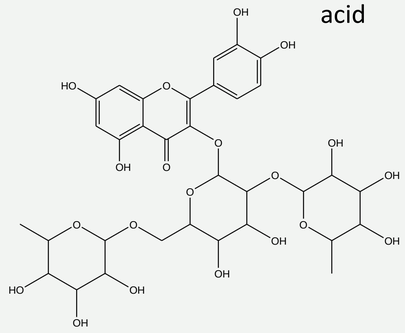
Gallic acid



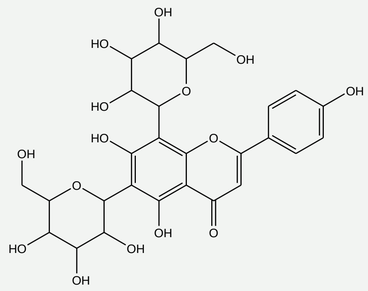
Eucomic acid



Vanillic acid glucoside



Quercetin 3-O-α-L-dirhamnopyranosyl-β-D-glucopyranoside

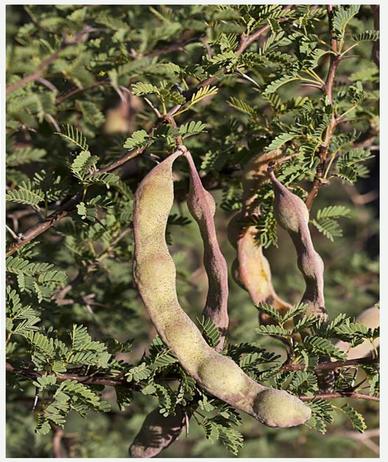


Apigenin 6,8-di-C-glycoside

Selected compounds identified from *Acacia gummifera*

Marrubium vulgare (White horehound)

Capparis spinosa (Caper bush)



Acacia gummifera (Photo by TrekNature)

Ammodaucus leucotrichus (Wooly cumin)

Some of the medicinal and aromatic plants being analysed

FURTHER WORK

- Crude extracts, purified compounds and formulations of various compounds and/or extracts selected from the different plants will be trialled in biostimulant and bioprotectant assays in the quest to develop a novel product for use in African agriculture.
- Later in the project, we shall explore the effect of genotype, environment, processing and other factors on the distribution of secondary metabolites and on the biostimulant and bioprotectant potential.

REFERENCES

[1] *Food and Energy Security* **2017**; 6(2): 48–60

[2] *Frontiers in Plant Science* **2017**; 7:2049

ACKNOWLEDGEMENT

This project is funded by OCP.

Cranfield University

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OCP

An Innovation Ecosystem Approach to the Agricultural Sector

Exploring Innovation and Co-creation of value in the context of UK Ecosystem

ROTHAMSTED RESEARCH

Abstract

Agriculture is the oldest industry in the world and has innovated throughout history to meet the growing needs of an ever-increasing world population. Challenges such sustainability, policy changes, increased urbanisation, and resource constraints, continues to drive the need to build ecosystems to delivering value and meaningful change within the sector. An Innovation Ecosystem approach requires multiple actors, stakeholders, activities, institutions and governments to deliver a networked approach to innovation across sectors to engage wide innovation led systems thinking. Agricultural Innovation Systems, on the other hand, tends to apply to specific country, sector or technology. Whilst the UK's technology ecosystem is recognised as being a world leader and has developed globally recognised clusters such as "Silicon Roundabout" and the "Golden Triangle", the agricultural sector seems to have been bypassed. This project aims to introduce the concept of an Agricultural Innovation Ecosystem and critically assess the UK against existing models globally.

Methodology

This work aims to provide a tangible outcome to inform UK policy makers and industry partners that can be used as the basis of a future action plan.

Data to back up the study will be collected via primary and secondary sources globally including from stakeholders involved in innovation ecosystems and agricultural innovation clusters:

- Literature searches
- Structured interviews
- Surveys and field-based research

Around the World

A successful AgTech ecosystem requires key elements across innovation, access to capital and access to talent. Australia's ranking compared to the six international AgTech players we investigated is provided here. While Australia is ranked highly on the 'Global Entrepreneur Index' and in terms of number of researchers, it lags by comparison across collaboration and access to capital.

Ranking not available *India ranking not available

UK

Government initiatives:

- £60m Agri-tech Catalyst Fund - financial contribution to collaborative industry-led research projects to support commercialisation
- £90m over 5 years to establish Centres of Agricultural Innovation to improve the levels of technology adoption through the agri-food supply chain. Research Centres are used as spaces for farmers to experience technology first hand on a demonstration farm.

Investors:

- Shake Climate Change: early-stage investment into agricultural innovation
- Cambridge Agritech: angel network investing in agritech

Research:

- NIAB
- Rothamsted Research
- James Hutton Institute

Ecosystem builders:

- AgritechE
- Farm491
- Ceres Agri-tech
- Agritech Centres (CHAP, Agri-EPI, Agrimetrics, CIEL)

Results to date

Innovation ecosystems have seen rapid growth over the past 15-20 years and whilst the agricultural sector continues to innovate, it seemingly lags behind in cross-sector and inter-actor connectedness. Historically, Agricultural Innovation System have been driven by a Country's need to innovate at scale (i.e. Industrial innovation) or the requirement for focus on specific technologies. The recent attention of agriculture's impact on climate change, the environment, and sustainability, has led to a renewed interest in "Agriculture 4.0" and the support an innovation revolution rather than evolution by government, academia and industry.

Some early lessons learned:

- Diversity and redundancy amongst actors are important to build value and resilience
- Connectivity is crucial to enable network effects
- Alignment and feedback provide a way to accelerate adoption
- Polycentric governance and decentralisation can deliver scale

Understanding the mechanisms of hypoxia tolerance in rice: Assessing the effect of different imbibition conditions on anaerobic germination of rice seeds- a role for ethylene priming?

Gavers Oppong[1,2], Hongtao Zhang[1], Darren Wells[2], Smita Kurup[1], Shalabh Dixit[3], Frederica Theodoulou[1]

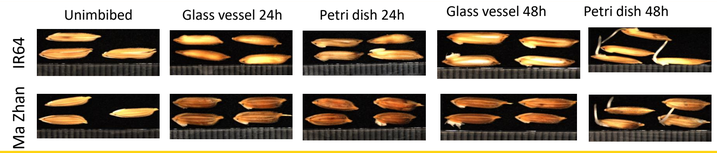
1 Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ
2 School of Biosciences, The University of Nottingham, Sutton Bonington, LE12 5RD UK
3 International Rice Research Institute, Los Baños, Philippines

INTRODUCTION

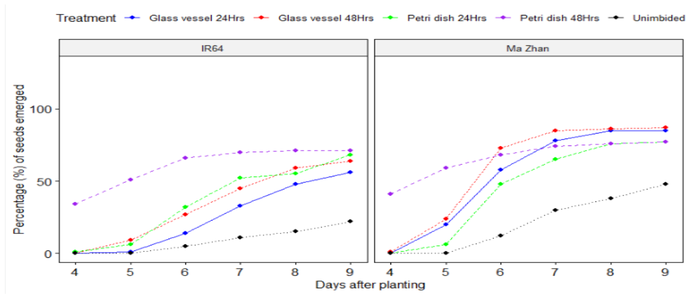
Due to rising labour costs, water scarcity and convenience, many farmers in sub-tropical and tropical regions are adopting **direct seeded rice (DSR)** over the traditional method of transplanting. However, most elite rice varieties fail to grow above flood waters, which is a major bottleneck to the widespread adoption of DSR. Fortunately, natural variation exists for **anaerobic germination (AG) tolerance**. However, the mechanisms behind anaerobic tolerance are poorly understood. Apart from genetic factors, pre-sowing practices such as seed priming and pre-soaking have been identified to increase tolerance to AG in some rice cultivars and other plant species such as Arabidopsis. This study forms part of a baseline investigation to determine whether the increase in AG tolerance after pre-soaking before planting is as a result of ethylene priming.

METHODS

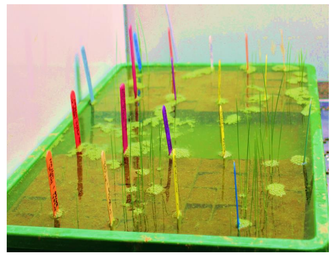
Two cultivars **Ma Zhan (AG tolerant)** and **IR 64 (AG sensitive)** were compared with two imbibition treatments for 24 or 48 h. Seeds were planted in soil, flooded, and emergence and survival rates measured. The images show treated seeds before planting.



RESULTS: Imbibition increased survival and emergence in both cultivars under flooding

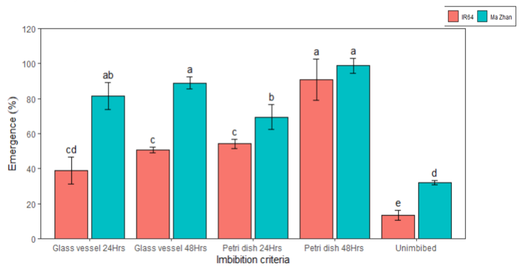


Effect of imbibition on seedling emergence rate
Data represent the average of four independent experiments (n=100)

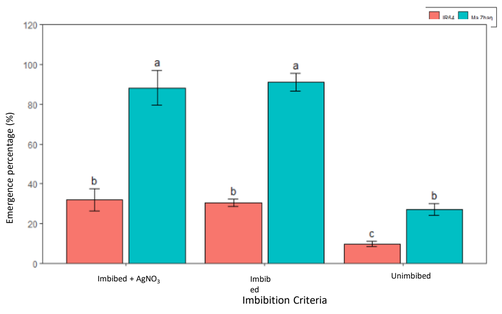


Ma Zhan and IR64 seedlings growing under submergence after imbibition treatment

- Is Ethylene involved in the tolerance seen after imbibition?



