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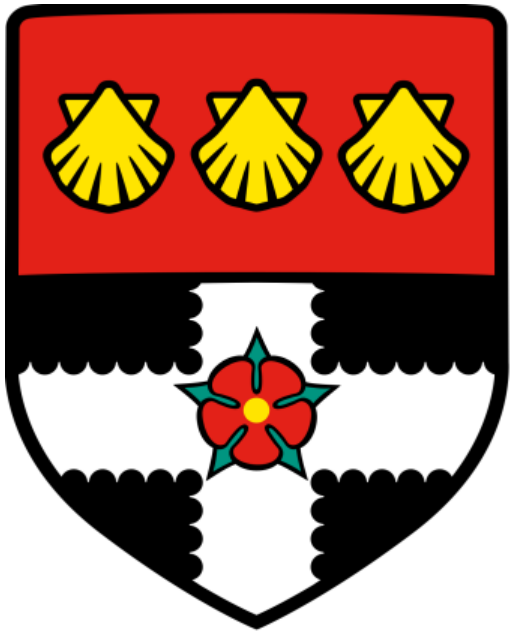

Genetic architecture of wheat yield responses to drought

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A thesis submitted to the School of Agriculture, Policy and Development

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Declaration of Original Authorship

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

Maintaining wheat grain yield under expected more frequent and maybe severe drought episodes requires identifying the drought tolerance traits as well as deciphering the genetic basis of these traits responses to drought, and utilising potential symbiotic endophytes to alleviate drought effects. The aim of this project was to conduct an in-depth study of the genetic architecture of wheat responses to drought, deciphering the genetic basis of both source and sink traits under field conditions, as well as, investigating the ability of the endophyte fungus *Piriformospora indica* to increase yield in both well-watered and drought conditions and identify QTL underpinning drought-resistance traits influenced by endophytic growth.

In the field trial, a representative subset of the elite eight-founder population, comprising 384 RILs, the founders and a check variety 'Kielder' were tested in rainfed vs irrigated field blocks, monitoring soil moisture content at different depth intervals. Field plots were phenotyped throughout the growing season using integrative drone-based and proximal sensing approaches.

The results showed maximum soil moisture deficit (SMD) peaking over 120 mm in the rainfed plots, with large deficits (>75 mm) from late April that coincided with tillering and more prolonged large deficits from mid-June to mid-July (>100 mm), significantly decreasing crop canopy indices at all measured dates post-irrigation and causing significant increase in canopy temperature of rainfed plots, all driving an average yield reduction of 32.8% which was significantly genotype dependent. Also identifying traits most significantly ($p \leq 0.001$) correlated with yield revealed grains.m^{-2} (r=0.68) and (r=0.72) and canopy temperature depression (CTD) (r=0.52) and (r=0.61) in rainfed and irrigated condition, respectively. QTL analysis for yield revealed a total of 16 novel QTL expressed commonly across both treatments explaining individually 1 to 4.5% as well as treatment dependent QTL. With remarkable examples of grain yield QTL collocating with major QTL such as grains.m^{-2} QTL on chromosome 3A and *Rht-D1* pleiotropic region on chromosome 4D and highlighting significant SNP-SNP epistatic interactions for yield occasionally coinciding with QTL for crop canopy indices.

Investigating the response of 200 MAGIC lines to *P. indica* inoculation showed the potential of the endophyte to significantly increase yield in well-watered and drought conditions, however, for most traits, there was significant difference in genotypes responses to

colonization. Several QTL unique to colonized plants were detected on most chromosomes and linked to measured traits under drought, Those QTL can be investigated as candidate genes governing the symbiosis between wheat and *P. indica*.

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1 Chapter 1: Introduction and literature review

1.1 Overview

Water availability is a fundamentally important resource in arable agriculture and in many important production areas (North American prairie, Caucasian steppe, semi-arid Mediterranean basin) is the single edaphic factor which most limits production. Even in the maritime climate of the UK, however, up to 30% of the wheat acreage is grown on drought-prone soils, resulting in a >10% annual loss in yield (Foulkes *et al*., 2007). In all parts of the globe, increased likelihood of extreme weather events/patterns driven by global warming make scientific progress towards understanding how to breed crops able to efficiently and stably yield food supplies on defined, low amounts of water more important than ever before.

Despite the importance of this topic, there is some confusion in the literature, stemming mainly from the paradox about which characteristics sustain better yield under stress, depending on the nature of the water limitation and plant ideotype targeted and although it is well understood that relevant genetic variation for several drought tolerance-related traits exists (Dodd *et al*., 2011), few have been the subject of QTL mapping.

The recent creation and validation of an eight-parent wheat mapping population resource (Mackay *et al*., 2014) offers new possibilities to achieve a far better understanding of the genetic and physiological trade-offs between sustainable crop water use, high yield potential and performance under limiting water conditions in wheat.

From a plant-fungus symbiosis perspective, recent studies of a novel fungal endophyte of wheat, *Piriformospora indica*, have shown a 40% increase in total seed weight of inoculated versus uninoculated control (unstressed) plants and up to 2.2-fold increase in total seed weight of inoculated versus uninoculated in droughted conditions (Hubbard *et al*., 2014).

This PhD thesis aimed to conduct an in-depth study of the genetic architecture of wheat responses to drought, deciphering the genetic basis of both source and sink traits under field conditions, as well as, investigating the ability of *P.indica* to increase yield in both well-watered and drought conditions and identify QTL underpinning drought-resistance traits influenced by endophytic growth.

To accomplish this, the winter wheat elite 8-founder MAGIC population was used, as it captures more than 80% of the allelic diversity found in the UK winter wheat elite breeding material, making it likely that the markers identified in this analysis are segregating in the UK population as a whole.

1.2 Wheat background

Wheat is one of the major food resources worldwide providing 20% of total consumed calories, grown globally on more than 218 million hectares with total production of 772 million tons in 2017. Throughout history, wheat was a prominent food source for both humans and livestock thanks to its adaptability and the success of growing under wide range of latitudes from 67 °No in Scandinavia and Russia to 45 °So in Argentina hosting highly diverse environmental conditions. Currently, only 5 % of world wheat production is accounted for by tetraploid durum wheat (*T. turgidum* L. ssp. *durum* (Desf.), mainly used for pasta industry with production hub and adaptability to semi-arid Mediterranean climate and 95% by hexaploid bread wheat (*Triticum aestivum* L.) (Shewry, 2009). Due to large differences in the availability of arable lands, cultivation resources and environmental stresses, the cultivated area in individual countries ranges from 4 hectares in Qatar to 24 million hectares in China, and variation in yields ranges from averages of 0.4 t/ha in Somalia to 10.2 t/ha in Ireland (FAO, 2019a).

Wheat flour is processed in a variety of ways producing several end products such as bread, cakes, biscuits, noodles and pasta depending on gluten content (Curtis *et al*., 2002, Shewry, 2009). The wheat grain is rich in vitamins B6, folate, thiamin and riboflavin and minerals such as manganese, phosphorus and zinc. Compared to other cereals, wheat has higher protein, fibre and fat content (Sramkova *et al*., 2009). Although different regions of the world have different end uses for wheat flour and thus measures of quality, all wheats can be classified as either ‘hard’ or ‘soft’ depending on the particle size distribution of wheat flower and the grains’ resistance to crushing. This is based on the functionality of puroindoline proteins, as the presence of both puroindolines (a) and (b) in their wild state results in soft wheat, while absence or lack of functionality of one of them determines hard texture (Morris, 2002), puroindolines are governed by the soft (Ha) and hard (ha) alleles on the short arm of chromosome 5D (Doekes and Belderok, 1976). In the UK, the National Association of British and Irish flour Millers (NABIM) classifies wheat cultivars into four groups: Group 1: these are the cultivars that produce consistent milling and baking performance with high protein content of 13%; Group 2: may be used by millers in ‘general purpose’, some perform

inconsistently while others are suited to specialist flours; Group 3: cultivars possessing soft milling characteristics, low protein, good extraction rates, and extensible but not elastic gluten, used for biscuit and cake; and lastly, Group 4: these cultivars can be either hard or soft but are unsuitable for milling and are grown mainly for animal feed (NABIM, 2019).

Bread wheat cultivars are classified based on their seasonal growth habits into spring and winter wheat, where winter wheat requires a period of low temperature exposure during winter to induce flowering known as the vernalization requirement, while spring wheat does not require vernalization to shift from vegetative to reproductive stage (Law, 1987).

Wheat belongs to the family *Poaceae* (subf. *Pooideae*), genus *Triticum*. There are four cultivated species in the *Triticum* genus; the diploid einkorn wheat (*Triticum monococcum*) representing the AA wheat genome is the oldest cultivated wheat species and was gradually replaced by emmer wheat due to its low yielding potential. Emmer wheat (*Triticum turgidum*) is a tetraploid species which arose from the domestication of wild tetraploid Emmer wheat. The only *Triticum turgidum* that is still commercially cultivated is the subspecies *durum*. Hexaploid bread wheat resulted from hybridization of diploid *Aegilops tauschii* (DD) and tetraploid *Triticum dicoccoides* (AA BB), and its high yield potential, broad adaptability and bread-making and nutritional properties led it to become one of the most significant crops in human history (Simons *et al*., 2006, Isidore *et al*., 2005).

Domesticated hexaploid bread wheat originated more than 10 thousand years ago near the upper reaches of the Tigris and Euphrates rivers, in present-day south-eastern Turkey and northern Syria, an area known as the Fertile Crescent (Lev-Yadun *et al*., 2000). The newly domesticated crop was transported through human trade and migration routes across the world in different directions, through Anatolia to Europe through Greece (8000BP), then across to Italy, France and Spain and north via Balkans to the Danube (7000BP), to reach Scandinavia and the UK (5000BP). Another route went through Iran to central Asia and finally China (3000BP), a third route to Africa happened through Egypt. Finally, wheat was introduced to the New World via Mexico in 1529 and to Australia in 1788.

1.3 Effects of drought stress on wheat production

Although variability in weather patterns and the consequent temperature and hydric stresses have always been a challenge to global agricultural production, with a rapidly growing population and an accelerating rate of greenhouse gas-driven global warming, the threat to global food security has never been higher (Godfray *et al*., 2010).

Drought is one of the main threats to sustainable crop production as changes in precipitation patterns accompanied by more drought episodes coupled with increased water demand due to agricultural intensification in many parts of the world make drought stress currently a significant and potentially devastating constraint to crop production (Mishra and Singh, 2010, Semenov and Stratonovitch, 2015). In 2009 Kenya was faced with an extremely dry year, reducing wheat yield 45 % compared to 2010's good crop season and yield losses of 46% were experienced in Australia during the 2010 growing season compared to the previous 50 years average (FAO, 2019b).

Field trials for spring and winter wheat in different locations and years emphasise the profound negative effect of different degrees of drought on grain yield. For example, Afzal *et al*. (2017) tested a diversity panel of 213 spring wheat lines and commercial varieties under naturally rainfed, investigating yield response in control and supplementary irrigated treatment in two consecutive years and reported an average of 62% yield loss in the rainfed field. Another trial using a CIMMYT association panel of 287 lines reported a yield reduction of 29% when tested under contrasting well irrigated and rainfed conditions, in a doubled haploid bi-parental population comparing full to limited water irrigation (Edae *et al*., 2014), grain yield was reduced by 39% in the limited water treatment (El-Hendawy *et al*., 2017). And a more recent field trial investigating 108 advanced wheat lines reported 44.6% yield losses under drought treatment. Under mild drought, Elfeki *et al*. (2018) tested a winter wheat doubled haploid population and reported a loss of 20% of its yield potential when the irrigation was limited, compared to full irrigation and in a different experiment testing three commercial cultivars, yield was reduced by 47% (Thapa *et al*., 2018).

Although rarely reaching the level of severe drought of the type experienced in Kenya or Australia, crop productivity is nonetheless limited in the UK as a result of drought episodes occurring at different stages of crop growth and development during spring and summer, as average rainfall is 130 mm less than average evapotranspiration (Jones *et al*., 1985). It is reported that only a minority of UK arable land planted with wheat is subjected to drought in an average year (Bailey, 1990), mainly because deep soils with fine texture are able to hold an adequate amount of moisture to compensate for this shortfall (Gales and Wilson, 1981) and brought to field capacity before spring, but in a dry year, the majority of arable soils might face various degrees of drought. Various experiments reported dry years to cause yield losses of 22-68%, depending on the level of drought that varied significantly among years and field

locations and genotypic response (Foulkes *et al*., 2001, Foulkes *et al*., 2002, Whalley *et al*., 2006, Foulkes *et al*., 2007, Dodd *et al*., 2011).

Drought stress occurring during different stages of wheat growth causes different effects; early season drought resulting in low establishment and reduced shoot and root length (KizilgeÇİ *et al*., 2017), whereas during the later vegetative stages it results in reduced tillering (Duggan *et al*., 2000, Foulkes *et al*., 2002), reduces pollen fertility during reproductive stage (Dong *et al*., 2017), subsequently reducing grains/ear (Foulkes *et al*., 2002, Senapati *et al*., 2019), while late season drought induces earlier senescence (Farooq *et al*., 2014) and reduces grain filling (Liu *et al*., 2017). All the above, either as isolated or multiple episodes lead to varying levels of reduction in grain yield.

1.4 Breeding for drought tolerance

Breeding for improved drought tolerance and understanding its genetic underpinnings is impaired by the fact that drought tolerance is a complex quantitative trait with low heritability, showing a high genotype by environment (G×E) interaction and in most cases it is confounded with and accompanied by other stresses such as heat (Fleury *et al*., 2010) and by complexity of plant responses to drought, which is amplified under field environment by significant variation in severity and timing of drought among years (Dolferus *et al*., 2019).

Improving and speeding up the breeding programs may be achieved via combining comprehensive understanding of target environments and knowledge about genetic control of drought through key physiological traits, nevertheless this approach is highly dependent on the genetic correlation of the physiological trait of interest with final grain yield, extent of genetic variability, level of heritability and extent of G×E interactions (Mir *et al*., 2012).

Various morphological and physiological traits were identified to contribute to yield stability under drought, such as stem-soluble carbohydrates (Foulkes *et al*., 2002), green flag leaf persistence and slow senescence rate (Foulkes *et al*., 2007, Lopes and Reynolds, 2012), CO_2 exchange rates, water use efficiency and stomatal conductance (Kimurto *et al*., 2009), thousand grain weight, grains/ear and tillers/plant (Afzal *et al*., 2017), canopy temperature depression (Thapa *et al*., 2018), increased root length density (Ehdaie *et al*., 2012), early flowering date escaping terminal drought (Blum, 2011) and presence of long awns (Taheri *et al*., 2013). However, the relative significance of particular traits depends on the severity, duration and timing of the drought, for example, the last two traits did not add significant value in case of mild and early season drought (Foulkes *et al*., 2007).