Cumulative seedling emergence 9 d after planting, Petri dish experiment. Data represent the average of four independent experiments (n=100). Values with different letters are significant at $P < 0.05$



Cumulative seedling emergence 9 d after planting, Petri dish experiment. Data represent the average of four independent experiments (n=100). Values with different letters are significant at $P < 0.05$

CONCLUSIONS

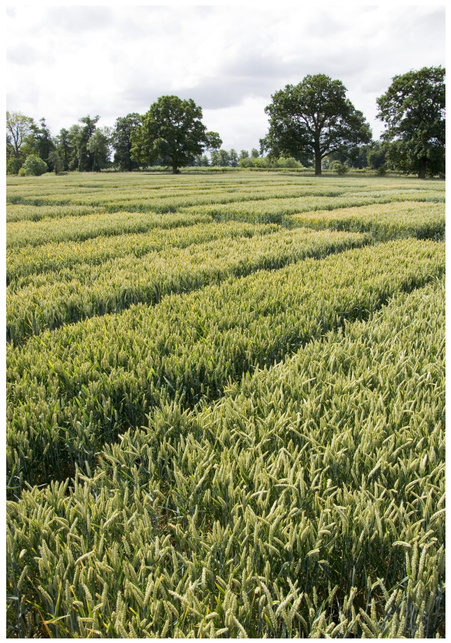
Different pre-sowing strategies can enhance rice germination and seedling survival. Such treatments can complement genetic AG tolerance. Knowledge about the physiological and metabolic or molecular bases for these observations will be imperative for the wider adoption of DSR. Therefore, the next step in this project will be to conduct further research to investigate the physiological and metabolic basis for the advantages conferred by imbibition, including a potential role for ethylene signalling.

Exploitation of beneficial root-associated bacteria in grain cereal-based cropping systems

ROTHAMSTED RESEARCH

MAHASSINE ARHAZZAL1,2; TIM MAUCHLINE1; IAN CLARK1; JIM HARRIS2, MARK PAWLETT2, ADNANE BARGAZ3

1, ROTHAMSTED RESEARCH, HARPENDEN. 2, CRANFIELD UNIVERSITY. 3, MOHAMMED VI POLYTECHNIC UNIVERSITY



INTRODUCTION

- The plant growth-promoting rhizobacteria (PGPR) are becoming a promising tool for sustainable agriculture. In fact, the microbiome plays an essential role in enhancing nutrient provision (e.g. P and K solubilization), abiotic stress tolerance (e.g. drought, high salinity), manipulation of plant hormone signalling, as well as disease suppression of foliar and root pathogens. In this work we investigate the ability of different wheat (*Triticum aestivum*) cultivars to select for a beneficial soil microbiome when grown under conditions of abiotic stress. Apogee seeds were planted on a sandy bare fallow soil amended with 30% perlite, with and without fertiliser addition. They were then watered with sodium chloride solutions at the concentrations (0,30, 60, 90, 120, 150, 300, 600, 900, 1200 mM) and kept at 40% WHC. The rhizosphere and rhizoplane have been sampled at the flowering stage. The bacteria will be then cultured, isolated and functionally screened through a suite of assays for nutrient solubilization (N, P, K, Zn, Fe solubilisation). Next generation amplicon sequencing methods will be used to assess the total microbiome.

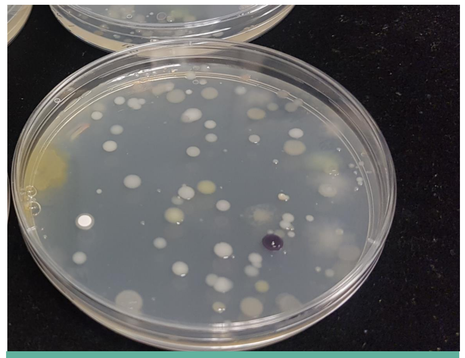
METHODS

- Experimental approaches will be based on a platform of plant culture in glass houses or under a controlled environment under specific conditions. This will encompass soil at low and high fertilization levels, drought, heat and salt stressed soil microcosms with different wheat cultivars.
- This soil and plant biomass from these plant cultures will be used to generate datasets and a microbiological isolate resource.
- To analyse the plant microbiome both culture independent and dependent methods will be adopted. The culture independent approach will involve next generation amplicon sequencing to assess the total microbiome. This will be done with 16S rRNA gene and internal transcribed spacer (ITS) based primers.
- The culture dependent approach will involve the isolation of microbes from the root systems of plants from the experiments. These will then be functionally screened through a suite of assays for nutrient solubilization (N, P, K, Zn, Fe solubilisation), drought, heat and salt tolerance. The microbial isolates will be identified by a 16S Sanger sequencing approach.
- A subset of promising isolates will have their genomic DNA extracted and this will be used for whole genome sequencing. Genomes will then be screened for plant growth promoting and stress tolerance genes.

RESULTS

Primary observations:

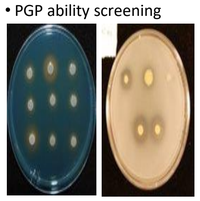
Wheat plants can no longer grow at 600 mM sodium chloride concentration.



Bacteria culture

• Bacteria isolation

• PGP ability screening



CONCLUSIONS

The findings of this work will identify what contribution the plant microbiome can make to enhance wheat growth under a variety of abiotic stress conditions. The work will also identify whether wheat cultivar choice is important, and if particular cultivars should be grown in areas that are prone to particular abiotic stresses or fluctuations in growing conditions.

Acknowlegments

This work was supported by OCP group.

Cranfield Environment and Agrifood

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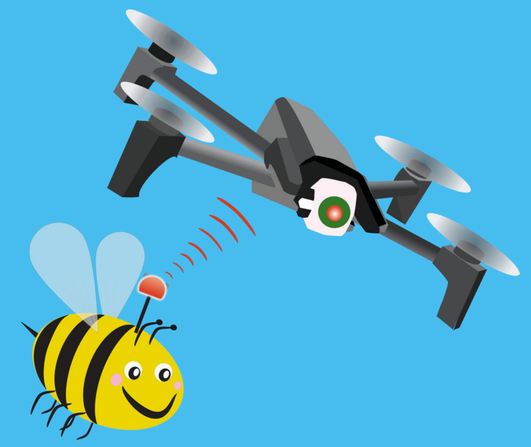
UKRI Biotechnology and Biological Sciences Research Council

ROTHAMSTED RESEARCH

Session Seven Thursday, 25th February 2021

Using a new **drone** technology to **track bees** during flight.



Using piezoelectric tags and autonomous drone technology to understand the space use of bees at a landscape scale.

Thomas R. Oliver, Joe Woodgate, James Makinson,, Lars Chittka, Andy Reynolds and Paul Cross.

INTRO

- Wild bee populations are suffering a decline.
- Understanding how bees use their environment can help to better inform agri-environment schemes to benefit wild pollinators.
- We present a novel method of tracking bees across a landscape-scale using a drone.

METHODS

1. Bees are equipped with a new lightweight tag.
2. Bees are released and allowed to forage normally.
3. The drone follows the bee and records location data multiple times a second.

DISCUSSION

This technology has a number of benefits over existing insect telemetry techniques:

- The tag does not need a battery and so is lightweight.
- The drone provides the longest range of current insect tracking techniques.
- All the equipment is comparably inexpensive to purchase and maintain.

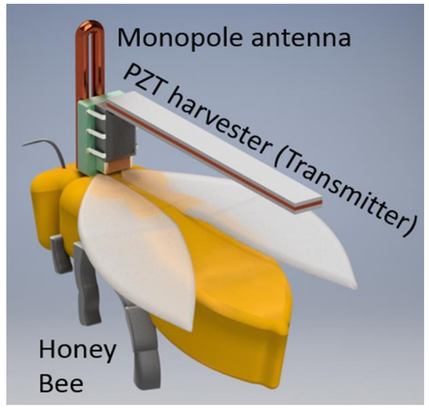


Figure 1. Energy harvesting tag attached to a bee. (Figure taken from Shearwood *et al,* 2017).

Figure 2. Energy harvesting piezoecltric tag attached to *Bombus terrestris* foragers visiting a feeder. (Photo taken July 2019)

Figure 3. Autonomous drone in-flight carrying antennae array (receiver). (Image courtesy of Jake Shearwood).

Figure 4. Multisource energy harvester (receiver) above bee hive. (Figure taken from Shearwood *et al,* 2017).

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ROTHAMSTED RESEARCH

Keep up to date with my research on Twitter!

ADSORPTION AND DESORPTION IN THE DIFFERENT LANDSCAPE POSITIONS IN AMHARA REGIONAL STATE, ETHIOPIA

MESFIN KEBEDE[12], STEVE MCGRATH[1], JAVIER HERNANDEZ[1], MARTIN BROADLY[2], KIRSTY HASSALL [1], STEPHAN M. HAEFELE[1]

[1] Rothamsted Research, Harpenden, UK. [2] University of Nottingham, Sutton Bonington, UK

INTRODUCTION

- **Ethiopian** farming is characterized by highly variable and different landscape positions (Figure 1)
- **Landscape positions** do vary with its associated soil factors and hence affecting the yield and quality of grain through retaining (high pH, OC, Clay content…) and release (low pH,….) of nutrients, especially Zinc for my focus
- Therefore, it is important to **identify the most dominant soil factors** driving adsorption-desorption characteristics of Zinc.
- This will help to devise fertilizer recommendations schemes.
- For example, highly adsorbed soil need relatively high fertilization by taking into account the potential of desorption

Figure 1. Farming system and Landscape positions

METHODS

- Sixty on-farm soils samples from the Geo-Nutrition Project sites in Ethiopia were used for this study (Four sites; Aba Gerima Tef, Aba Gerima Maize, Debre Mewi and Markuma)
- Standard procedures followed with six levels of Zinc stock solutions (0, 2, 5, 10, 15 and 30 mg L^{-1}) for adsorption and 0.01M $CaCl_2$ Solution for desorption
- Fitness to Langmuir and Freundlich Isotherm
- Important to have **Predictive Models** for these soil factors. Sticking with the most universally dominant soil factors (pH, OC, eCEC), taking all soil factors and removing those which are not significant , forward selection forcing the model to have (1), and backward selection, but first removing high VIF
- Finally, **making predictions** using these models. Put one from each adsorption and desorption

RESULTS

- Adsorption and desorption of Zn^{++} increased with increasing Zn^{++} concentration at equilibrium solutions to soils of the study sites (Figure 2) though the rate of adsorption gets its peak at 5 and 10 mg L-1 while rate of desorption decreases with increasing initial Zn concentrations.
- The separation factor R_L for adsorption ranges between 0 and 1 across the sites which indicates that the situation is favourable for the adsorption and desorption process as $R_L > 1$ and close to 0 is unfavourable and non-reversible processes, respectively.
- Freundlich isotherms were found to fit well for adsorption (Figure 3) and desorption (Figure 4) while Langmuir fits only to desorption (Figure 5) for all locations and landscape positions.
- Different soil parameters were identified as influential factors in governing the adsorption and desorption at different locations. Soil pH, eCEC and OC involved in many ways to influence adsorption and desorption.
- **Four** models were developed for adsorption and desorption (Equation 1-4) and predictions (Figure 6)

Multiple regression models for Adsorption:

Eq 1 = -2.19+0.74pH+0.0051eCEC-0.048 (pH^2)+0.018 (Org.C^2), [0.94]

Eq 2 = -0.92+0.26pH+0.03OC, [0.90]

Eq 3 = -1.03+0.16pH+0.008eCEC+0.08OC+4*10^{-4}Al+4*10^{-5}Ox_Mn, [0.94]

Eq 4 = -0.96+0.21pH+0.088OC+3*10^{-6}Al-2.5*10^{-5}Ox_Al+0.09EX_K+ .02EX_Mg; [0.95]

Multiple regression models for Desorption:

Eq 1= 0.89-0.11pH-0.03OC, [0.69]

Eq 2= 0.81-0.11pH-4*10^{-4}Cu-7*10^{-6}Mg+8*10^{-4}Zn+0.58EX_Na,[0.77]

Eq 3= 0.84-0.11pH-1.2*10^{-5}Ox_Al–3.3*10^{-4} Cu+1.1*10^{-3}Zn,[0.77]

Eq 4= 0.98-0.13pH-3.5*10^{-4}Cu+1.6*10^{-4}Na+6*10^{-4}Zn-1.4*10^{-5}Ox_Al,[0.79]

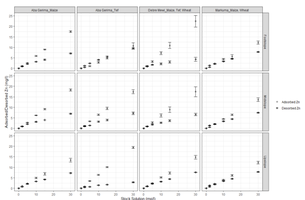


Figure 2. Adsorbed and desorbed Zn

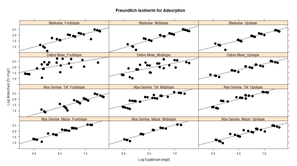


Figure 3. Adso_Freundlich Isotherm

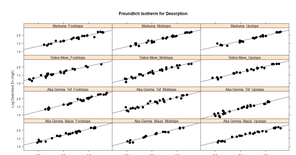


Figure 4. Deso_Freundlich Isotherm

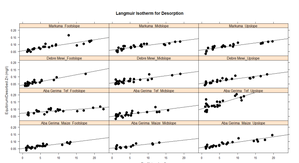
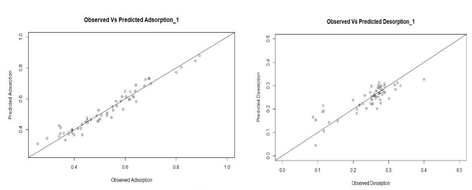


Figure 5. Deso_Langmuir Isotherm



a b

Figure 6. Predictions for adsorption and desorption (a,b)

CONCLUSIONS

- It can be concluded that the most probable reasons for the widespread Zn deficiency in the study area could be due to high rate of adsorption with less desorption or vice versa.
- Hence, in areas where the soil has high adsorption with less desorption (Aba Gerima Maize and Debre Mewi), they might need an application of high doses of Zn fertilizer to compensate for the adsorbed Zn^{++} while in low adsorbed soils with high desorption (Aba Gerima tef), needs low doses of Zn fertilizers to minimize the effect of toxicity and further accumulation by taking into account the amount of desorbed Zn^{++}
- The **models** will help to quantify the amount of adsorbed and desorbed Zn^{++} and help to devise stratified Zinc fertilizer recommendations for these site and even landscape position.

Acknowledgements

University of Nottingham for financing this study and Rothamsted Research SAS-H (AfSIS lab) for allowing the laboratory facility for this study is highly acknowledged.

Unlocking the potential of the pod

Increasing seed yields by altering seed number and seed size

In a world facing climate change and increasing population, increasing crop seed yields for the future is a crucial avenue for current research [1]. Increases in seed yield can be achieved by either altering seed size or by adjusting seed number. Although some studies suggest that seed number and seed size are inversely correlated, recent studies have demonstrated that seed number and seed size are governed by mostly non-overlapping QTLs [2], suggesting the two factors could be uncoupled and seed number could be altered without experiencing trade-offs in seed size and vice versa.

Mollie Langdon, SWBio DTP PhD student

Based at Rothamsted Research (Harpenden) and at the University of Bath

Lead Rothamsted supervisor: Dr Smita Kurup ; Lead University supervisor: Dr James Doughty

Acknowledgements: All group members including Dr Pete Eastmond, Dr Guillaume Menard and Dr Laura Siles-Suarez, the Bioimaging team, the Statistics team, and the CE and GH staff at Rothamsted Research.

Increasing seed number

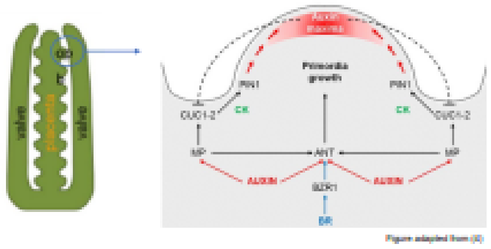


Figure adapted from (3)

Seeds develop from fertilised ovules in the gynoecium (the female plant reproductive organ), therefore increasing ovule number could offer an unexplored route to increasing seed number and subsequently seed yields. Ovule initiation is a poorly understood mechanism, however the current model suggests several hormones are involved including auxin, cytokinins and brassinosteroids (BRs) [3,4]. Previous research found manipulating genes in the BR biosynthesis pathway can affect seed number [5,6] however, the effect of BR manipulation on ovule number and seed yield was not explored. Therefore, brassinosteriods have been considered an interesting hormone to manipulate to potentially achieve increases in seed yield through alterations in ovule and seed number.

This PhD project aims to address two objectives to increase seed yields by **altering seed number**, as follows:

Aim 1:

Ectopically express brassinosteroid-related genes under a 'optimised' gynoecium-specific promoter in *Arabidopsis thaliana* to investigate changes in **ovule** and **seed number**

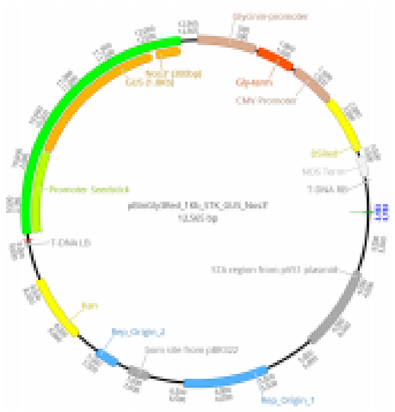
Aim 2:

Investigate the effect of silencing potentially novel **ovule number**-related genes using *Arabidopsis thaliana* T-DNA knockout mutants

Preliminary work was undertaken to express brassinosteroid-related genes under a gynoecium-specific promoter known as SEEDSTICK (STK) in *Arabidopsis thaliana* [10]. As this preliminary research yielded promising results, work has begun to optimise the length of the STK promoter used to govern gene expression.

Work so far on Aim 1: Three varying lengths of the STK promoter were generated and fused to the GUS (β-glucuronidase) reporter gene. *Arabidopsis thaliana* plants were floral dipped with *Agrobacterium tumefaciens* transformed with the different length STK-GUS-DSRed constructs, using DSRed as the transformation event screenable marker.

Once the transgenic plants have matured, transformants will be analysed to determine the optimal STK promoter length for gynoecium specific expression, by identifying GUS expression exclusively located in the gynoecium. Following promoter optimisation, transgenic *Arabidopsis* lines expressing brassinosteroid-related genes governed by the STK promoter will be generated and resulting homozygous lines will be analysed for effect on ovule number and overall seed yield .



Increasing seed size

Alternatively, increases in seed yields could be achieved by altering seed size. Following double fertilisation, the ovule develops into a seed and undergoes several developmental stages including the growth of the endosperm and the enlargement of the embryo [7,8]. During these stages, the integuments surrounding the ovule develop into the final seed coat and act as an upper boundary limiting seed growth [7,8].

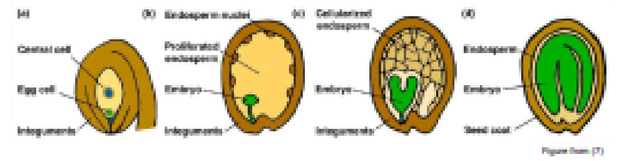


Figure from (7)

Previous research in *Arabidopsis* revealed that a mutation in an auxin transcription factor known as ARF2 produced larger seeds, as a result of extra cell divisions in the integument [9]. However, research has yet to be performed to determine whether downregulation of similar ARF2 orthologs have a similar effect in a crop species. Therefore, ARF2 has been regarded as an interesting seed size regulator that could be manipulated to potentially increase seed yield.

This PhD project aims to address two objectives to increase seed yields by altering **seed size**, as follows:

Aim 3:

Assess the result of truncating the ARF2 protein in *Brassica oleracea* through CRISPR-Cas9 gene editing on **seed size**

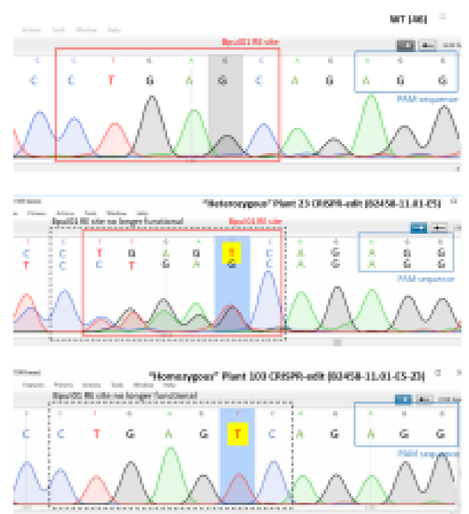
Aim 4:

Investigate the impact of silencing *ARF2* expression in *Oryza sativa* through RNA-inference on **grain size**

Work so far on Aim 3: Genome editing of the *ARF2* gene in *Brassica oleracea* was performed by BRACT (JIC). DNA from 46 lines was analysed for CRISPR editing. One line was found to be heterozygous for a '+T' insertion, resulting in a premature STOP codon and a predicted truncated ARF2 protein. This heterozygous CRISPR-edited line was taken through to the next generation.

Several lines in the T2 generation were subsequently identified as both homozygous for the '+T' CRISPR edit and Cas9-free (as it is important to segregate away the Cas9 T-DNA cassette to assess the effect of the CRISPR insertion independent of any further editing occurring).

The homozygous CRISPR-edited Cas9-free lines have been taken through to the T3 generation where the effect of knocking out the *ARF2* gene is being assessed. Seed and ovule samples at different developmental timepoints are being analysed by microscopy to investigate cell number in the integument. In addition, Q-PCR will be performed with seed and leaf tissues to confirm the predicted truncated *ARF2* transcripts. Following this, randomised blocked experiments will be performed with promising CRISPR-edited lines that possess alterations in integument cell number to examine how *ARF2* manipulations impact seed size and total seed yield.