With such a wide palette of genetically determined traits that could potentially mitigate drought stress, the key to an accurate mapping of the most relevant traits is adoption of high throughput and precise phenotyping in target environments (Tuberosa, 2012). The idea of being able to phenotype large experiments with massive numbers of lines in a short time with high repeatability and at low cost could be attained using less-complex tools such as RGB high resolution cameras for rapid (and time series) assessment of vegetation indices, ground cover, or plant establishment counts (Mullan and Reynolds, 2010).

Several researches have indicated the repeatability and high heritability of spectral reflectance indices; Babar *et al*. (2006) reported similar evidence for high accuracy on Simple Ratio (SR), Water Index (WI) and Normalized Water Index-1 (NWI-1) measured in wheat subjected to different water regimes.

The availability of commercial devices that measure spectral index Normalized Difference Vegetation Index (NDVI) and estimate ground cover, biomass and senescence rate made it feasible to phenotype of large mapping populations successfully (Lopes and Reynolds, 2012).

In trials carried out by Tattaris *et al*. (2016), imposing different combinations of heat and drought stresses on wheat, significant correlations were found between canopy temperature and NDVI and key measured phenotypes such biomass and yield, with higher correlations when using drone-based imagery compared to proximal ground-based ones. Air-born NDVI and thermography were found to be repeatable and suggested as a reliable guide to selection for root depth and grain yield as reported by Li *et al*. (2019b).

Since yield is a complex trait that needs to be explained by various phenotypic traits measured throughout the growing season (under both optimum and water stressed conditions), identifying QTL governing high throughput measured traits such as canopy temperature (as a proxy for root distribution and/or depth) and "NDVI" (stay green) became a necessity. Pinto and Reynolds (2015) carried out field experiments testing spring wheat under drought and/or heat stress and found that optimal root distribution where roots are growing near the surface in hot and irrigated conditions while they proliferate deeply under drought, is to be associated with QTL related to cooler canopies. In a different field trial, Gao *et al*. (2015) tested 246 F_8 RILs for NDVI at different time points and unravelled eight QTL for NDVI explaining 4.0–9.8% of the phenotypic variances with heritability of 0.8-0.94.

1.4.1 Genetic bases of quantitative traits in wheat

Traits with significant importance tend to show a complex mode of inheritance and to be governed by several genes with additive and/or epistatic nature in addition to strong response to environmental factors.

A basic definition for quantitative trait locus is a genomic region that is statistically associated with the genetic variation of a complex trait (Geldermann, 1975). Genotype-phenotype associations are identified when different classes of marker alleles show statistically different trait values, due to linkage disequilibrium between the tested marker positions and genomic loci that govern trait variation (Lynch and Walsh, 1998). Reliable QTL detection requires high quality replicated phenotypic data, a trait of high heritability (Abiola *et al*., 2003) and a mapping population of desired type and size (Singh and Singh, 2015).

Mapping populations are generated by crossing two or more genetically diverse lines and handling the progeny in a definite fashion, for a long time the most common type of experimental population to be used for quantitative genetics was the biparental cross, combining the genomes of two parents with contrasting trait(s) in order to decipher genomic regions governing these traits. They are developed in various forms as summarized in Table 1.1, but due to the narrow genetic base of these kind of populations, all that is captured is a small snapshot of QTL affecting the traits, offering the possibility to only detect genomic regions that differ among the two founders and alleles occurring in high frequency (Jannink, 2007).

To overcome these drawbacks, association mapping was introduced as a complementary mapping approach, whereby sampling from distantly related individuals that make up a typical association panel offers a wide view for the whole population and captures greater diversity than any bi-parental population. Genome wide association studies (GWAS) genetically dissects complex phenotypes using the pattern of linkage disequilibrium (LD) existing in collections of diverse germplasm (Yu and Buckler, 2006).

GWAS could generate finer mapping resolution in comparison to QTL mapping with bi-parental populations, as the latter can only rely on the informative meiosis accumulated during population development (Morgante and Salamini, 2003). However, a very large number of lines is needed to ensure sufficient power for detecting target genomic regions and confounding associations due to population structure and linkage disequilibrium need to be carefully controlled (Huang *et al*., 2015).

Nested Association Mapping Population (NAM) was then proposed to combine the advantages of both association and linkage mapping approaches. NAM populations are created by crossing a set of diverse founder parents to one or two common parents and a set of RILs from each of these crosses is generated using the single seed descent (SSD) method (Yu *et al*., 2008).

The Multi-Parent Advanced Generation Inter-Crosses (MAGIC) is believed to overcome some weaknesses of the aforementioned designs, increasing simultaneously the power, diversity and resolution of detecting genomic regions associated with different traits. In this schema, multiple inbred founders are intermated for several generations before deriving the inbred lines each inheriting a unique fine-scale mosaic genome of contributions from all founders, all together making a diverse population (Figure 1.1). The MAGIC approach offers high accuracy and fine resolution for detecting QTL, facilitated by the extensive breakdown of LD that follows from the relatively high number of crossovers during the inter-crossing generations (Flint *et al*., 2005).

The MAGIC concept was developed originally by (Mott *et al*., 2000) in order to fine map small-effect QTL in mice. The idea was subsequently adopted by plant scientists due to its advantages (Cavanagh *et al*., 2008) and MAGIC populations were developed in several crops with 4-8 parents such as Arabidopsis (Kover *et al*., 2009), spring wheat (Huang *et al*., 2012), chick pea (Gaur *et al*., 2012), rice (Bandillo *et al*., 2013), winter wheat (Mackay *et al*., 2014, Stadlmeier *et al*., 2018), maize (Dell'Acqua *et al*., 2015), tomatoes (Pascual *et al*., 2015), barley (Sannemann *et al*., 2015), Cotton (Li *et al*., 2016), Durum wheat (Milner *et al*., 2016) and cowpea (Huynh *et al*., 2017).

Table 1.1 Summary of the features of some common mapping populations (Singh and Singh, 2015)

	Mapping population			
Feature	**F_2**	**Backcross**	**RIL**	**NIL**
Perpetuation	ephemeral	ephemeral	perpetual	perpetual
Genetic composition	Homo & heterozygotes	homo & heterozygotes	homozygotes	homozygotes
A genotype represented by	One plant	One plant	One line	One line
Generations needed	Two	Two	7–8 or more	8–10
Number of crosses	One (F_1)	Two (F_1 & backcross)	One (F_1)	6 or more (F_1 & backcrosses)
Selection during development	None	None	None	Yes
Recombination rounds	One	One	About two	1+ backcrosses
Segregation ratio markers	Different	Different	Same	Same
Suitable for:				
(i) Oligogene mapping	Yes	Yes	Yes	Yes
(ii) QTL mapping	No	No	Yes	Yes
(iii) Fine mapping	No	No	No	Yes
(iv) Mapping of heterosis	Yes	No	No	No
(v) Positional cloning	No	No	No	Yes
(vi) Assessment of QTL X genotype interaction	No	No	Yes	Yes
Minimum QTL X QTL interaction	No	No	Yes	Yes
Mapped loci belong to	either parent	either parent	either parent	donor parent
Analysis covers	whole genome	whole genome	whole genome	genomic segment

1.4.2 Haplotype reconstruction in MAGIC populations

A prerequisite to carry out genetic analysis of multi parent-population is defining the parental origin of marker alleles along the fixed chromosomes, known as haplotype reconstruction, then deploying the appropriate computational tools that can process the allelic segregation patterns associated with marker genotypes and setting the framework which is better fitting the associated founder probabilities, in order to construct multi-parental linkage map and mapping of quantitative traits.

Two major factors cause various degrees of ambiguity in inferring the parental origin of allelic information. the first one is caused by information acquired from the bi-allelic SNPs markers system, which could be either fully informative or non-informative in a simple cross, a SNP marker system will never be totally informative in a multi-parent population. In theory there might be alleles equal to number of founders segregating at each genomic locus, but a SNP marker system can't capture more than two of them, making it impossible to differentiate between alleles that are Identical By State (IBS) from those that are Identical By Descent (IBD), unless we apply probabilistic models accounting for founder haplotype probabilities. The second factor is the severe bottlenecks that characterise crop history, implying genomic regions to show IBD stretches and hindering the power to detect the parental origin of marker alleles.

Several statistical tools were developed to reconstruct the genome of each RIL as a mosaic of the founder haplotypes and trace its allelic information back to the founder lines. For example, R package R/HAPPY (Mott *et al.*, 2000) which assumes all ancestry combination to be equally possible, ignoring the pedigree information. R/QTL developed by Broman *et al.* (2003) includes multipoint probabilities computed conditionally on the observed marker data at flanking markers, resulting in more accurate inference. More recently, R/mpmap (Huang and George, 2011) was released to analyse MAGIC population data combining and extending functions of R/HAPPY and R/QTL.

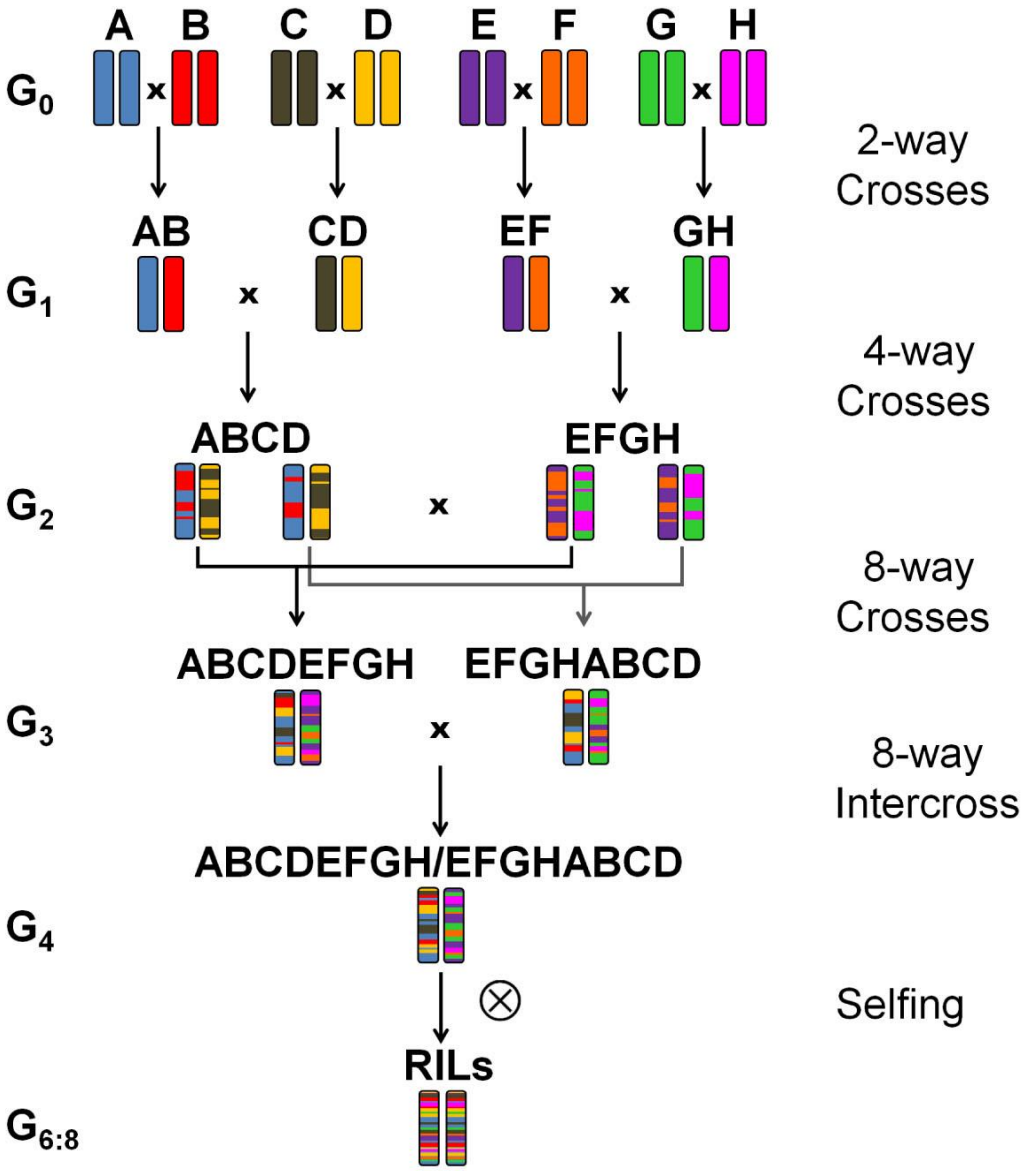

Figure 1.1 Generic 'funnel'-based crossing scheme for an eight founder MAGIC population. Reproduced from (Cavanagh *et al*., 2008).

Table 1.2 Quantitative trait loci (QTL) regions identified for drought tolerance

Trait	Chromosome	Mapping populations	Reference
Seedling vigour	6A	RILs	(Spielmeyer *et al*., 2007)
Chlorophyll content	3A,3B,3D,4B,6A	RILs	(Kumar *et al*., 2012)
Stomatal conductance	2B,2D,4A,6D	Doubled haploid	(Wang *et al*., 2015)
Water soluble carbohydrates	1A, 1B,1D, 4A	Association panel	(Ovenden *et al*., 2017)
Root diameter & surface area	1D, 2A, 2B, 2D, 3A, 4A, 4B, 5B,5D, 6D, 7A, 7D	Advanced backcross population	(Ibrahim *et al*., 2012)
Flowering date	1B,1D,2B,4A,4D,5A,7A	RILs	(Mathews *et al*., 2008)
	1B,3A,3B,4B,5A,6B	Doubled haploid	(Gahlaut *et al*., 2017)
Plant height	1B,1D,2B,3A,4A,5A,5B,6B	RILs	(Mathews *et al*., 2008)
	3D,4B,5A,6A	Doubled haploid	(Gahlaut *et al*., 2017)
Leaf temperature	1A,1D,3A,3B,5B,6A	RILs	(Kumar *et al*., 2012)
CT	1A,5A,6A,6B,6D	Association panel	(Li *et al*., 2019a)
Stay green	1A, 3D, 7B	RILs	(Joshi e*t al.*, 2010)
Grains/ear	1A,2A,3A		(Xu *et al*., 2017b)
Tillers/m^2	1B,5A	Doubled haploid	(Gahlaut *et al*., 2017)
Thousand-grain weight	2A	Association panel	(Ahmad *et al*., 2014)
	1B, 4A, 4B, 7A, 7D	Association panel	(Nezhad *et al*., 2012)
Grain yield	1D, 2B, 3A, 3B, 4A,4B,5B, 6A,6D,7A, 7B	RILs	(Alexander *et al*., 2012)
	3B	Doubled haploid	(Bennett *et al.*, 2012)
	1B,2B,3D,5A,5B,5D,6A,6D	Association panel	(Qaseem *et al*., 2019)

Since drought tolerance traits are mostly polygenic, the advances in QTL mapping approaches and the availability of well-structured mapping populations provide the perfect pipeline to unravel the genetic underpinnings of such complex traits with the possibility of more robust findings by applying high throughput phenotyping (Kulkarni *et al*., 2017), In the recent past, many studies have been conducted to identify QTL governing morphological and physiological traits under various degrees of drought (Table 1.2).