References: (1) Long et al., 2015. *Cell*, 161, 56-66; (2) Gnan et al., 2014. *Genetics*, 198, 1751-+; (3) Galbiati et al., 2013. *The Plant Journal*, 76, 446-455; (4) Cucinotta et al., 2014. *Frontiers in Plant Science*, 5, 117; (5; Choe et al, 2001. *Plant Journal*, 26, 573-82; (6) Wu et al., 2008. *The Plant Cell*, 20, 2130–2145. (7) Sun et al., 2010. *Current Opinion in Plant Biology*, 13, 611-620; (8) Berger et al., 2008. *Trends in Plant Science*, 13, 437-443; (9) Schruff et al., 2006. *Development*, 133, 251-261; (10) Langdon and Kurup, unpublished. Icons taken from https://www.flaticon.com.

ROTHAMSTED RESEARCH

Session Eight
Thursday, 25th February 2021

BIO-PROSPECTING FOR PLANT-GROWTH-PROMOTING MICROBES IN BROADBALK SOILS

ROTHAMSTED RESEARCH

BASF We create chemistry

Owen Thornton – SAS-H

The potential to promote plant growth is harboured by many soil microbes but where are we most likely to isolate agriculturally relevant strains?
...and how can we extract them?

BROADBALK! The single most important field site on Earth in which to dissect the agricultural relevance of the wheat root microbiome and identify the determinants of microbial plant-growth-promotion. Change my mind...

- **Large numbers of experimental plots** enabling the identification of a 'core' microbiota by filtering out transient associations and prioritising microbes reliably associated with wheat roots
- **Wide range of treatment combinations** enabling the identification of context dependent microbial associations potentially of benefit to plant growth and eventual yield
- **Long term experimental continuity** reinforcing relevant associations over 175+ years of cropping
- **Why would you want to work anywhere else?**

Dilution-to-extinction culturing! Using dilute liquid media in microwell plates with long incubations.

- Statistically ensures the purity of cultures by physically separating microbial cells by dilution
- Removes competition between co-cultured microbes that inhibits slow-growers
- Avoids time-consuming spread-plating and colony picking steps
- Enables automation with basic liquid handling robotics

Ask me how this robot ~~stole my job~~ creates larger, less biased microbial culture collections.

PHENOTYPING THE NUTRITIONAL STATUS OF CROPS USING REMOTE SENSING TECHNOLOGIES

DANIEL K. CUDJOE[1,2], FRANK G. OKYERE[1,2] MALCOLM J. HAWKESFORD[1], NICOLAS VIRLET[1], POURIA SADEGHI-TEHRAN[1], PETER BUCHNER[1], ANDREW RICHE[1], FADY MOHAREB[2], TOBY WAINE[2], MANAL MHADA[3] AND MICHEL GHANEM[3]

ROTHAMSTED RESEARCH

[1]Plant Science Department, Rothamsted Research, Harpenden, AL5 2JQ, UK
[2]School of Water, Energy and Environment, Cranfield University, Cranfield, Bedfordshire, MK4 0AL, UK
[3]Mohammed VI Polytechnic University (UM6P), Lot 660 Hay My Rachid, 43150, Benguérir, Morocco

Cranfield University

1. Background

Low soil fertility is one of the factors most limiting agricultural production particularly in developing countries such as Africa. However, mineral fertilizers that are applied to avert this production constraint comes with an economic cost to the farmer with low income and further cause threats to human health and environmental pollution to ecosystems [1]. Therefore, more restricted and reasonable use of fertilizers is critically essential. Improved agronomic practices in combination with breeding (i.e., phenotyping) of more nutrient efficient crops are necessary especially in the low productive regions to achieve food security. Remote sensing has become an important methodology for the application of agricultural monitoring and to improve precision and throughput in phenotyping [2]. In this project, we will explore drone technologies, satellite imagery, gantry scanalyzer systems as well as low-cost portable phenotyping tools for controlled and field evaluation of crop performance in Africa, specifically in Morocco for locally important crops with a specific emphasis on optimizing the use of fertilizers

Fig. 1. Unmanned aerial vehicle

Fig. 2. The gantry scanalyzer at Rothamsted

2. Current Work

Nutritional pot experiments at RRes and Cranfield University

- 4 nutrient treatments
- 5 replicates/treatment
- Structured in RCBD
- Measurement parameters included imagery based on RGB, hyperspectral, 3D laser and thermal infrared as well as manual physiological data

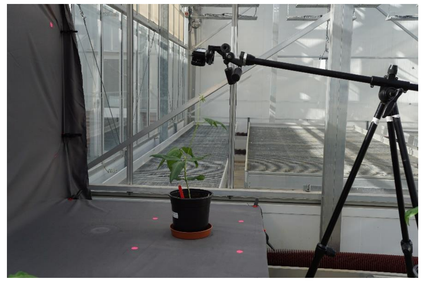


Fig. 3. RGB Imagery at RRes glasshouse

Fig. 4. Crop establishment involving quinoa and cowpea under the scanalyzer for imaging

Fig. 7. Evaluation of morphological traits of cowpea (A) and quinoa (B) based on nutrient treatments

3. Preliminary Results

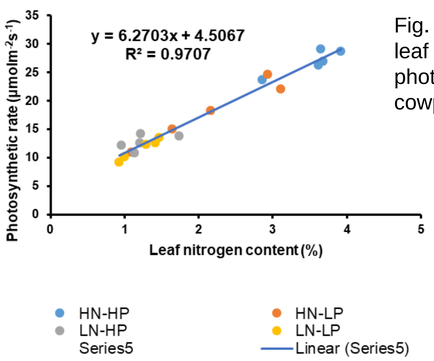


Fig. 8. Correlation between leaf nitrogen content and photosynthetic rate of cowpea

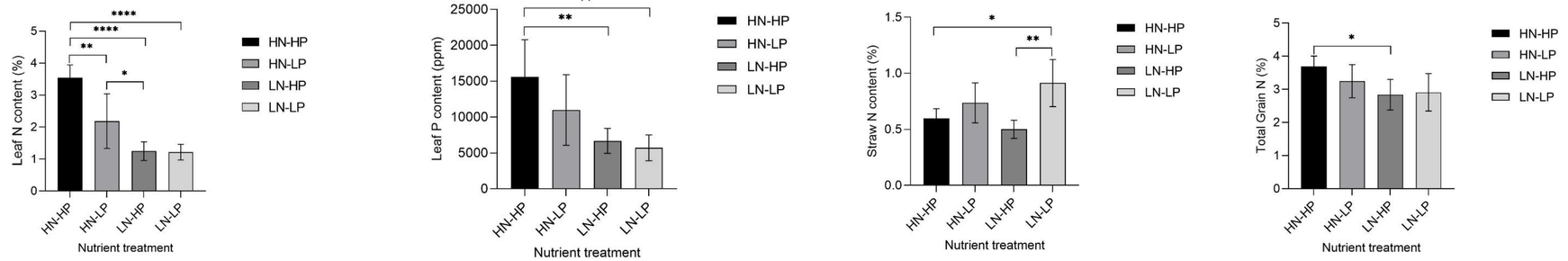


Fig. 9. Chemical analysis of quinoa grown under varying nutrient compositions for nutritional status estimation Error bars represnt mean ± SD (n=5). One-way ANOVA was used for mean comparisons between treatment conditions with significance difference at $p < 0.05$. Multiple mean comparisons analysis was done using Tukey's post-hoc test.

4. Conclusion

Nutrient availability impacts on quinoa and cowpea growth and nutritional status. This can be quantified manually and via quantitative analysis of images. Future work will focus on image-based derived indices (remotely sensed data) and correlations with manual ground-truth measurements and spectral data. Knowledge and methodologies from the current experiment will be transferred and simulated in field conditions in Morocco. The ultimate aim is for high-throughput phenotypic trait analysis

[1] Ahmed, M., Rauf, M., Mukhtar, Z., and Saeed, N. A. (2017). Excessive use of nitrogenous fertilizers: an unawareness causing serious threats to environment and human health. *Environ. Sci. Pollut. Res.* 24, 26983–26987
[2] Fiorani, F. and U. Schurr. (2013). Future scenarios for plant phenotyping. *Annual Review of Plant Biology* 64(1): 267–291

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OCP
MOHAMMED VI UNIVERSITY
UKRI Biotechnology and Biological Sciences Research Council

Competition Between an Invasive and Endemic Species of Armyworm in Africa

Ruth Carter
Prof Kenneth Wilson- Lancaster university
Dr John Caulfield- Rothamsted Research

Why? - The background

Most of the world's food is grown on smallholder farms. 98% of African farmers are smallholders. Poor smallholder farmers have less access to crop protection products, as these come at some expense. The larvae of both migratory moth species are a risk to food security including maize, pasture, rice, sorghum and cash crops. Maize plants can compensate for most foliage damage. But the larvae can cut the central shoot causing 'dead heart' and damage the cobs.

African Armyworm (*Spodoptera exempta*) - Native to Africa. It causes periodic outbreaks. In eastern Africa, larval densities can reach up to 1,000 larvae/m^2.

Fall Armyworm (*Spodoptera frugiperda*) - Native to the Americas. Invasive in Africa since 2016. Estimated maize yield reduction in Africa of 8-16 million tons per year. The estimated value of loss of maize in Africa is US$2,400-$4,800 million per year.

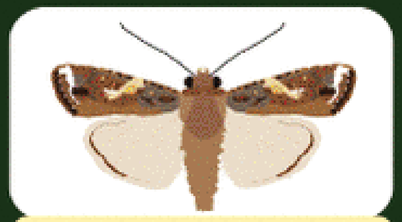
What? - Control

Biopesticides have been suggested as an alternative to broad-spectrum pesticide because of rising concerns over synthetic pesticides cost, availability and safety to farmers and environment. Biopesticides include entomopathogens (insect-specific viruses, bacteria, fungus), parasitoid and predators, botanical extracts ect.

Lancaster University · ROTHAMSTED RESEARCH · Envision — Developing next generation leaders in environmental science

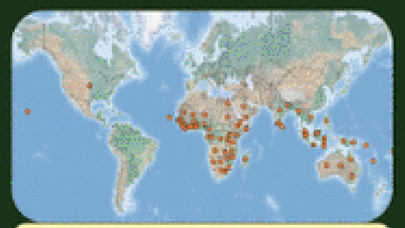
Can a new study guide the development of biopesticides ?

Adult *Spodoptera exempta*

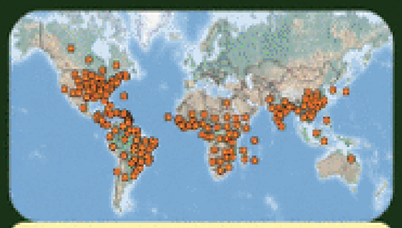


Adult *Spodoptera frugiperda*

What happens to native pest when you get a new invasive pest ?