1.5 Symbiotic Fungus *Piriformospora indica*

Endophytes are microorganisms that spend part(s) of their life cycle within living host tissues inter/intracellularly without giving rise to obvious damage to the plant (Sun *et al*., 2014). The behaviour of fungal endophytes varies from mutualistic (White and Torres, 2010) to pathogenic (Tellenbach *et al*., 2011), when the relation includes long term interaction between the two species with one of them living inside the other, it is known as 'endosymbiosis', where mycorrhiza is the best studied species for such interaction beside other non-mycorrhizal fungi (Weiss *et al*., 2011).

Increased attention is being paid to the growth enhancing effect of endophytes on plants driven by the hope that they may effectively increase crop productivity by means of boosting the plant tolerance to abiotic stresses such as heat (Hubbard *et al*., 2012, Ismail *et al*., 2018), drought (Hubbard *et al*., 2014, Khan *et al*., 2015), high salinity (Halo *et al*., 2015, Ghaffari *et al*., 2016, Molina-Montenegro *et al*., 2018) and heavy metals (Dourado *et al*., 2015). Fungal endophytes are known to be capable of increasing resistance to biotic stresses, such as insects (Hammer and Van Bael, 2015, Lopez and Sword, 2015) and microbial pathogens (Waqas *et al*., 2012, Dutta *et al*., 2014, Rabiey *et al*., 2015, Rabiey and Shaw, 2016).

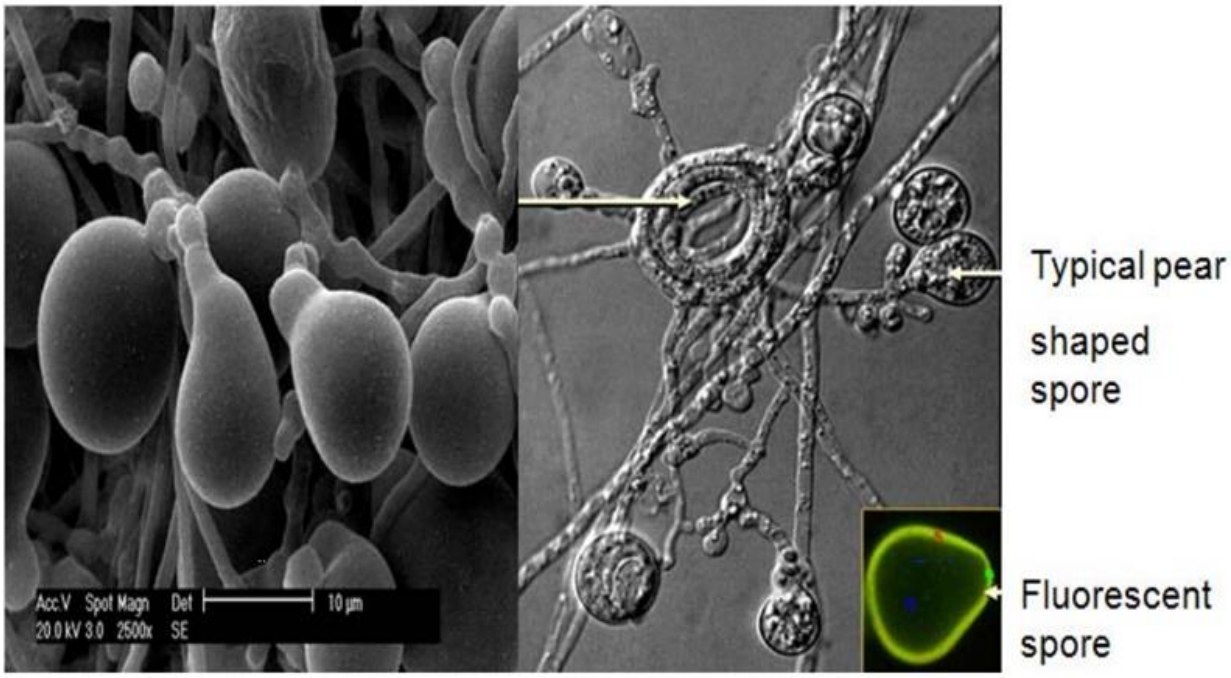

Figure 1.2 Coiled hyphae and pear shaped chlamydospores (right) and electron microscopic view of spores (left). Reproduced from (Varma *et al*., 2012).

Piriformospora indica (*Serendipita indica*) was isolated for the first time as a contaminant during isolation of arbuscular mycorrhizal fungus *Funneliformis mosseae* spores from the rhizosphere of *Prosopis juliflora* and *Ziziphus nummularia* in the sandy desert soils of Rajasthan, northwest India. After extensive molecular genetics and ultrastructural analyses, it was described as a new species following the order Sebacinales, Basidiomycota. Its name was derived from the pear-like chlamydospores "*piriformospora*" and the geographic territory where it was isolated for the first time "*indica*" (Verma *et al*., 1998). *P.indica* thin walled hyphae (0.7 to 3.5 µm) typically appear as coiled cord of highly mingled threads, with mostly vitreous or white mycelia (Figure 1.2). By maturity, mycelia produce either single or clustered distinctive pear-shaped chlamydospores (Varma *et al*., 2012). It was found to grow successfully using different synthetic media, with improved aspergillus medium ranking the best among them (Hill and Käfer, 2001).

P.indica has a very wide range of host plants (that were tested in the last years under field and/or controlled environment conditions) exceeding 50 different species which encompass monocots, dicots, perennial and annual plants (Kost and Rexer, 2013) mainly via direct

manipulation of phytohormone signalling pathways during mutualism with plants (Varma *et al*., 1999).

Root colonization by *P.indica* follows the procedure of intracellular germination of chlamydospores, which then form intracellular hyphae to penetrate the rhizodermal and cortical tissues, this biotrophic colonization does not trigger defence responses in the host tissues indicating that it does not rely on cell death for successful colonization (Jacobs *et al*., 2011). It depends on the potential for suppressing innate immunity in roots involving phytohormones such as glucosinolate, salicylic acid (SA) -related defence pathways and JA-mediated suppression of early immune responses, Moreover the ability to significantly reduce the secretion of immunity-associated proteins (e.g., PR1 and PRRs) is promoting disturbance of endoplasmic reticulum (ER) integrity by *P.indica*, thereby facilitating colonization (Schäfer *et al*., 2009, Qiang *et al*., 2012).

P. indica is the archetype for the mutualistic symbiosis between fungi and Angiosperms, conferring growth promotion and inducing tolerance to various stresses (Varma *et al*., 2012). *P.indica* has a reported role in nutrient mobilization. Phosphorous mediation and translocation from culture medium to the host plant as an energy dependent process by Varma *et al*. (2001), and *P.indica* was found to facilitate access to the necessary amounts of complex, condensed or insoluble forms of phosphate by producing significant amounts of acid phosphatases (Archana *et al*., 2000, Ngwene *et al*., 2015).

Inoculation with *P.indica* improved germination and survival rates of *in vitro* grown chickpeas, soybean and peas, to be near 100% while it was about 50% in the corresponding controls. And increased plant height of chickpeas and mung beans by 35.7 and 14.2%, respectively and increase of fresh weight of 9 and 11% respectively in inoculated plants compared to controls (Pham *et al*., 2008). Following the same trend, Varma *et al*. (1999) tested four weeks old plants of maize, tobacco and parsley grown in pots and found inoculated plants to have increased total shoot and root biomass by 100%.

In terms of its effect on disease tolerance of its host plant, *P.indica* is reported to mediate reductions in symptoms severity caused by root rot (*Fusarium culmorum*), soil-borne take-all disease (*Gaeumannomyces graminis* var.*tritici*) and stem rot (*Pseudocercosporella herpotrichoides*) in wheat (Ghahfarokhy, 2011). Also, it was found to induce systemic resistance in barley leaves against the powdery mildew *Blumeria graminis f.sp. hordei* and in *Arabidopsis thaliana* against the powdery mildew *Golovinomyces orontii* (Waller *et al*., 2005,

Stein *et al*., 2008). Panda *et al*. (2019) found *P.indica* to induce systemic resistance against *Alternaria solani* the causative agent of tomato blight.

In recent studies *P.indica* showed the potential to reduce the severity of crown rot at seedling stage and Fusarium head blight diseases under simulated UK weather conditions in addition to significantly increasing thousand grain weight, total grain yield and above ground biomass (Rabiey *et al*., 2015, Rabiey and Shaw, 2016). Anwar *et al*. (2019) reported *P.indica* to significantly decrease disease severity and area under the disease progress curve, when two susceptible wheat varieties to leaf rust were inoculated with the endophyte under disease stress.

A number of studies have shown that *P.indica* could boost abiotic stresses tolerance. In Arabidopsis subjected to different levels of drought at different stages, *P.indica* was reported to increase germination rate, give a threefold increase in the fresh weight and double the chlorophyll content of 18 day old seedlings (Sherameti *et al*., 2008). Inoculation with *P.indica* mitigated thylakoid protein and chlorophyll degradation in drought-challenged Chinese cabbage (Sun *et al*., 2010).

Xu *et al*. (2017a) conducted an *in vitro* maize experiment, where drought was imposed using polyethylene glycol (PEG-6000). *P.indica* colonized plants had increased leaf area, chlorophyll, antioxidative activities of catalases and superoxide dismutase, proline content and dry weight, while reducing indicators of membrane integrity, such as malondialdehyde (MDA).

In barley, *P.indica* conferred a significant increase in yield explained by increasing shoot and root dry weight of plants under both ambient and drought stress conditions stressed by 300 mM NaCl (Ghabooli, 2014). *P.indica* enhanced plant growth under such stress by differentially expressing 254 genes either up or down regulation (Ghaffari *et al*., 2016) and mitigated the drought stress effect by promoting significant accumulation of proteins protective of photorespiration, primary metabolism and energy modulation, in addition to enhancing the electron transfer chain and photosystem activity. Moreover, the number of altered abundance proteins under severe drought for inoculated and control treatments was 144 and 462, respectively (Ghaffari *et al*., 2019).

P.indica has shown an increase of 1.2 to 2.2 and up to 5 fold in total seed weight in ambient, droughted and heat stressed conditions respectively and significantly increasing the photosynthetic efficiency F_v/F_m under heat and drought stress (Hubbard *et al*., 2014).

Yaghoubian *et al*. (2014) tested the responses of wheat to *P.indica* inoculation under drought and found reduced lipid peroxidation rate and hydrogen peroxide level in inoculated wheat plants and increased leaf chlorophyll content antioxidant enzymes activity such as CAT, APX and POD.

1.5.1 Basis of plants response to *P. indica*

The earliest and most persistent responses to plants interaction with *P.indica* are molecularly and physiologically directed by altered levels of phytohormones and intracellular calcium (Ca^{2+}), inducing beneficial mechanisms (Gill *et al*., 2016), with a complex network of responses depending on the conditions of the host plant (Figure 1.3).

Root growth as *P.indica* colonizes the host plant was widely explained by auxin (IAA) produced by the fungus, as well as upregulating auxin gene expression (Sirrenberg *et al*., 2007, Schäfer *et al*., 2009, Lee *et al*., 2011). Another plant growth strategy was promoted via fungal interference with ethylene signalling (Barazani *et al*., 2007).

Evidence has been reported for the role of the phosphate transporter (PiPT) in *P.indica* for mediating phosphorus to the host plant, after isolation and functional characterization of high affinity phosphate transporters (Yadav *et al*., 2010). In addition to significant production of acid phosphatases, making insoluble, condensed and complex forms of phosphate more accessible (Archana *et al*., 2000). Inoculation of tobacco and Arabidopsis with *P.indica* showed significance increase in one of the vital nitrate acquisition enzymes NADH-dependent nitrate reductase (NR) which induced more transfer of nitrogen to the shoots (Sherameti *et al*., 2005).

Abiotic stress mitigation by *P.indica* was explained by significant promotion of shoot and root growth by means of increasing the activity of superoxide dismutase, peroxidases and catalases in the plant leaves. It significantly reduced the degradation of chlorophylls and thylakoid proteins, in addition to upregulating the expression of drought related genes such as DREB2A, CBL1, ANAC072 and RD29A and modulating several members of ROS system and antioxidant defence enzymes such as monodehydroascorbate reductase (Waller *et al*., 2005, Sherameti *et al*., 2008, Sun *et al*., 2010, Hamilton *et al*., 2012, Xu *et al*., 2017a).

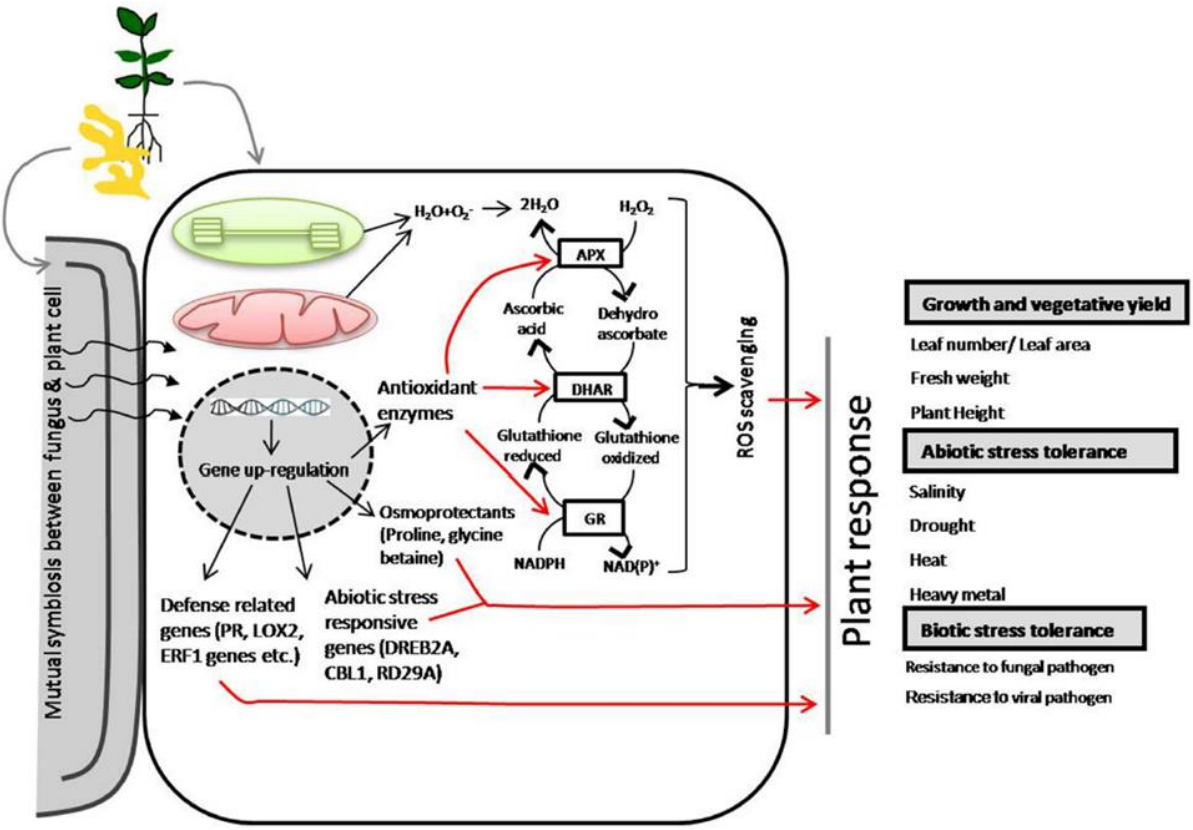

Figure 1.3 Schematic representation of symbiosis between *P.indica* and host plant. Reproduced from (Gill *et al*. 2016).

Biotic stresses resistance was reported as a result of *P.indica* colonization, as various defence related genes in the host plants were upregulated, including ethylene (ERF1) signalling genes, jasmonate JA(VSP, PDF1.2, LOX2) and pathogenesis related PR genes (Molitor *et al*., 2011).

1.5.2 Commercializing *P. indica*

As reports accumulated about enhancing effects of the *P.indica* on various plants under different environments including stresses, conducted in field conditions or controlled environments, there were some steps to mass produce it as inoculum on a large scale and was tested on after Guar, rice and sugarcane (Smriti and Ajit, 2014).

1.6 Project Outline:

This PhD project aims to assess genotypic variation in wheat responses to drought stress by testing a representative subset of the 8-founder elite MAGIC population under field conditions and responses to the endophyte fungus *Piriformospora indica.*

1.6.1 Hypotheses:

1. The ability of a given wheat genotype to withstand limited periods of drought results from multiple interacting quantitative traits expressed throughout the life cycle including, but not limited to, phenology, canopy development and architecture, and of course regulation of photosynthesis, evapotranspiration, canopy temperature in response to fluctuating environmental conditions.

2. There are significant heritable differences in the phenological and developmental traits between MAGIC genotypes which cause heritable differences in the final yield under contrasting water regimes.

3. The extent to which *P. indica* may buffer a particular wheat genotype against drought stress is conditioned by a specific set of *P.indica* response QTL, understanding of which would contribute to a better mechanistic understanding of mutualistic symbiosis.

1.6.2 Objectives

1. Quantifying associations among measured traits and association with yield under contrasting water regimes.

2. Identifying Quantitative Trait Loci (QTL) and genetic interactions associated with various phenotypic traits under both rainfed and irrigated conditions in order to describe the optimal genetic architecture of genotypes that can sustain yield potential under drought stress.

3. Assessing wheat responses upon inoculation with *P.indica* under both drought stress and well-watered conditions and identifying QTL governing these responses.

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2 Chapter 2: Characterization of spatial variability of wheat responses to water limitation

2.1 Abstract

The effect of a prolonged period of drought on soil moisture deficit, soil temperature and performance of high yielding winter wheat variety 'KWS Kielder' throughout the growing season (2016/17) was assessed in a field experiment on a free-draining deep sandy loam soil. 'KWS Kielder' was tested under rainfed/irrigated treatment using integrative drone-based and proximal sensing approaches of time series green canopy development and multispectral indices as well as plant height, flowering time, above ground biomass and grain yield. The results showed soil temperature to diverge by 2°C between the treatments and maximum Soil Moisture Deficit (SMD) peaking over 120 mm in the rainfed plots, with large deficits (>75 mm) from late April that coincided with tillering and more prolonged large deficits from mid-June to mid-July (>100 mm), significantly decreasing green area index and (Normalized Difference Vegetation Index (NDVI) at all measured dates post irrigation. Thousand grain weight and grains/ear were not significantly suppressed by drought, opposite to flowering time, plant height and above ground biomass decreased, all resulting in a significant ($p \leq 0.001$) decrease in grain yield of 31.5%. Concluding the reliability of using such set of soil and time series crop measurements in detecting the dynamic effect of drought on grain yield and its source traits of winter wheat under UK conditions.

Keywords: Wheat; drought; soil moisture deficit; soil temperature; multispectral indices; canopy temperature.

2.2 Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops supporting the global population, accounting for 29% of world cereal production (FAO, 2019). The continued growth in the world population is projected to necessitate an approximate doubling of the food supply (especially of grain crops) by 2050 to ensure food security against the challenge of accelerating climatic change likely to include more frequent drought episodes. Even before factoring in the possible impacts of climate change in the future, drought is already a very real limitation on crop productivity in the UK, with the estimated shortfall between average evapotranspiration and average rainfall during spring and summer at about 130 mm (Jones *et al*., 1985).

Against this, soils are typically brought to field capacity before spring, allowing deep soils with fine texture to hold an adequate amount of moisture to compensate this shortfall (Gales and Wilson, 1981). This means that only a minority of UK arable land planted to wheat is subjected to drought in an average year (Bailey, 1990). However, rainfall patterns are variable, and most wheat crops are expected to be subject to a degree of water limitation in a dry year. The most recent and reliable estimate of the magnitude of UK wheat production losses to water limitation suggests that 30% of the UK wheat arable area faces annual losses of 1-2 tones/ha due to insufficient water availability at sensitive stages of wheat development such as stem elongation, anthesis and grain filling (Foulkes *et al*., 2001).

Under UK conditions, because of the sporadic nature of profound drought episodes during the growing season, few studies comparing rainfed and irrigated regimes have been conducted in open field conditions, though those that have been conducted highlight the potential for dramatic changes in phenology and productivity. For example, when Foulkes *et al*. (2001) and Foulkes *et al*. (2002) compared irrigated to rainfed treatments over three consecutive seasons, soil moisture deficit was found to exceed 140 mm during the growing season, with prolonged episodes over 75 mm which restricted canopy expansion and decreased canopy area at late grain filling, advanced flowering date by up to 9 days, lowered total above-ground biomass between 4.2 t/ha and 6 t/ha and lowered grain yield by between 1.38 t/ha and 4.55 t/ha.

Whalley *et al*. (2006) found that grain yield of wheat variety ‘Claire’ was reduced by 16.6 and 35.5% in 2003 and 2004, respectively and emphasised the role that physical restriction of root growth in drier (and hence stronger) soils was a significant factor in limiting yield, while another experiment in well-drained, sandy soil using six elite UK winter wheat lines showed grain yield reduction comparing rainfed to irrigated treatment of 68% and 29% in 2009 and 2010, respectively (Dodd *et al*., 2011). Foulkes *et al*. (2007) reported 22-27% loss of potential yield due to insufficient water availability in two doubled haploid populations.

Penman (1970) proposed the concept of limiting deficit (LD), which is the value of potential soil moisture deficit (SMD) above which the grain yield diminishes. LD is highly dependent on the soil’s available water capacity and on the critical growth stage of the crop; for example, LD was 40 mm for spring wheat before ear emergence. Under different soils and environmental conditions in New Zealand, Jamieson *et al*. (1995) found LD to be 262 mm for winter wheat subject to late season drought.

Most studies testing high temperature impact on crop response almost always consider air temperature, while the counteractive effect of high soil temperature at root zone on crop performance was poorly studied. Wraith and Ferguson (1994) compared the effect of two soil temperature treatments using cover materials and reported up to 65 mm difference of cumulative soil water depletion between treatments and significantly accelerated soil water depletion in warmer soil.

The advances and availability of commercial devices that measure the spectral index Normalized Difference Vegetation Index (NDVI) and estimate ground cover, biomass and senescence rate made it feasible to phenotype large crop populations on a wide scale successfully (Lopes and Reynolds, 2012). A bottleneck in screening big fields for multispectral indices and canopy temperature, is the sensitivity of these measurements to environmental parameters and hence the need to be collected in a short time span, which can be attained by integrating UAV measurements. For example, Tattaris *et al*. (2016) imposed different combinations of heat and drought stresses on wheat, significant correlations were found between canopy temperature and NDVI and key measured phenotypes such as biomass and yield, with higher correlations when using drone-based imagery compared to proximal ground-based ones. Li *et al*. (2019) found air born NDVI and thermography to be repeatable and suggested them as a reliable guide to selection of root depth and grain yield.

Few studies have aimed to trace the dynamic effect of extended dry episodes on time series canopy development using non-destructive approaches, including canopy development over time and multi-spectral indices using integrative proximal sensing and drone-based measurements under the UK conditions.

The objectives of this study were to: (a) update the literature on limitation of yield under drought using a contemporary high-yielding wheat variety (b) ascertain the magnitude of divergence in water availability between large rainfed versus irrigated blocks by quantifying rainfall, soil temperature and soil moisture deficit; (c) test the effect of spatial variation of the field on the measured traits; and (d) detect the differential effect of divergent water availability regimes on crop canopy development, plant height, flowering time, biomass and grain yield using high-throughput phenotyping techniques that could be used in parallel to characterize genetic responses of an entire multi-parent mapping population.

2.3 Materials and methods

2.3.1 Plant Materials

This experiment is part of a larger field trial accommodating 1600 plots of nearly 400 recombinant inbred lines of the winter wheat multiparent advanced inter-cross (MAGIC) population (Mackay *et al*., 2014). The analysis of genetic architecture of drought responses will be reported In Chapter 3. This report focuses on the variety 'KWS Kielder' (AFP 1/2076 bred by KWS UK Ltd), which was used as a highly replicated control in the field trial during the growing season 2016-2017.

2.3.2 Site and experimental treatments

Field work was carried out in the University of Reading's Sonning farm, Sonning, UK (0°54' W, 51°29' N), where the soil is a free-draining deep sandy loam. The experiment was designed as a Randomized Complete Block (RCB), with two main blocks (reps) and 16 plots within each block, each 0.5m x 2m KWS Kielder plot was located within a 50 m^2 sub-block which were included as covariates in the statistical analysis to account for spatial variation in the field (which spanned 2 hectares). Seeds were drilled mid-October in 0.5 m x 2 m plots and seeding rate of 350 seeds/m^2. Plots were maintained free of weeds and disease with the appropriate herbicides and fungicides and received standard nutrient regime.

Two of the four replicates were managed to receive supplementary irrigation from T-tapes running through the gap between rows 2 and 3 of a 5-row plot at the rate of 3.7 mm/day. Irrigation started on 26th April 2017 and was terminated on the 26th June 2017 giving a total supplementary irrigation of 222 mm.

To quantify the water availability in soil at different depths among the two water regimes, 1-meter deep fiberglass access tubes (ALT-1 Access Tube, Delta T Devices, Cambridge, UK) were inserted at 27 of the 32 plots shortly after emergence. Soil moisture measurements were recorded from early May at 10-day intervals.

Soil moisture deficit (SMD) was calculated using the field-level model IRRIGUIDE with onsite rainfall measurements and other daily weather data taken from the on-site weather station (Silgram *et al*., 2007).

To obtain frequent time series data quantifying soil temperature, 16 I-button Hygrochrons (DS1923) were inserted in soil at 50 cm depth in 16 of the 27 plots with the soil moisture

access tubes in January 2017 and soil temperature was recorded each hour from 1st of February up to harvest time.

2.3.3 Crop measurements

Red-Green-Blue (RGB) images of high quality were captured at fixed height using a Canon EOS 6D camera with a resolution of 5472 x 3648 pixels, mounted on a proximal sensing cart (Supplementary material, Figure S2. 1) similar to that described by White and Conley (2013). Custom software was then used to estimate the Green Area Index (GAI) from the high-resolution pictures by measuring the proportion of green pixels in an image (supplementary material, Figure S2. 2). RGB images were taken from seedling emergence almost once a month until onset of irrigation at late April where it was taken every 10-15 days depending on weather conditions until partial lodging of a small proportion of plots after a summer storm at the end of June closed the gaps between rows and prevented further use of the proximal sensing cart.

Time series GAI data were then used to infer secondary traits from a spline curve model that interpolates data between points and extract potential growth indicators for biomass accumulation: canopy duration (CD) was measured as the number of thermal degree days spent above 50% of maximum value and maximum green value (MGV) using R package AUC (Ballings and Van den Poel, 2013). To overcome difficulty of counting tillers, the number of ears/unit area was counted as the total number of visible ears in the captured RGB images which had a fixed field of view covering 0.16 m^2.

Canopy temperature (CT) was captured once by thermal camera around solar noon (13:04 GMT) 19th June 2017 which was notable as one of the hottest days during the entire growing season, where air temperature at the adjacent weather station peaked at 32.1°C during the imaging session. Multispectral reflectance (NDVI) was recorded four times after the irrigation treatment started.

Plant height was measured using the rising plate (Sharrow, 1984) as the average of two measurements per plot at grain filling stage. Flowering time (FT) was recorded as number of days from 1st of May to Zadok's growth stage (GS67) where about 75% of anthers are extruded in more than 50% of the plants in the plot. Final grain yield was estimated at harvest time by recording plot harvest weight and adjusting for plot length area and grain moisture content. Two days prior to harvesting, four ears were sampled from each plot to estimate thousand grain weight (TGW) and number of grains/ear.

At harvest time the plots were manually trimmed above ground, weighed (fresh weight) as total above-ground biomass before being threshed and seed yield calculated in t/ha.

2.3.4 Statistical analysis

All descriptive statistics (mean, variance, standard error, distribution) and Analysis of Variance (ANOVA) were carried out in R software R 3.3.4 (R development core team, 2017).

2.4 Results and discussion

2.4.1 Level of drought in rainfed conditions.

The spring of 2017 was exceptionally dry in the United Kingdom by historical standards. Rainfall anomaly charts published by the UK Met Office showed that across large areas of SE England, where this investigation was carried out, the total rainfall for the month of April was less than 20% of the 1981-2010 average (supplementary material, Figure S2. 3).

Rainfall data from the Sonning Farm meteorological station from which records are available for 60 years showed that April 2017 monthly rainfall (6.8 mm) was just 13.6 % of the 1981-2010 average of 50 mm. Against this backdrop of unusually low levels of natural rainfall, it was possible to produce dramatic differences in soil moisture deficit by applying irrigation to half the replicate blocks in the experiment. Indeed, within our experimental field, soil moisture content measured by the access tubes showed the irrigated plots to have almost double the moisture content of the rainfed plots at all measuring dates across the soil profile (10-100 cm), except for the first measurement in early May that was immediately preceded by a rainfall event (supplementary material, Figure S2. 4).

Supplementary irrigation started in late April (at around 1300 degree-days from sowing), in the middle of a prolonged period of almost no rainfall where cumulative precipitation had reached a plateau. The plateau in cumulative precipitation persisted in the rainfed plots while cumulative precipitation progressively increased in the irrigated block (Figure 2.1c). Supplementary irrigation lead to a rapid divergence in SMD reaching 30 mm difference between treatments within ten days and reaching 93 mm by mid-June.