Spodoptera exempta distribution



Spodoptera frugiperda distribution

Can *S. frugiperda* and *S. exempta* be managed in the same way?

larvae of Spodoptera exempta

larvae of Spodoptera frugiperda

How? – method plan

Direct interference competition

Interspecific predation -*Spodoptera frugiperda* and *Spodoptera exempta*

Plant defences - compare volatiles of Cynodon grass (*Cynodon dactylon*), Maize (*Zea mays*) and Cassava (*Manihot esculenta*)

Indirect Exploitation competition

Shared pathogens, apparent competition - Fungus and virus infected larvae

Host plant overlap - Cynodon grass (*Cynodon dactylon*), Maize (*Zea mays*) and Cassava (*Manihot esculenta*)

References

illustrations are author's own
https://www.cabi.org/isc/datasheet/29810 & https://www.cabi.org/isc/datasheet/29809
Butt, T. M. *et al.* (2016) 'Entomopathogenic Fungi: New Insights into Host-Pathogen Interactions', *Advances in Genetics*. Academic Press Inc., 94, pp. 307–364. doi: 10.1016/bs.adgen.2016.01.006.

ENHANCED NITROGEN USE EFFICIENCY (NUE) OF UREA FERTILISERS FOR SUB-SAHARAN AFRICA

Authors: *Marieme Drame, Tom Misselbrook, Sigrid Heuer, Alison Carswell, Guy Kirk, Mark Pawlett*

INTRODUCTION

Urease inhibitors (UIs) and nitrification inhibitors (NIs) have been shown to effectively reduce ammonia (NH_3) and nitrous oxides (N_2O) emissions from surface applied urea, respectively. However, the stability and longevity of these nitrogen (N) cycle inhibitors are influenced by several factors including soil moisture, pH and temperature. Particularly for temperature, inhibitors are more stable and have a longer lifespan at lower temperatures. However, very few studies have studied inhibitors efficacy at high temperatures (above 25 °C) in the highly weathered and nutrient deficient soils found in Sub-Saharan Africa.

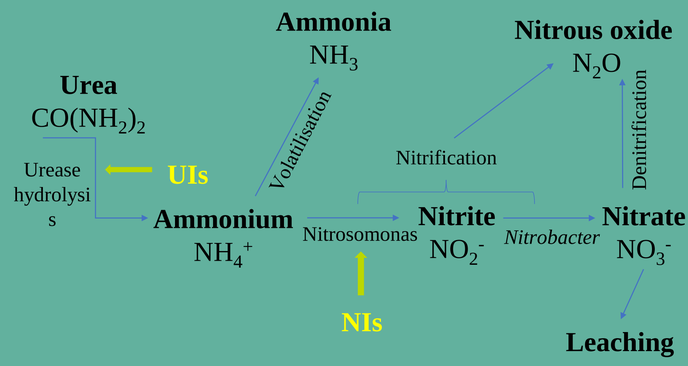


Figure 1: Simplified nitrogen cycle

OBJECTIVES

- Conduct literature review of fertiliser-NUE in SSA, focussing on the capacity of UIs and NIs to reduce NH_3 and N_2O emissions and improve crop NUE
- Conduct incubation experiments to elucidate the influence of temperature and soil moisture on the effectiveness of UIs and NIs for reducing NH_3 and N_2O emissions on selected SSA soils
- Conduct rice/wheat growth experiments to determine the effect of inhibitors on fertiliser-NUE, leaf-N storage compounds and assimilation genes
- Assimilate findings to provide recommendations on enhanced efficiency fertiliser (EEF) use under SSA conditions

ROTHAMSTED RESEARCH

METHODS

EXPERIMENT LOCATION

Rothamsted Research, North Wyke and Harpenden using arable soil from Tanzania, Ivory Coast and Madagascar.

AMMONIA AND NITROUS OXIDE MEASUREMENT

NH_3 volatilisation: cylindrical chambers with an air throughflow system, according to Misselbrook et al. (2005)
N_2O emissions: cylindrical chambers with closed headspace, method described by Klein and Harvey (2013)
Treatments: urea, urea with UI, urea with NI, urea with both UI and NI and a zero N control
Incubation temperatures: 15, 25, 35 °C

Figure 2: Purpose-designed laboratory chamber system

PLANT INCUBATION EXPERIMENT

NUE indices: biomass growth, N concentration, N uptake and N recovery
Leaf-N storage components: NH_4^+, NO_3^-, protein, glutamine
N losses: via volatile NH_3 using glutamine synthetase (GS) enzyme activity and apoplastic NH_4^+ as a proxy
Expression of N-related genes: GS, nitrate and ammonium transporter genes

PROGRESS

- Conducted literature review to find research gaps
- Started soil incubation experiment using UK soil
  Soil type: Dystric Cambisols
  Temperature: 15 and 25°C
  Moisture: 60% water filled pore space
  Treatment:(1) urea; (2) urea with UI N-(n-butyl-thiophosphoric triamide (NBPT); (3) urea with two UIs (NBPT and N-propylyl-phosphorothioic triamide (NPPT); (4) a zero N control.
  Measured emission: NH_3 , N_2O
  Experimental setup: randomized complete block design (RCBD) with three replications

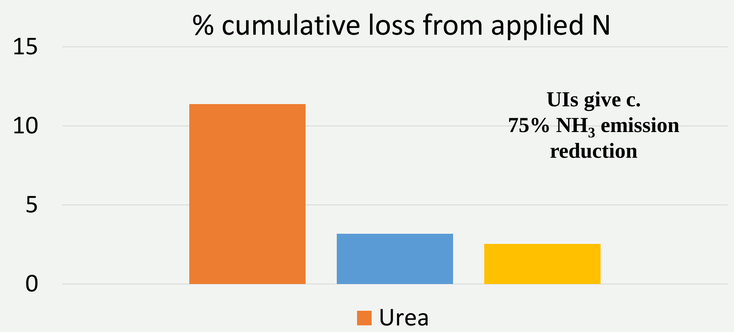


Figure 3: NH_3 percent cumulative loss over fourteen days period (25°C)

FUTURE WORK

- Conduct soil incubation experiment at 35 °C with soil moisture 60% WFPS using UK Soil
- Determine soil physicochemical characteristics
- Stablish plant incubation experiment using wheat varieties
- Conduct incubations with representative SSA soils

References:
Misselbrook, T. H., Powell J. M., Broderick G. A., and Grabb. J. H. (2005). Dietary Manipulation in Dairy Cattle: Laboratory Experiments to Assess the Influence on Ammonia Emissions. J. Dairy Sci. 88:1765–1777
Harvey, M., Klein C.D., C.D., and Chadwick D., D. (2013). Nitrous Oxide Chamber Methodology Guidelines edited December 2012, 146p. http://www.cppse.embrapa.br/redepecus/sites/default/files/principal/publicacao/Chamber_Methodology_Guidelines_Final-2013.pdf

Reduced free asparagine in wheat grain resulting from a natural deletion of *TaASN-B2*

Joseph Oddy, Mark Wilkinson, Sarah Raffan, J. Stephen Elmore, Nigel Halford

Plant Sciences, Rothamsted Research, Harpenden, UK

Background

- Asparagine is an amino acid involved in nitrogen mobilisation in many plants (Fig. 1) and is the major precursor to acrylamide in wheat.
- Free (soluble, non-protein) asparagine levels in wheat grain are likely controlled to a large extent by asparagine synthetase 2 (*ASN2*) expression.
- The B genome homoeolog of *ASN2 (TaASN-B2)* is variably present across wheat varieties but has not been fully characterised before.

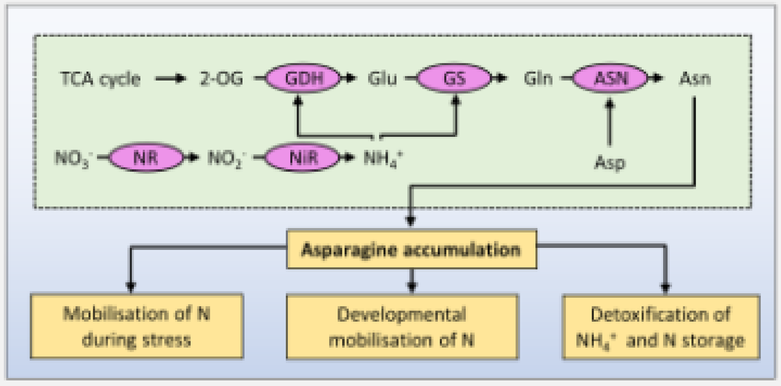


Figure 1. Synthesis and functions of asparagine in plants.

Results

- *TaASN-B2* is located near a large terminal repeat retrotransposon (Fig. 2a) and has a much smaller first intron than *TaASN-A2* and *TaASN-D2* (Fig. 2b).

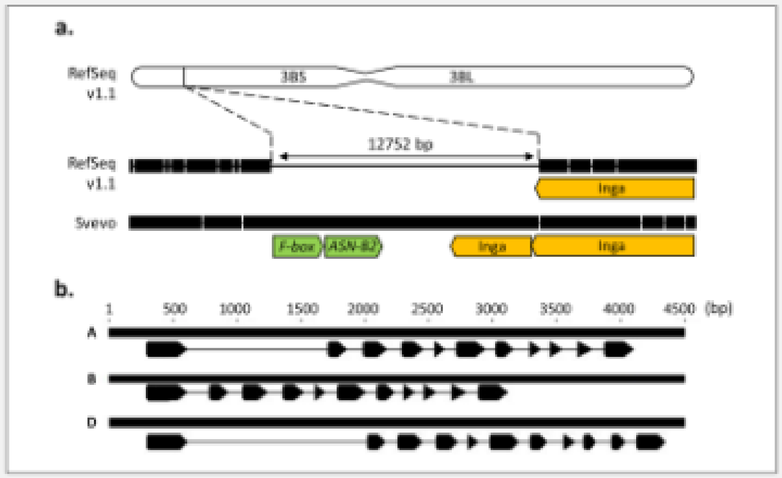


Figure 2. Genomic location (**a.**) and genetic structure (**b.**) of *TaASN-B2*.

Results

- *TaASN-B2* was not present at high frequencies across a collection of 63 UK wheat varieties, but it varies between different commercial groups (Fig. 3).