Maximum soil moisture deficit (SMD) in the rainfed blocks peaked over 120 mm, with large deficits (>75 mm) from late April that coincided with tillering and more prolonged large deficits from 13th June to 18th July (>100 mm) that covered most of the grain filling stage (Figure 2.1d), all imposing significant stress on crop development and performance, illustrating that potential SMD exceeded the limiting deficit LD (Penman, 1970), as observed

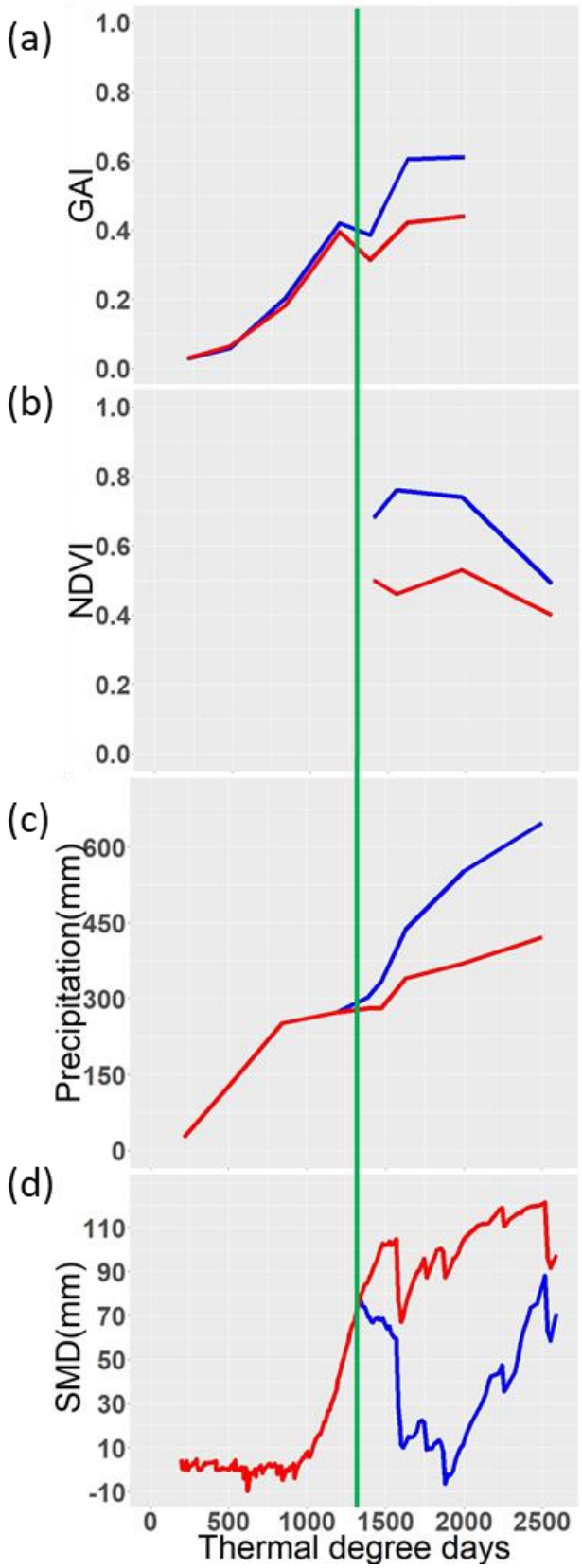

Figure 2.1 Changes in (a) GAI; (b) NDVI, (c) cumulative precipitation and (d) soil moisture deficit, from germination to late grain filling over thermal time (expressed in degree days). Blue and red lines show irrigated and rainfed treatments respectively. The green vertical line indicates the time of onset of supplementary irrigation.

in significant reduction in grain yield.

Soil average daily temperature at 50 cm depth was found to differ between the two treatments from late April onwards, with irrigation driving a difference >1°C from mid-June to mid-July and occasionally exceeding 2°C. A reduction in soil temperature as a consequence of irrigation was previously reported (Dong *et al*., 2016, Karandish and Shahnazari, 2016) and later senescence of winter wheat was detected in cooler soils (Wraith & Hanks 1992), moreover, Dong *et al*. (2016) found that during the hot summer months, top layer soil temperature peaks in early evenings which if prolonged and repeated results in hindered plant growth and yield of maize and that well watering at night reduced soil temperature, increasing root length and marginally increasing grain yield by 10%.

2.4.2 Spatial variation

The means, standard error of mean (S.E.) and significance of treatment response were calculated for 22 traits (Table 2.1).

The possible impact of spatial variation was investigated by ANOVA with block and sub-block as random effects. These showed that the main blocks had no significant impact on any

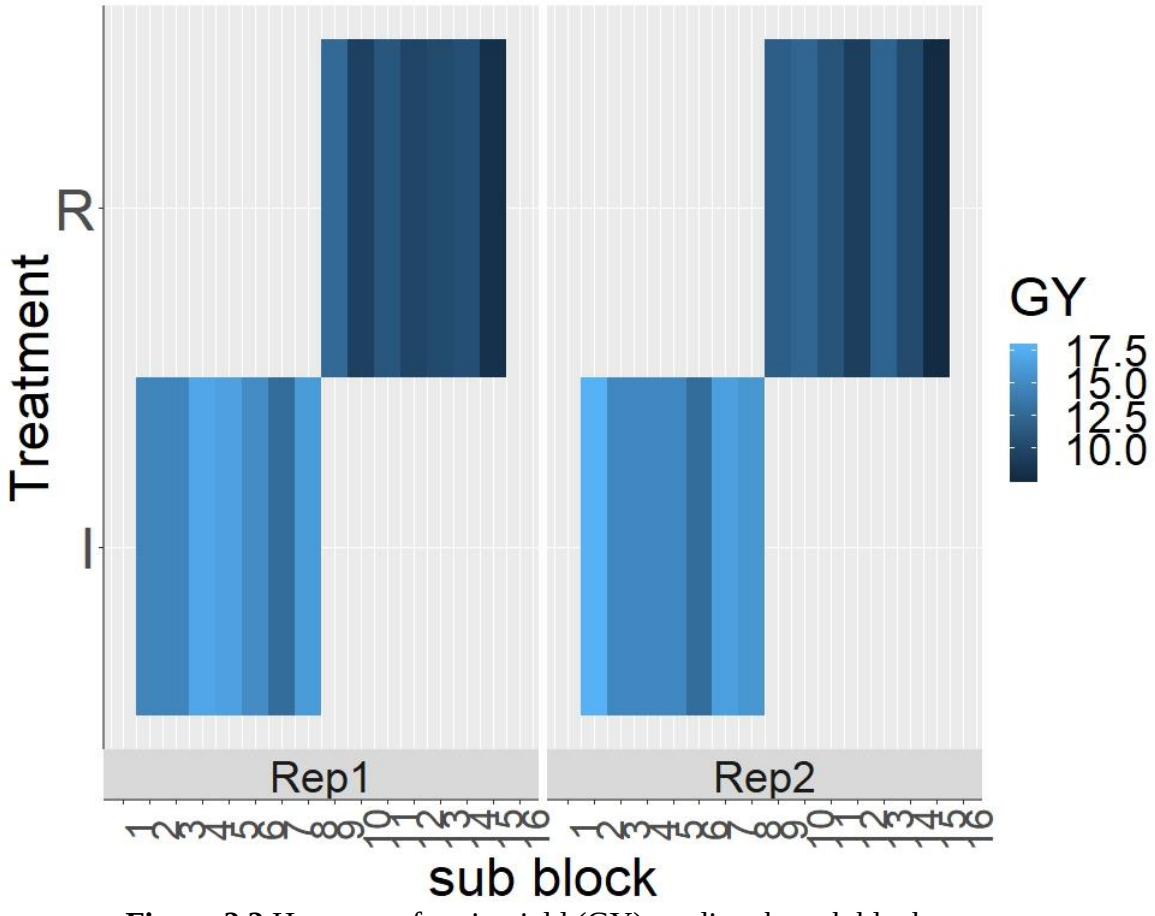

Figure 2.2 Heatmap of grain yield (GY) gradient by sub blocks.

of the measured traits, as did sub block/block (Figure 2.2), except for NDVI 4th and 16th of May and plant height, where the overall blocks showed no significant difference, but the individual sub blocks/block appeared to differ significantly. To illustrate this, (supplementary material, Figure S2. 5 and Figure S2.6) show the locations of 'KWS Kielder' plots in a map of the field trial and heat maps for sub block effect for the measured traits.

The vanishingly low significance of block or sub-block variation in comparison to marked treatment effects is crucial to the wider objective of being able to precisely quantify responses of a large number of individual genotypes physically distributed over a two hectares land area

Table 2.1 *P*-values and means of measured phenotypes under the two treatments. Canopy duration and maximum green value were estimated as one value/block.

Trait	*P*-value			s.e	Mean	
	Treatment	Block	Sub block/block		irrigation	rainfed
GAI.213(dd)	0.091	<0.001***	0.079	0.001	0.02	0.03
GAI.486(dd)	0.081	<0.001***	0.076	0.007	0.06	0.08
GAI.841(dd)	0.264	0.006**	0.636	0.012	0.23	0.24
GAI.1192(dd)	0.127	0.912	0.778	0.016	0.50	0.45
GAI.1388(dd)	<0.001***	0.232	0.130	0.014	0.48	0.38
GAI.1471(dd)	<0.001***	0.520	0.095	0.016	0.55	0.41
GAI.1630(dd)	<0.001***	0.117	0.926	0.024	0.67	0.46
GAI.1994(dd)	<0.001***	0.360	0.965	0.026	0.64	0.42
NDVI.1409(dd)	<0.001***	0.543	0.005**	0.021	0.76	0.58
NDVI.1555(dd)	<0.001***	0.175	0.011*	0.026	0.82	0.57
NDVI.1976(dd)	<0.001***	0.119	0.778	0.014	0.78	0.64
NDVI.2553(dd)	<0.001***	0.232	0.869	0.013	0.54	0.40
CT	<0.001***	0.411	0.037*	0.467	29.32	34.34
FT	<0.001***	0.842	0.969	0.295	38.06	35.38
Pht	<0.001***	0.163	0.016*	0.709	70.40	63.94
CD	0.029*	0.540	ND	35.400	846.50	713.50
MGV	0.025*	0.422	ND	0.060	0.66	0.46
Biomass	0.009**	0.643	0.227	0.873	29.73	21.58
TGW	0.223	0.936	0.732	0.680	46.37	44.54
Grains/ear	0.809	0.046*	0.593	1.705	84.60	85.33
Ears/area	0.002**	0.311	0.658	2.392	92.12	76.53
Grain yield	<0.001***	0.981	0.524	0.499	15.28	10.48

*, **, *** Statistically significant at $p \leq 0.05$, 0.01, 0.001, respectively.

ND. Not determined.

sufficiently homogeneous treatment effect to be able to accurately map genetic factors underpinning the differences in response.

2.4.3 Trait responses

The treatment means of GAI did not differ until late April (prior to applying irrigation) showing homogeneity of the replicates designated for both water treatments (Figure 2.1a), GAI development in both treatments was suppressed by late April as a result of large SMD but was significantly mitigated in the irrigated treatment leading to significant divergence since imposing supplementary irrigation up to late season. This result agrees with Foulkes *et al*. (2001), who found significant increase in GAI as a result of irrigation in dry year at growth stages GS 39 and GS 61.

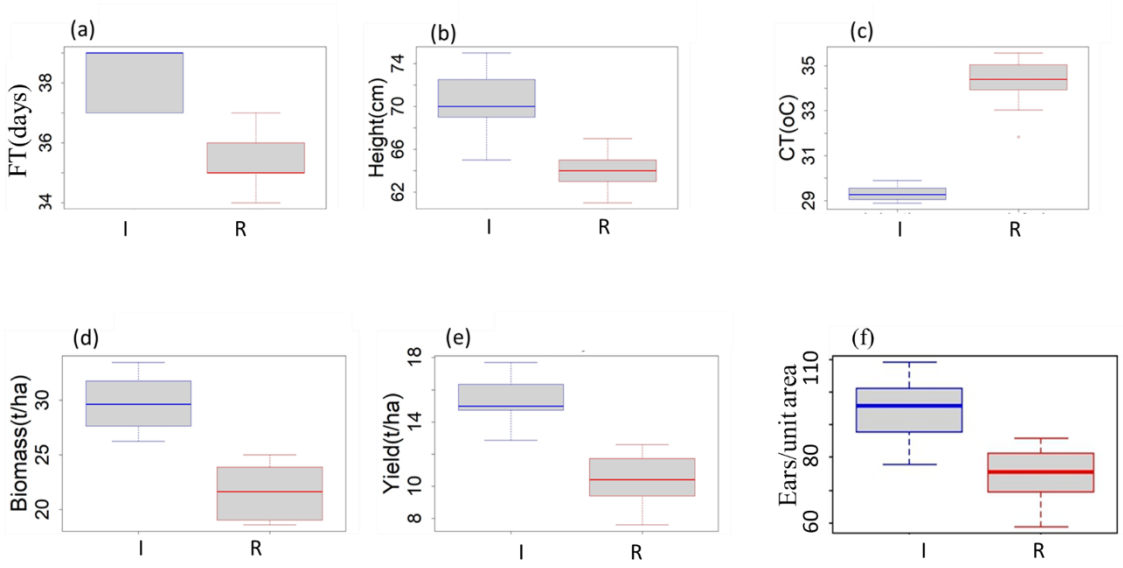

Figure 2.3 Phenotypic responses to irrigation. (a) flowering time (expressed in days from 1st May), (b) plant height in cm, (c) canopy temperature in °C, (d) above-ground biomass in t/ha , (e) grain yield and (f) ears/unit area. In each panel, blue box and whiskers denotes the irrigated (I) treatment and red the rainfed (R) treatment.

The biological parameters obtained from time series GAI; Canopy duration and Maximum green value were both significantly suppressed by the drought, showing the impact of these stress episodes on means of solar radiation interception and subsequently on grain yield.

NDVI measurements recorded between 4th May and 14th July always showed a significant effect of the irrigation treatment in stressing the plants under rainfed conditions (Figure 2.1 b) and indicating later senescence in the irrigated blocks, where Foulkes *et al*. (2001) found post anthesis drought to advance senescence by 7 days.

Drought induced a small but significant effect on flowering time, advancing it on average by 2.68 days (Figure 2.3a); this limited effect of drought might be explained by the alleviating effect of mid-May rainfall, this finding agrees with Foulkes *et al*. (2007) who detected an average of 1 day advanced anthesis in drought treatment, while it was found to be significantly advanced by up to 9 days in Foulkes *et al*. (2001), which might be explained by seasonal differences of timing and duration of the drought treatment relative to anthesis.

In contrast to the small size effect on flowering time, canopy temperature (CT) was significantly higher in the rainfed than the irrigated plots by an average of 5°C (Figure 2.3 c) which is suggested to be one of the main influencers of reduced yield under stress, as Crain *et al*. (2016), Thapa *et al*. (2018) found a significant negative correlation between CT and grain yield, Li *et al*. (2019) reported wheat with cooler canopies to have 7-19% higher yield than warmer ones after testing three mapping populations.

Plant height was significantly reduced by the drought on average of 6.46 cm between the two treatments (Figure 2.3 b) in agreement with previous studies reporting the suppressive effect of drought on wheat height (Liu *et al*., 2017, Zhang *et al*., 2018).

Above ground biomass was compared with rainfed/irrigation treatments and showed that rainfed plot lost significantly an average of 8.2 t/ha, which is likely to be the result of the suppression in plant height and canopy development caused by the drought stress at different stages (Figure 2.3 d). This effect agrees with Foulkes *et al*. (2001), Foulkes *et al*. (2002) and Varga *et al*. (2017) findings on winter wheat.

More importantly, for all traits which were measured both in the KWS Kielder control plots and across the wider experiment, there was full agreement both in terms of direction and magnitude of phenotypic responses (data shown in chapter 3), which means that the demonstration of limiting soil moisture in rainfed conditions in the control plots and their consequences for rates of growth, development and ultimately yield can be extrapolated to the experiment as a whole.

2.4.4 Yield and yield components

Grain yield was 4.8 t/ha lower in rainfed compared to irrigated plots (Figure 2.3 e). As shown in Table 1, neither TGW nor number of grains/ear were significantly affected by the drought episodes, indicating that they had the least contribution to differential yield gain, on the other hand number of ears/unit area (Figure 2.3 f) was significantly reduced by 17% in the rainfed part of the field, which shows that yield response in this experiment might have been more dependent on tillering which was the phase of first drought episode on season. Foulkes *et al*. (2002), (Foulkes *et al*., 2007) found that number of ears/m^2 was significantly reduced only in years where drought occurred pre-anthesis, as was the case in this study. On the other hand, they found significant parallel reductions in TGW and number of grains/ear driven by high SMD during grain filling phase, whereas SMD during grain filling was lower in this study.

2.5 Conclusion

The results of this study show that UK wheat fields experience drought spells during the growing season that was detected quantitatively in terms of acute and prolonged SMD and higher soil temperature in rainfed plots, which in turn lead to high canopy temperature and suppression of time series crop canopy indices, plant height, biomass, number of ears/unit area and grain yield. Moreover, the spatial effect of the field on the measured traits was not significant given a total area of the experimental field of 2 hectares. These findings indicate the reliability of such experimental design, environmental measurements and water limitation magnitude to test big size mapping population response to drought and dissect genetic underpinnings of these responses.

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2.7 Supplementary material

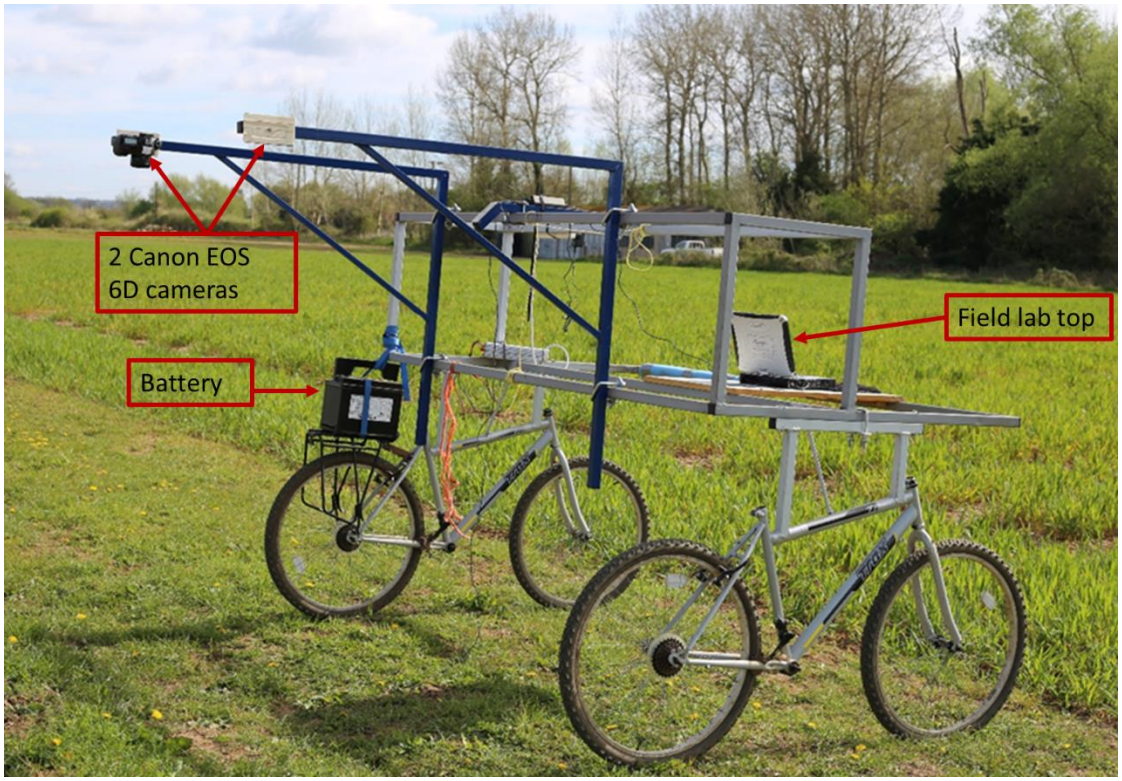


Figure S2. 1 Proximal sensing cart used for tracking canopy cover development.

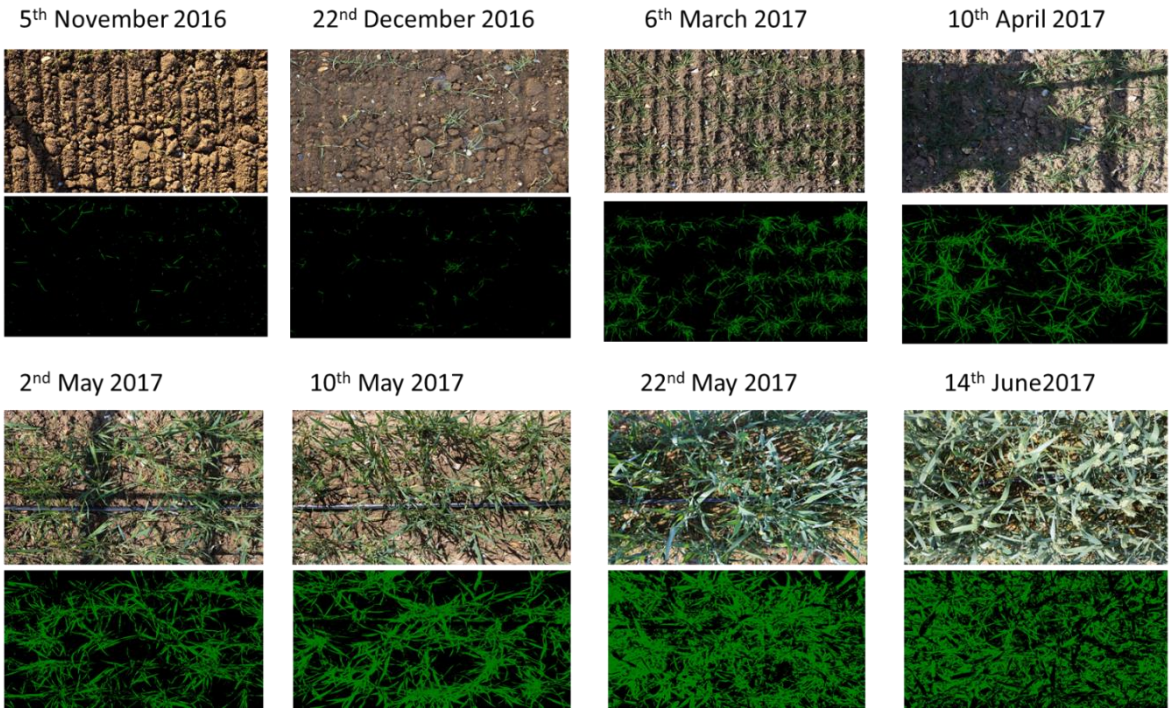


Figure S2. 2 Custom software (Pheno-harvesrt) processing of green pixels on time series pictures, capturing crop canopy cover development.

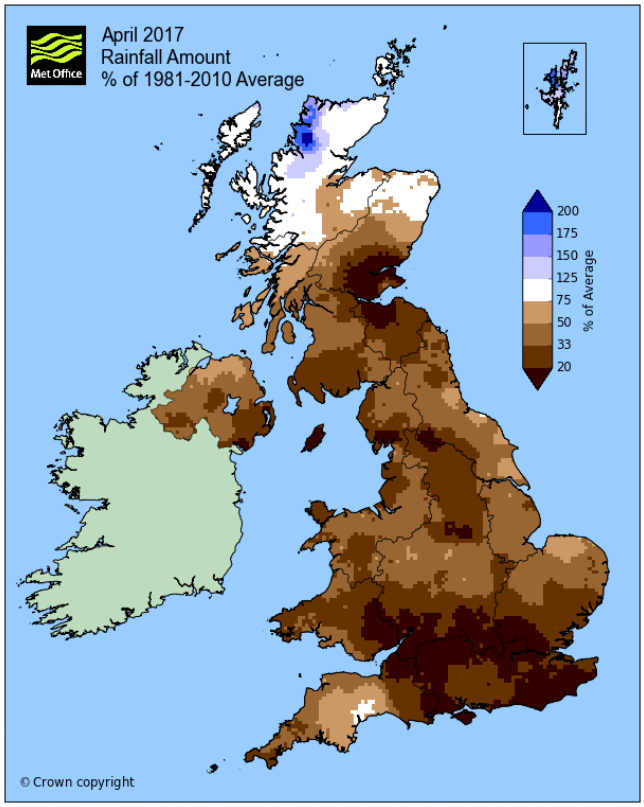


Figure S2. 3 UK rainfall anomaly map for April 2017 (Met office 2017)

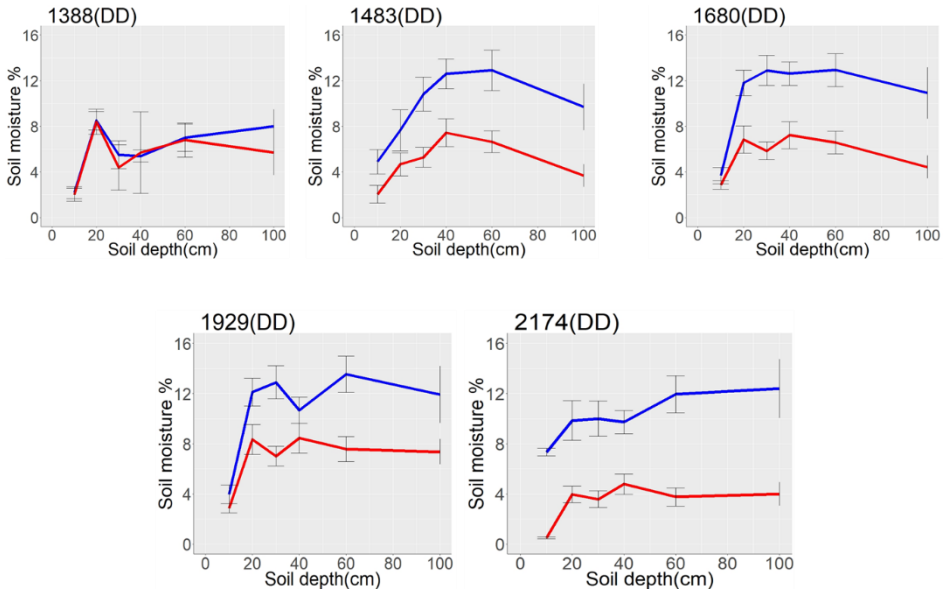
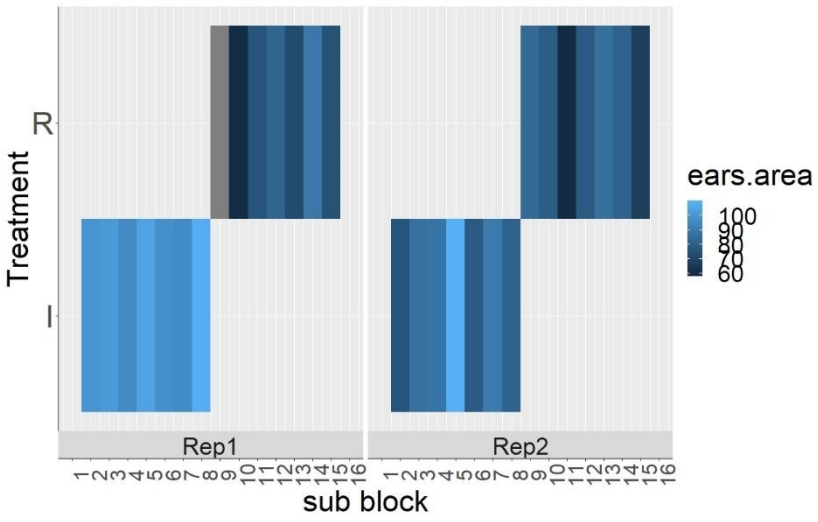


Figure S2. 4 Average soil moisture within 'Kielder' field plots under the two water availability regimes. Blue and red lines correspond to irrigated and rainfed treatments, respectively.