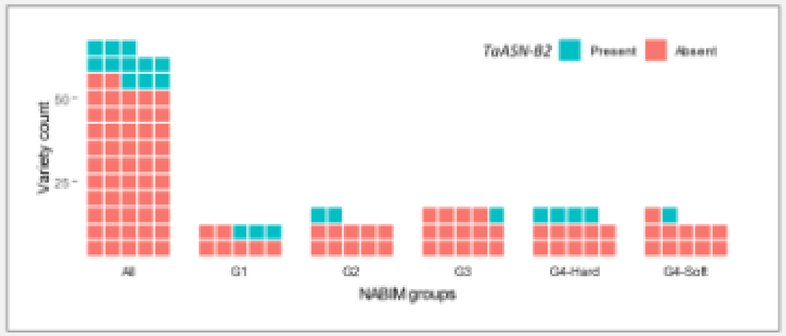


Figure 3. Frequency of varieties possessing *TaASN-B2* across UK commercial wheat groups.

- *TaASN-B2* is expressed more than *TaASN-D2*, but less than *TaASN-A2*, when present (Fig. 4a, 4b). Expression is absent when it is deleted (Fig. 4c, 4d).

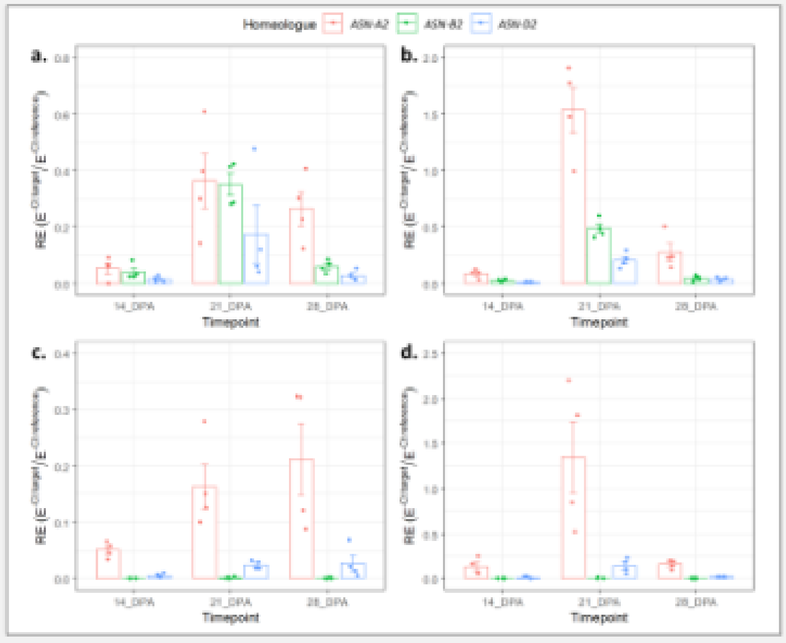


Figure 4. Relative expression of the 3 *ASN2* homeologs in embryo tissue in varieties possessing *TaASN-B2* (Cadenza (**a.**) and Duxford (**b.**)) and lacking *TaASN-B2* (Claire (**c.**) and Spark (**d.**)).

Results

Table 1. Significance values for ANOVA and REML analyses of field trial data.

	2011-2012 ANOVA	2012-2013 ANOVA	Both year's REML
Treatment	0.040	0.009	<0.001
ASN-B2	<0.001	0.270	0.040
ASN-B2*Variety	<0.001	0.002	0.003
ASN-B2*Treatment	0.195	<0.001	0.012
ASN-B2*Variety*Treatment	0.068	0.008	0.100
Year			<0.001
Year*ASN-B2			0.948
Year*Treatment			<0.001
Year*ASN-B2*Variety			0.439
Year*ASN-B2*Treatment			0.009
Year*ASN-B2*Treatment*Variety			0.326

- The deletion of *TaASN-B2* is associated with a significant reduction in free asparagine under field conditions (Table 1, Fig. 5).

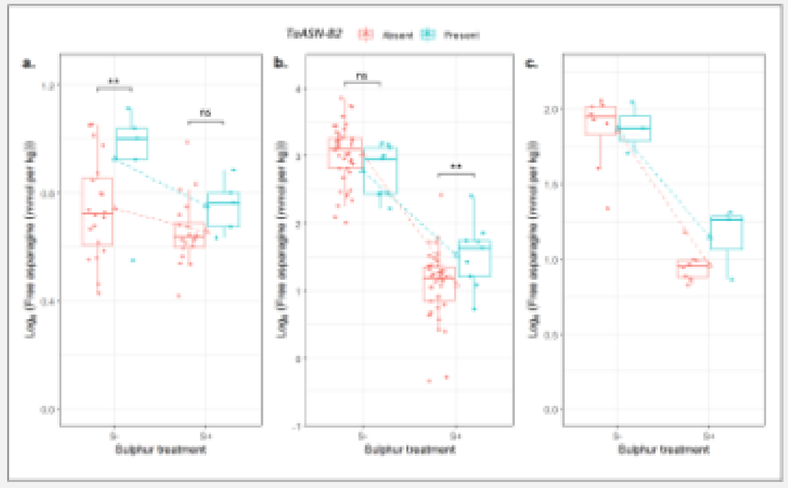


Figure 5. Effect of the *TaASN-B2* deletion on free asparagine levels in varieties grown during the 2011 – 2012 (**a.**) and 2012 – 2013 (**b.**) growing seasons, and for those varieties grown in both seasons (**c.**). Post-hoc Bonferroni tests for comparisons (ns > 0.05, * < 0.05, ** < 0.01).

Conclusion

- Selection for the *TaASN-B2* deletion in wheat breeding germplasm can improve wheat quality by reducing average levels of free asparagine.

References

Oddy, J., Raffan, S., Wilkinson, M.D., Elmore, J.S., Halford, N.G. Stress, nutrients and genotype: understanding and managing asparagine accumulation in wheat grain. CABI Agric Biosci. 2020.

Xu HW, Curtis TY, Powers SJ, Raffan S, Gao RH, Huang JH, Heiner M, Gilbert DR, Halford NG. Genomic, biochemical, and modelling analyses of asparagine synthetases from wheat. Front Plant Sci. 2018.

ROTHAMSTED RESEARCH

Session Nine
Friday, 26th February 2021






FLIGHT-TO-LIGHT & DECLINE OF BRITISH MOTHS

Ishbel Hayes[1,2], James Bell[1], Kevin Gaston[2], Jon Bennie[2]
[1]Rothamsted Research & [2]University of Exeter

INTRODUCTION

UK moth abundance has declined by 31% since 1968[1]. This has largely be attributed to habitat loss and climate change[2]. However light pollution is associated with high insect mortality and has negative effects on moth development, reproduction and foraging[3] so may have contributed to declines.

MEASURING LIGHT POLLUTION

Images from the new satellite Luojia1-01 were geo-corrected and processed to removed noise and clouds to create a new 130m resolution light pollution map of the UK.

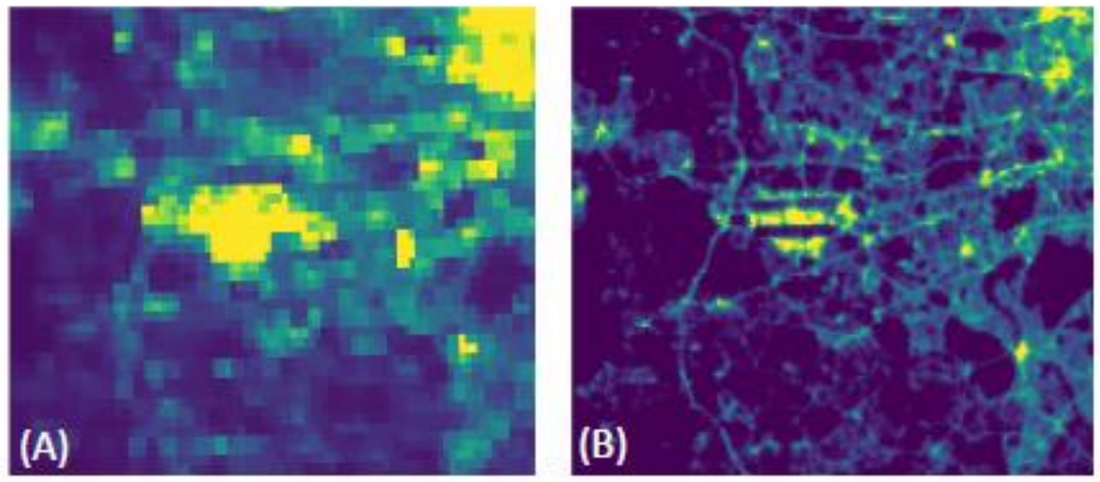


FIGURE 1: Night-time satellite imagery of west London (scale 1:250,000) from existing light pollution data (A) and the new high resolution light pollution map (B).

PRELIMINARY MODELLING

Data from the Rothamsted Insect Survey light trap network was modelled for six woodland site to compare the impact of light pollution on moth trends.

FIGURE 2: Moth abundance for four light polluted traps (green) and two dark-sky sites (navy). Solid lines show significant trends; dashed lines are non-significant.

FURTHER WORK

- Further modelling of moth trends between lit and unlit sites using satellite and light sensor data (SQMs)
- Field work to establish impact of lunar phase on moth activity
- Experiments on impact of wavelength on flight-to-light behaviour

REFERENCES
[1]Bell et al., (2020); [2]Owens et al., (2019); [3]Fox et al., (2013);

DIGITAL IMAGE ANALYSIS FOR HIGH THROUGHPUT PHENOTYPING OF QUINOA PLANTS IN A GLASSHOUSE

Frank G. Okyere[1], Daniel K. Cudjoe[2], Pouria Sadeghi-Tehran[2], Nicolas Virlet[2], Daniel Simms[3], Michel Ghanem[4], Manel Mhada[4] ,Fady Mohareb[3], Malcolm J. Hawkesford[2*],

[1,2] Rothamsted Research, Harpenden, United kingdom, [3] Cranfield University, Milton Keynes, United kingdom, [4] University of Mohammed VI Polytechnic, Morocco

INTRODUCTION

Tools to phenotype quinoa plants and analyse its growth with fertilizer inputs are not readily available. Hence a study was aimed at developing an automated tool(pipeline) to phenotype and analyse the growth dynamics of quinoa with respect to the variability in nutrient application. The phenotyping is done on quinoa plants from growth stage BBCH19(develop nine pair of leaves) – BBCH59(inflorescence visible) as documented by Sosa-Zuniga et al(2017)

Figure 1. Experiment setup in the greenhouse

MATERIALS AND METHODS

Four treatments: High Nitrogen High Phosphorus (NHNP), High Nitrogen Low Phosphorus (HNLP), Low Nitrogen High Phosphorus (LNHP) and Low Nitrogen Low Phosphorus (LNLP), each with five replicates were set up in a controlled glasshouse.

An imaging platform was set up to capture plant images (top view and side view) twice weekly. Computer vision techniques utilizing scientific libraries such as OpenCV, SciPy, Scikit learn etc is applied to build a robust automated pipeline to phenotype quinoa plants.