Treatment
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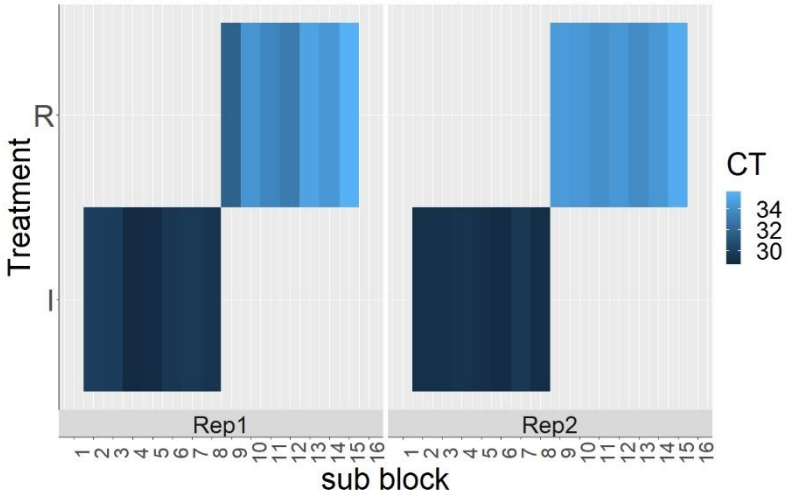
I
Rep1
Rep2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
sub block
ears.area
100
90
80
70
60


Treatment
R
I
Rep1
Rep2
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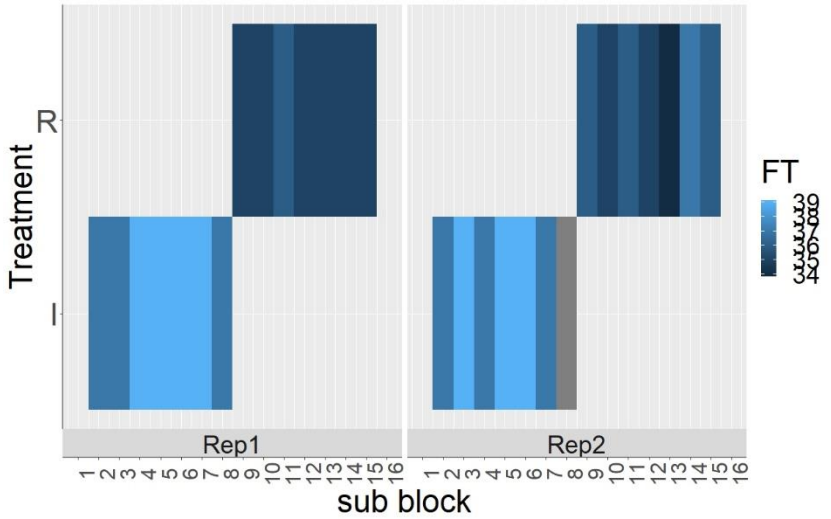
sub block
CT
34
32
30


Treatment
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Rep1
Rep2
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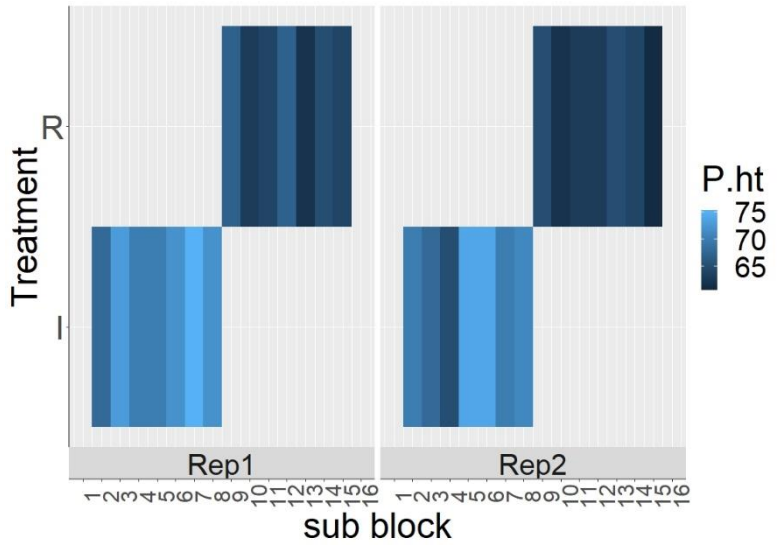
sub block
FT
39
38
37
36
35
34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I
Rep1
Rep2
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sub block
P.ht
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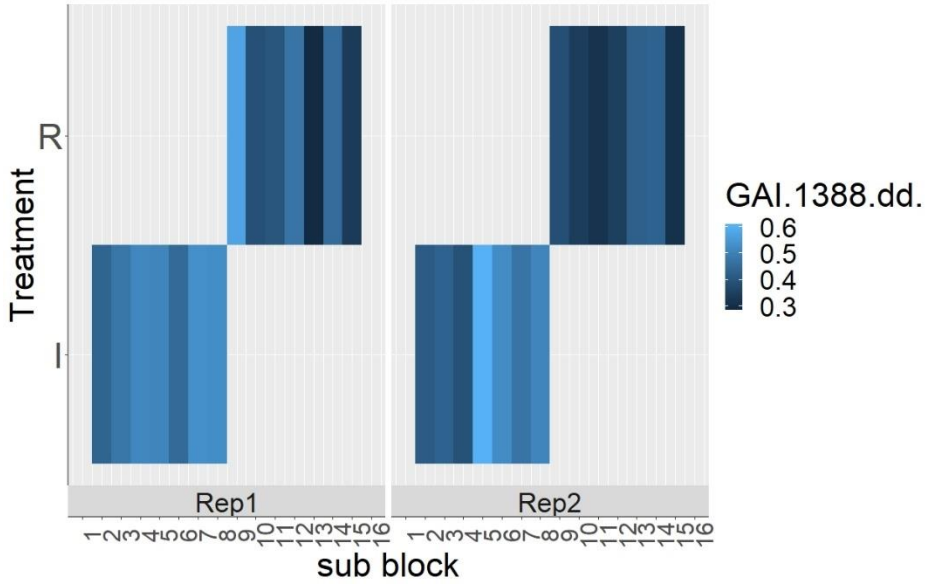
Treatment
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I
Rep1
Rep2
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sub block
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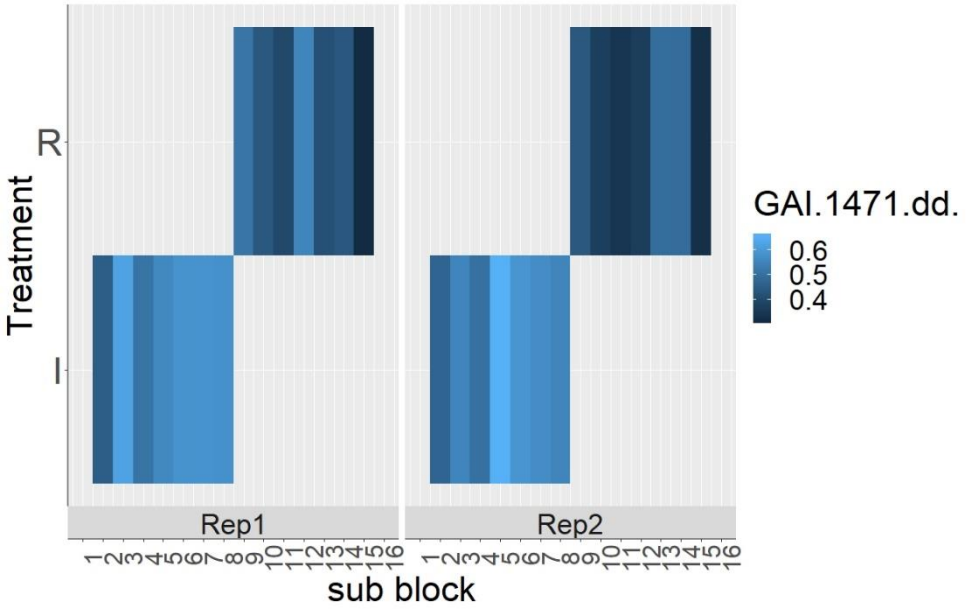
0.3


Treatment
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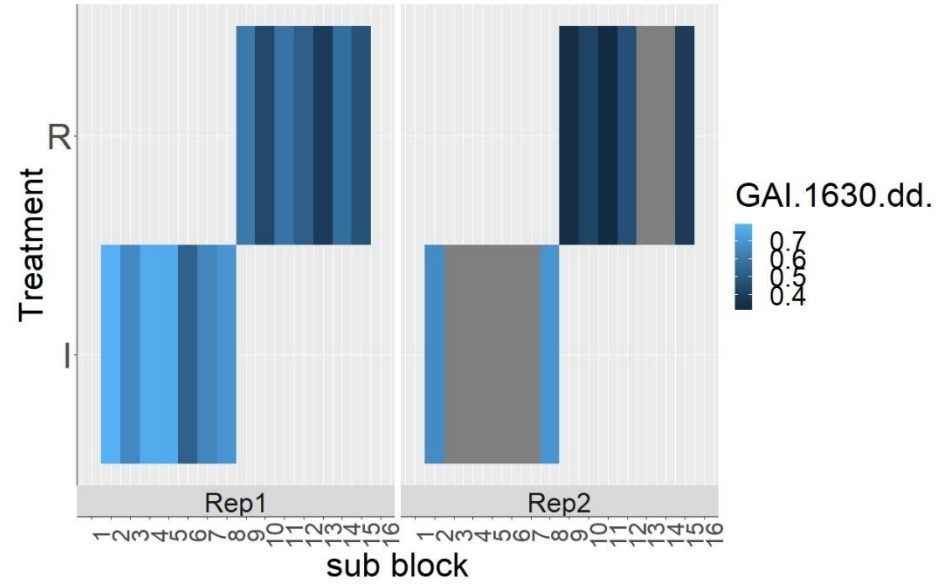
Treatment
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Rep1
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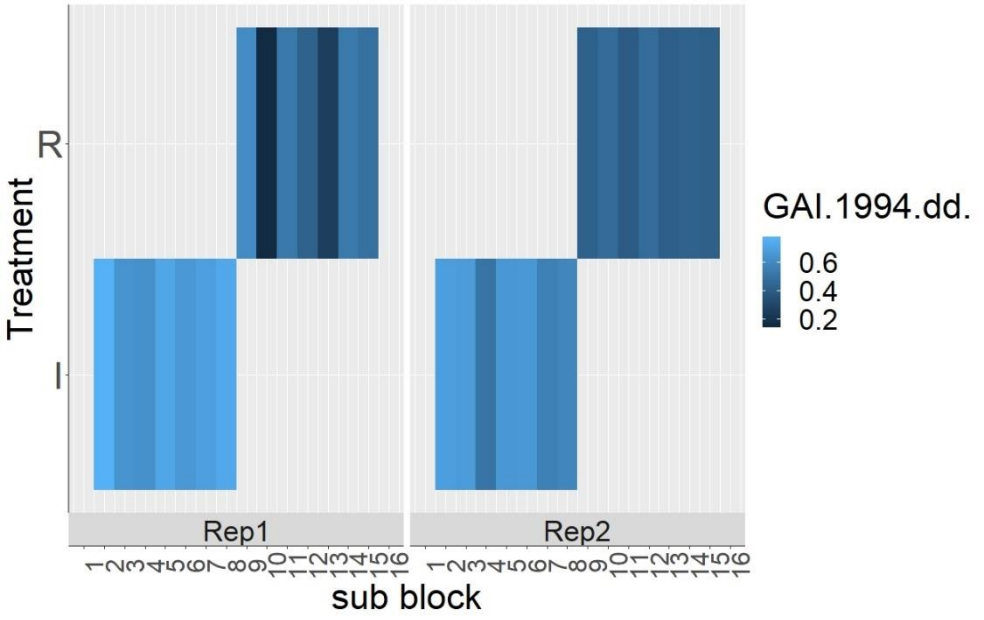
Treatment
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sub block
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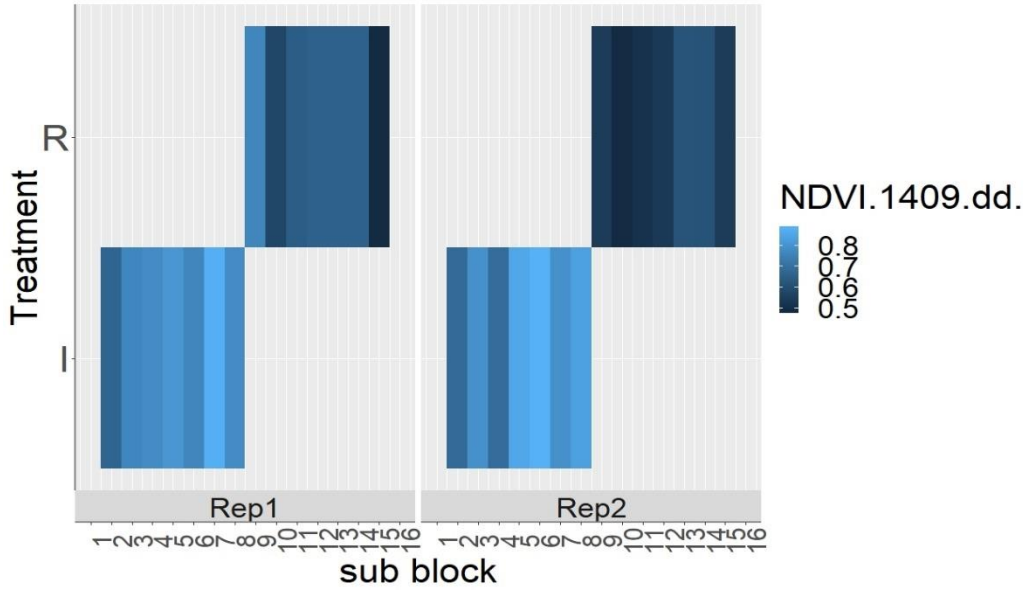
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I
Rep1
Rep2
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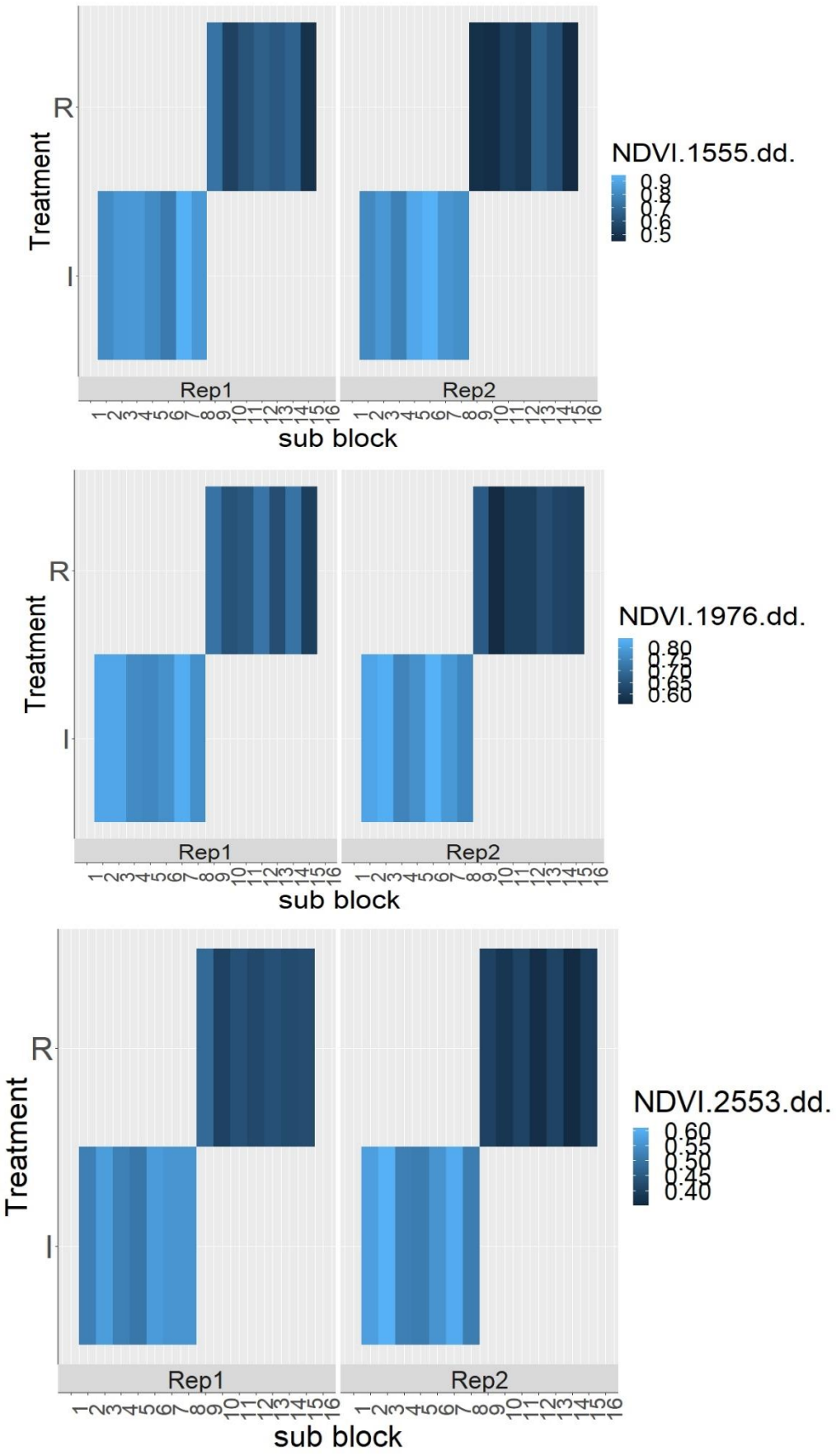