This tool will be used to classify plants based on their nutrient content, predict chlorophyll content, estimate plant shoot height and width, analyse plant growth rate with respect to the variation in nutrient application

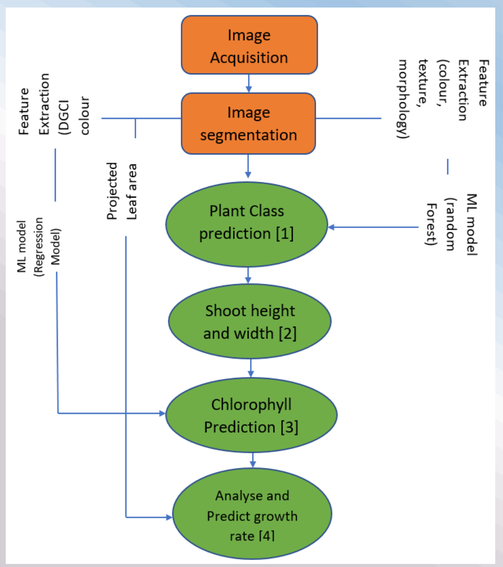


Figure 2. Flowchart of the pipeline for automated phenotyping

Original Image Segmented Image Image for feature Extraction

Figure 3. Image analysis Process

RESULTS AND DISCUSSIONS

As part of the pipeline, a machine learning model(random forest) has been trained to classify plants into HNHP, HNLP, LNHP and LNLP based on their nutrient content. The model with a 98% accuracy(Figure 5) can make more accurate class treatment predictions on unknown data

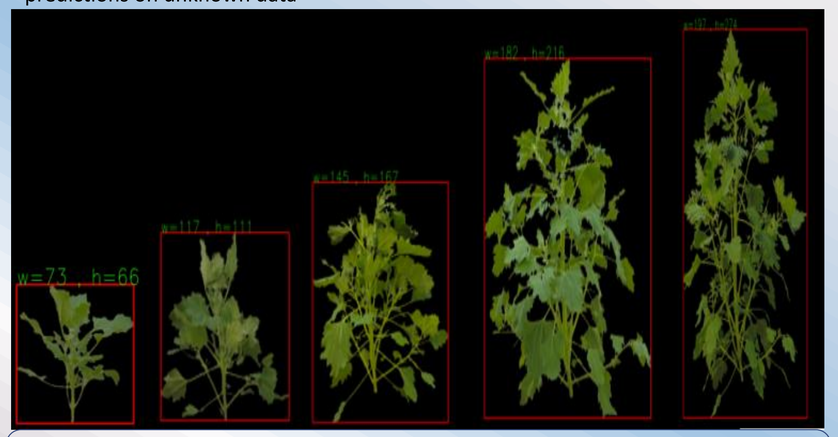


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Figure 4. Plant shoot height and width

As a part of the tool, a script for analysing the plant shoot height and width as well as its canopy area cover has been developed. The tool when used will be able to estimate plant height and canopy area at any given growth stage of the plant (Figure 4).

Script to predict and analyse the crop chlorophyll has also been developed. Colour indices were extracted and correlated with the ground truth. With an R^2 (0.53), a PCR model could predict with 0.38 error the plant chlorophyll. (Figure 6)

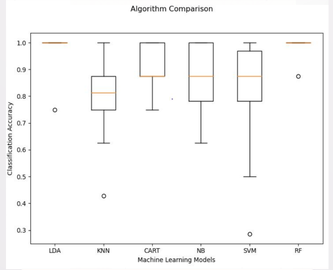


Figure 5. Plant treatment classification

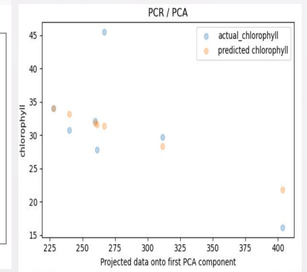
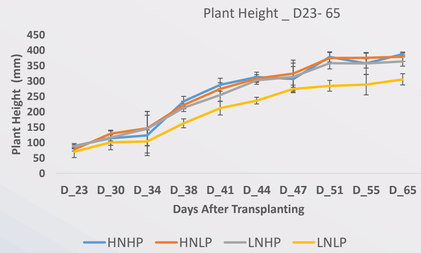


Figure 6. PCA/ PCR for chlorophyll prediction



The HNHP and HNLP treatments had high plant heights comparably with the LNLP plants having the lowest plant height from Day_23 to Day_55

Figure 6. Plant height dynamic analysis

CONCLUSIONS: An experiment is ongoing for developing an automated pipeline/ tool for phenotyping the vegetative growth stage of quinoa plants. This tool when fully developed will be used in batch processing of quinoa plants to predict plant class based on nutrient level, plant shoot height, width and canopy area at the vegetative growth stage. In addition, the chlorophyll content as well as the growth dynamics due to nutrient deficiency will be evaluated.

REFERENCE: Sosa, Viviana (2017) Phenological growth stage of quinoa (*Chenopodium quinoa* Wild) based on the BBCH scale, Annals of Applied Biology 171(124). Doi.10.1111/aab.12358

QUANTIFYING THE TRUE COSTS OF FARMING SYSTEMS

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The environment, and services it provides, must be properly valued in order to prevent further degradation. This is especially true in agricultural systems as agriculture is extremely dependent upon natural capital and ecosystem services and occupies 40% of the earth's terrestrial land surface, so is disproportionately responsible for environmental degradation. Therefore, agricultural systems are a key target for sustainable improvements and there is a need for research into understanding the interdependencies between agricultural land use and management, natural capital and ecosystem service delivery to develop sustainable agricultural systems for the future.

THE LARGE SCALE ROTATIONAL EXPERIMENT - LSRE

Treatment structure and design

- **Main plot treatments:**
  Crop rotation x3: 3, 5 and 7 year rotations with a gradient of functional diversity
  Cultivation x2: Ploughed vs. No/Min-till
  Crop protection x2: Conventional vs. Smart
- **Sub-plot treatments:**
  Nutrient inputs: Cover cropping, green compost and straw incorporation vs. no inputs and straw removal
- **Multi-site**
  Brooms Barn, Suffolk est. 2017 and Harpenden est. 2018
- **60 different 24 x 24m plots, split into two equal sub-plots** per site encompassing all treatment combinations every year

Aerial photograph of the LSRE plots at Brooms Barn, Suffolk

METHODS AND MONITORING

The following variables will be measured on the LSRE which will contribute towards a set of sustainability metrics used to "value" the different treatment/management systems.

Natural capital accounting
Biodiversity: Soil entomology, Soil microbiology, Pollinators and winged insects, Weed diversity, Weed-seed predation, Pest pressure
Soil health: Aggregate stability, Bulk density, Porosity, Infiltration rates, Compaction, Nutrient Status, Microbial diversity, Earthworms, Decomposition rates
Losses from the system: GHG emissions, Leaching of agrochemicals

Conventional accounting
Crop production: Crop yields, Straw yields, Crop quality, Sale prices/premiums
Operational costs: Seed costs, Inputs (agrochemical and organic), Logistics (labour) and Required capital (machinery, grain storage etc.)

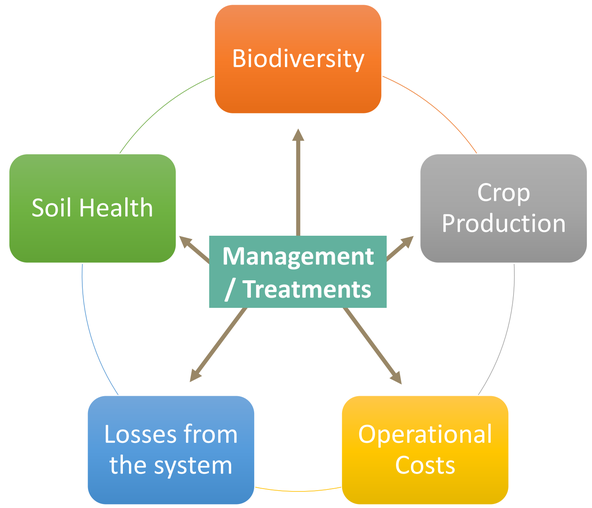

Figure 1: Proposed set of sustainability metrics which are interdependent, with positive and negative effects in response to different treatments/management systems captured in the LSRE.

AIMS AND HYPOTHESIS

Aims

- Decide on a set of sustainability metrics to be measured on the LSRE, which capture the "complete picture"
- Develop practical methods of monitoring natural capital in agricultural systems with application for widespread monitoring on farms
- Identify trade-offs and synergies between different components of the systems
- Place an economic value on natural capital measured and incorporate it into a conventional farm economic model in order to work out the "true cost" of the different farming systems captured in the LSRE

Hypothesis

- As the treatments on the LSRE diverge, initially see a reduction in yield in the more environmentally focused systems but as the natural capital and ecosystem services, such as beneficial insects, build up over time these systems will catch up as the more conventional profit-driven systems become degraded.
- When accounting for environmental degradation and valuing natural capital and ecosystem services the more environmentally focused systems will be more "profitable"

ROTHAMSTED RESEARCH

Lawes Agricultural Trust

University of BRISTOL

UKRI Biotechnology and Biological Sciences Research Council

AGENT-BASED MODELLING OF CROP MANAGEMENT

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UNLOCKING FARMERS' COLLECTIVE INTELLIGENCE TO CREATE WEALTH

Collaborative and co-designed research with farmers is an essential component for developing strategic policies

CONTEXT

- Agriculture is a critical sector for the Moroccan economy (12% of the GDP (MEF*, 2019)).
- Unpredictable and severe climate.
- Domination of smallholder farmers.

RESEARCH GAP

Models of behaviour generally assume that the agents are rational and will act to increase profit but often other social factors come in to play.

RESEARCH OBJECTIVE

Develop an agent-based approach for modelling the dynamics of farming stakeholders' behaviour, in a space-time context and explore how cooperation among them can improve the efficiency of sustainable agricultural production.

METHODOLOGY

1. Literature revue
2. Data collection and analysis
3. Development of agent-based models for case studies
4. Exploration of different scenarios
5. Validation

IMPLICATIONS

Explain how regularities observed at the macro-level can emerge from the interactions of agents at the micro-level.

Develop models to identify the influences on farmer behaviour and how these can be used to improve the sustainability of agriculture.

Explore how cooperation is a key element for delivering multiple ecosystem services

Funded by OCP

**Ministry of Economic and Financial Affairs in Morocco.*

Managing concurrent evolution of resistance to fungicides

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AHDB

Biotechnology and Biological Sciences Research Council

Fungicides are used to protect wheat crops against pathogens that cause disease, maintaining yield and quality

Septoria leaf blotch causes up to 50% yield loss

Fig. 1: Septoria leaf blotch on a wheat leaf.

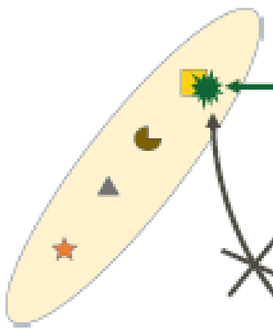


Fig. 2: A simplified representation of a pathogen cell.

What are single-site fungicides?
Single-site fungicides, such as azoles and SDHIs, target only one part of the pathogen's biology, e.g. an enzyme. This slows down the pathogen's growth.

What causes fungicide resistance?
A mutation in the pathogen target-site gene can lead to pathogen strains with resistance to a single-site fungicide.

Why does resistance spread?
When the fungicide is applied, the percentage of the pathogen population that is resistant increases, as resistant strains grow faster.

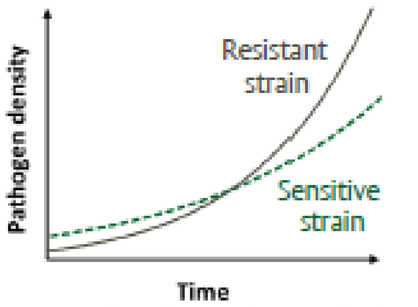


Fig. 3: When fungicide is applied, resistant strains outcompete sensitive strains.

What is concurrent evolution of resistance?
Septoria is currently evolving resistance to both SDHI and azole fungicides at the same time. This is known as 'concurrent evolution of resistance'. Can this process be slowed down?

What is 'resistance management'?
Choosing fungicide programmes that aim to keep selection for resistant strains as low as possible, whilst maintaining control of disease.

A balancing act

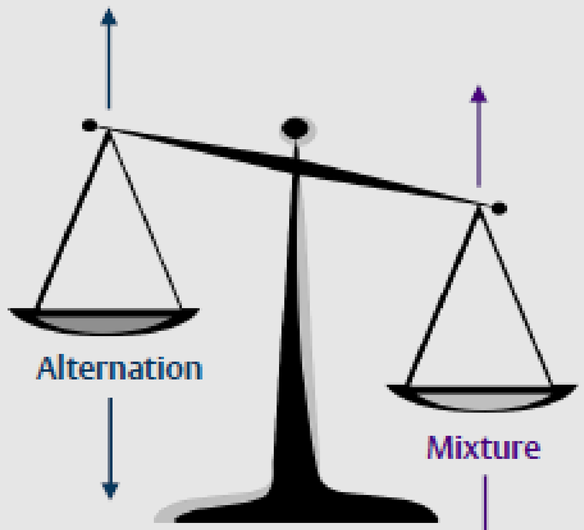
What is the best way to combine two single-site fungicides in a programme? Strategies include alternation or applying fungicides in mixture. Each option introduces trade-offs, and so could either increase or reduce the selection pressure overall.

Increased Selection Reduced

× No mixture effect

× Higher dose of each fungicide per application

× Longer exposure time for both fungicides



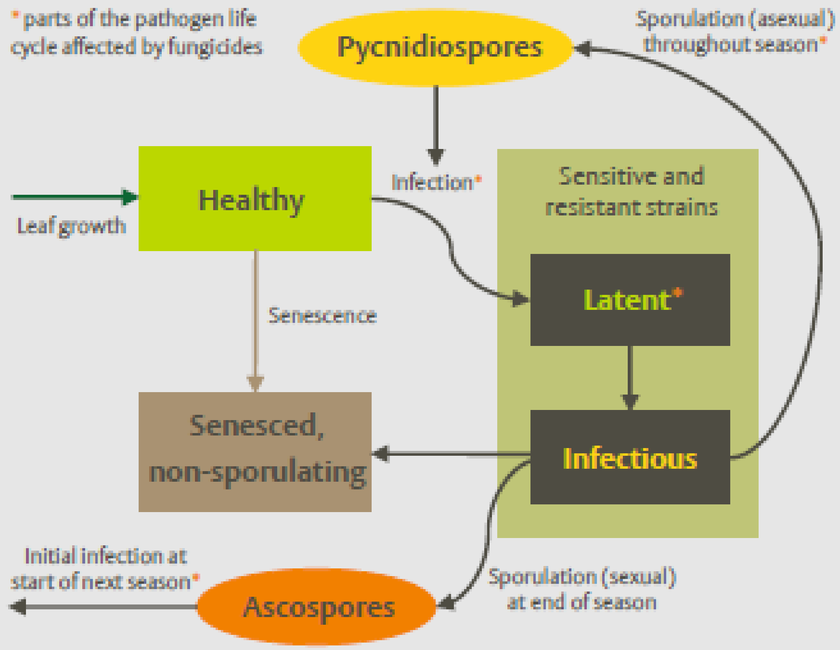
✓ Shorter exposure time for both fungicides (one application each)

✓ Mixture effect

✓ Can use lower dose of each fungicide per application to achieve same level of control

I will use models to run 'what-if' scenarios, testing a large number of possible strategies with the aim of informing and improving resistance management tactics.

Fig. 4: A schematic diagram of a fungicide resistance model for septoria.



References

1. Hobbelen, P.H.F. et al. (2011). Derivation and testing of a model to predict selection for fungicide resistance. Plant Pathol. 60: 304-313. 2. van den Bosch, F. et al. (2011). The dose rate debate: does the risk of fungicide resistance increase or decrease with dose? Plant Pathol. 60: 597-606. 3. van den Bosch, F. et al. (2014). Governing principles can guide fungicide-resistance management tactics. Annu. Rev. Phytopathol. 52: 175-195

Acknowledgements

- Studentship funding provided by AHDB and the Chadacre Agricultural Trust.
- Fig. 1 M. Shaw; Fig. 4 adapted from van den Bosch et al.[2]; Graphics CC BY-NC.

THE ECONOMIC AND ENVIRONMENTAL VALUE OF AGRICULTURAL MODEL-BASED DECISION SUPPORT TOOLS FOR WHEAT CROPS IN MOROCCO

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Precision agriculture (PA) has become increasingly important to farmers particularly in resource-poor and risk-prone settings in the developing world. However, due to cost and technical constraints, deploying PA infrastructure as decision support systems (DSSs) in smallholder farming settings is often hindered.
Remote sensing revealed the potential of assisting several DSSs and improving farmers' decisions in terms of both productivity and the environment.

METHODS

I. SCOPING WORKSHOPS AND SURVEYS :

- Identify DSSs used in Morocco.
- Identify farmers standard practices .
- Define appropriate study cases.

II.DSS ANALYSIS:

- Investigate models behind each DSS.
- Define the list of parameters needed for each DSS.

III.DSS CALIBRATION AND EVALUATION:

- Run DSS simulations with default parameters.
- Calibrate DSS using remote sensing and data assimilation methods.
- Evaluate and assess the value of the DSS.

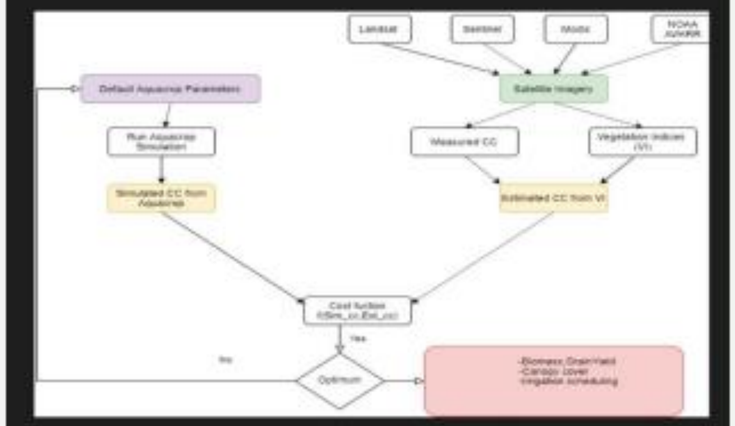

Figure 1 : Aquacrop calibration approach

RESULTS

1.KNOWLEDGE GAP ANALYSIS

- Data assimilation of remote sensing and crop models.
- Necessary datasets are identified.
- Supporting/auxiliary datasets :
  - rainfall and Actual Evapotranspiration (ERA5)
  - Biomass canopy cover and yield
  - Satellite imagery (Modis, Landsat5,Landsa 7,NOAA AVHRR)

2.DATA EXTRACTION:

- Necessary datasets are identified.
- Supporting/auxiliary datasets :
  - rainfall and Actual Evapotranspiration (ERA5)
  - Biomass,canopy cover and yield
  - Satellite imagery (Modis, Landsat5,Landsa 7,NOAA AVHRR)

3.DATA PREPROCESSING:

Figure 2: Landsat 7 Scan Line Corrector (SLC) sensor failure

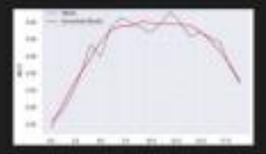

Figure 3: Smoothed NDVI time series using Savitzky-Golay filter

4.CANOPY COVER ESTIMATION FROM VEGETATION INDICES:

Figure 4: .Canopy cover for a calibration field during the 2002/2003 cropping season.

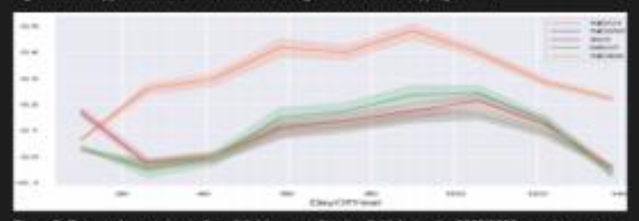

Figure 5: Extracted vegetation indices (VIs) from a calibration field during the 2002/2003 cropping season.

CONCLUSIONS

In this study,data assimilation algorithms were used to improve the estimation accuracy of Aquacrop for wheat production in Morocco.The conclusions are as follows:

- Several vegetation indices were highly correlated with canopy cover.
- Canopy cover estimated using Landsat achieved more accurate estimations than Modis data .

FUTURE WORK

- Conservative and non-conservative parameters of the first DSS to b calibrated (AquaCrop) are identified and will be collected from Moroccan partners.
- Vegetation indices are extracted from satellite data with different resolutions,regression relationships with biomass and canopy cover will be analysed and the best regression model will be determined .

Acknowledgements: We gratefully acknowledge the support of OCP Group through the 'Next generation of agricultural research scientists for Africa' project for funding this work.

ROTHAMSTED RESEARCH

Thank you for viewing the PhD Symposium posters