Figure S2. 5 Heatmap of phenotypes gradient by sub blocks

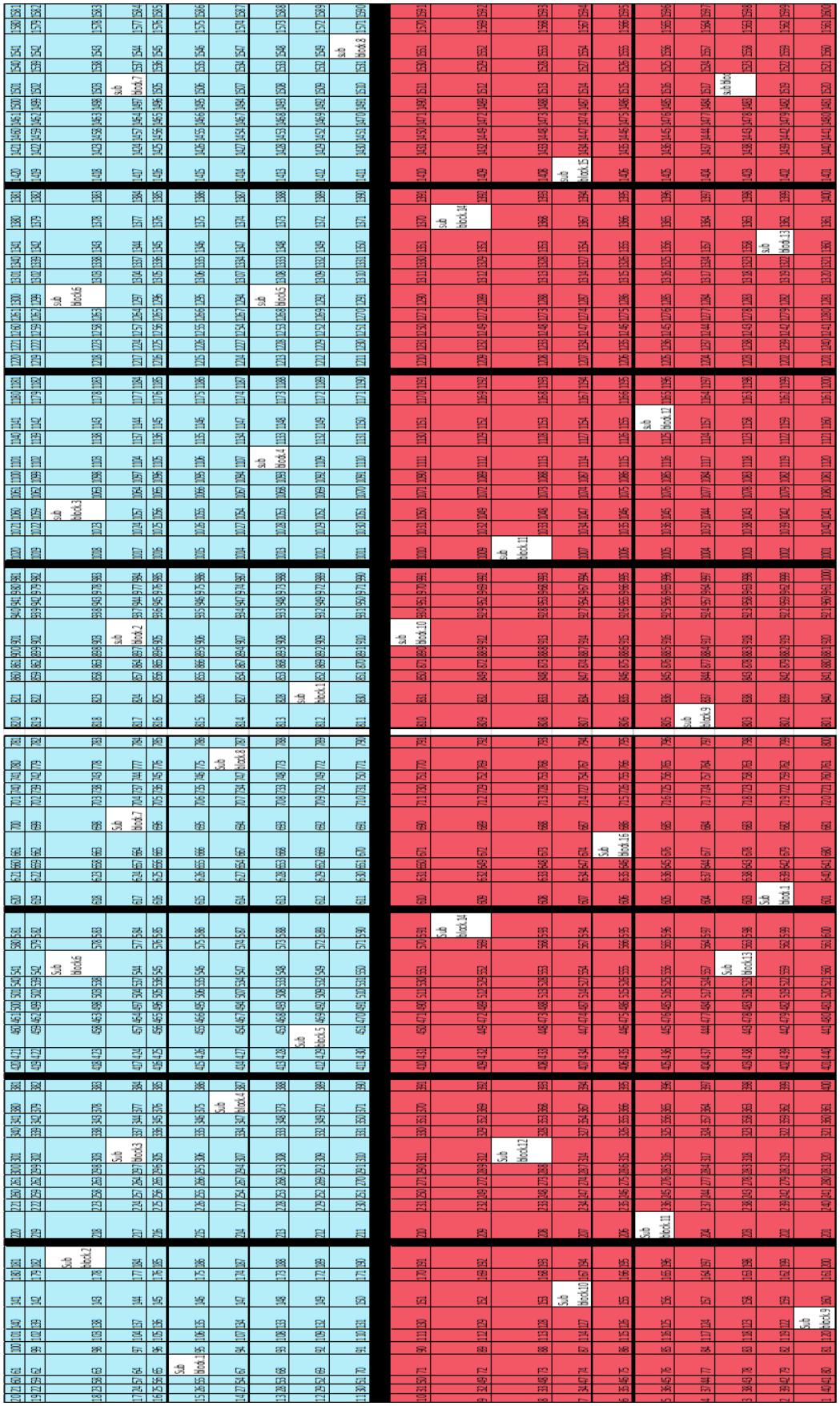


Figure S2.6 Field design showing 'Kielder plots' (in white) representing subblocks within the whole MAGIC panel. Blue and red parts of the field indicate irrigated and rainfed blocks, respectively.

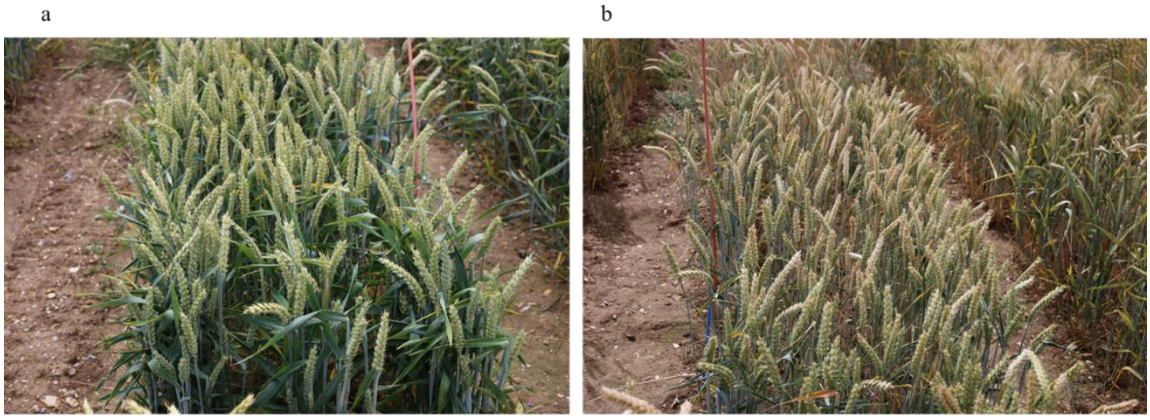


Figure S2. 7 'Kielder' plots (mid-grain filling) a: irrigated, b: rainfed.

3 Chapter 3: Genetic underpinnings of MAGIC wheat response to drought

3.1 Abstract

Sustaining yield potential under drought stress could result from a combination of phenotypic responses that accumulate over time. Therefore, characterising significant phenotypes throughout the growing season and dissecting their genetic basis should be a useful approach to describe the phenological and genetic architecture of wheat responses to drought. In this study, we conducted detailed above ground phenotyping combining proximal sensing and drone-based approaches of 392 RILs and founders of the eight-way "elite MAGIC" winter wheat population in a field trial with contrasting water availability regimes. The traits most significantly ($p \leq 0.001$) correlated with yield were grains.m^{-2} (r=0.68) and (r=0.72) and canopy temperature depression (CTD) (r=0.52) and (r=0.61) in rainfed and irrigated condition, respectively. QTL analysis for yield revealed a total of 16 novel QTL expressed commonly across both treatments explaining individually 1 to 4.5% as well as treatment dependent QTL, with remarkable examples of grain yield QTL collocating major QTL such as grains.m^{-2} QTL on 3A and *Rht-D1* pleiotropic region on chromosome 4D. A search for two-way SNP-SNP epistatic interactions for yield, identified significant pairs of alleles occasionally coinciding with QTL for crop canopy indices. The results of this study demonstrate how key phenotypes might explain yield under given environmental parameters and reveal novel QTL for drought tolerance associated traits that could be utilized in wheat breeding for resistance to such stress.

Keywords: wheat, QTL, drought stress, MAGIC, canopy indices

3.2 Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops supporting the global population, accounting for 29% of world cereal production (FAO, 2019b). Expecting future population growth and a doubled need of food production ensuring food security, this should be fulfilled while expecting climatic changes such as more frequent drought episodes which is currently affecting about 30-49% of the global wheat growing area in various degrees (FAO, 2019a). Despite the efforts put into wheat breeding program, yield genetic gain does not exceed 1% per year (Reynolds *et al*., 2012), with reports that this increase is expected to

be reduced due to climatic variability (Wiebe *et al*., 2015) and in Europe, genetic gain is predicted to be partially counteracted by high temperature during grain filling and drought during stem elongation (Brisson *et al*., 2010).

Winter wheat in the UK faces 1-2 t/ha yield annual losses in 30% of the UK wheat arable area, due to the limited water availability at drought sensitive developmental stages such as stem elongation, anthesis and grain filling (Foulkes *et al*., 2001). This necessitates breeding for cultivars capable of sustaining yield potential under possible drought episodes during the growing season and having a comprehensive understanding of the physiological and morphological traits required for such breeding programs, in addition to defining the genetic architecture reflecting this ideotype.

Few field trials designed to explicitly examine the impacts of drought in open field conditions have been conducted in the UK due to the unpredictability and sporadic nature of drought episodes during the wheat growing season. Nonetheless Foulkes *et al*. (2001) and Foulkes *et al*., (2002) found soil moisture deficit (SMD) to exceed 75 mm for long period and peaks over 140 mm, driving grain yield loss of 1.38 t/ha and 4.55 t/ha while other studies reported that wheat grown on well drained/sandy soils and facing prolonged dry episodes, showed a significant reduction in yield in the non-irrigated treatment of between 16.6 and 68% (Whalley *et al*., 2006, Dodd *et al*., 2011).

Morphological and physiological traits that mitigate the drought stress effect vary according to the severity and timing of the drought episode. Under the UK drought, which is usually mild and sporadic in nature, highly correlated with yield under drought were early flowering high GAI, water use efficiency and stem soluble carbohydrates (Foulkes *et al*., 2001, Foulkes *et al*., 2002). Foulkes *et al*. (2007) found persistence of green flag leaf area to be a trait with most significant correlation with yield under drought and reported presence of awns and flowering time to have neutral effects in the absence of terminal drought.

Given the massive amount of genotypic data that became available due to advances in genomics technology, one of the main constraints of crop breeding programs currently is accurate and efficient phenotyping and it becomes more challenging if the aim is to phenotype large number of lines for time series non-destructive traits (Tuberosa, 2012). To tackle this bottleneck, several researchers developed high-throughput phenotyping platforms to gather time series of red-green-blue (RGB) and multispectral images, plant height and canopy temperature, either proximal sensing (Busemeyer *et al*., 2013, Crain *et al*., 2016) or unmanned

aerial vehicles (UAV) (Gracia-Romero *et al*., 2019, Guan *et al*., 2019). Moreover, the advances in spectral index devices and thermal cameras made it feasible to phenotype large crop populations on a wide scale successfully (Lopes and Reynolds, 2012).

Highly significant correlations between yield and spectral reflectance indices with high repeatability and heritability were reported, for example, Guan *et al*. (2019) found significant correlation (r= 0.6-0.8) between NDVI and grain yield. NDVI and canopy temperature were reported to have (r= 0.3-0.39) and (r= -0.25, -0.52) correlation with root depth. Tattaris *et al*. (2016) found a significant correlation between canopy temperature and NDVI and key measured phenotypes under heat and drought stress with higher correlations when using drone-based imagery compared to proximal ground-based ones.

The Multi-Parent Advanced Generation Inter-Crosses (MAGIC) is believed to overcome the drawbacks of both biparental populations and association panels, increasing simultaneously the power, diversity and resolution of detecting genomic regions associated with different traits. In this schema, multiple inbred founders are intermated for several generations before deriving the inbred lines each inheriting a unique fine-scale mosaic genome of contributions from all founders, all together making a diverse population. A number of studies have already been reported using the same population used in this experiment, Thepot *et al*. (2015) used it to detect QTL controlling anthesis and were able to identify markers tagging *Ppd-D1* gene and identifying genetic elements that influence the timing of developmental stages until senescence was carried out by Camargo *et al*. (2016). Gardner *et al*. (2016) constructed the first eight-parent wheat MAGIC genetic map for wheat using 643 F_5 RILs, comprising 18601 SNP markers and concluded that the elite MAGIC population has captured > 80% of the genetic diversity of the UK wheat germplasm after comparing the SNP markers to those of the Wheat Association Genetics for Trait Advancement and Improvement of Lineages "WAGTAIL" association panel (520 varieties).

Recent studies using crop MAGIC populations demonstrated not only their suitability to discover novel QTL-QTL interactions (Mathew *et al*., 2018) but to do so amongst a selection of alleles that are representative of the gene pool from which the multiple founders are drawn, as is the case with the elite wheat MAGIC population that contains 74% of alleles found in a panel of varieties representing the UK winter wheat gene pool (Mackay *et al*., 2014).

The aims of this study were (1) quantifying associations among measured phenotypes and association with yield under contrasting water regimes, (2) identifying quantitative trait loci

(QTL) and genetic interactions associated with various phenotypic traits under both rainfed and irrigated conditions in order to describe the optimal genetic architecture of genotypes that can sustain yield potential under drought stress and to investigate for transgressive segregation among the MAGIC genotypes with the potential of sustaining yield under drought stress.

3.3 Materials and methods

3.3.1 Plant material

The germplasm is a collection of F_7, 384 recombinant inbred lines (RILs), as a representative subset of the >1000 RILs winter wheat elite eight-founder MAGIC population (Mackay *et al*., 2014), in addition to the eight founder varieties (Supplementary material, Table S3. 1) and a check variety 'Kielder' (KWS UK Ltd). The RILs and parent varieties seeds were all obtained from on-site multiplication field plots that were set the previous year.

3.3.2 Field trial

The genotypes panel was evaluated during 2016–2017 growing season at the Reading University Crops Research Unit, Sonning, UK (0°54'W, 51°29' N), where the soil is a free-draining deep sandy loam. A split plot design was employed in partially randomized complete blocks. Consisting of two blocks (replicates) and 8 sub-blocks/block. The whole plot was assigned for the irrigation/rainfed treatment, while the split plots were dedicated for the genotypes. In each whole plot there were 384 MAGIC RILs, the 8 parents and 8 replicates of 'KWS Kielder' (allocated randomly once in each sub-block). Seeds were drilled mid-October in 0.5 x 2 m plots and seeding rate of 350 seeds/m^2. Plots were maintained free of weeds and disease with the appropriate herbicides and fungicides and received standard nutrient regime.

Two of the four replicates were managed to receive supplementary irrigation from T-tapes running through the gap between rows 2 and 3 of a 5-row plot at the rate of 3.7 mm/day. Irrigation started on 26th April 2017 and was terminated on the 26th June 2017 giving a total supplementary irrigation of 222 mm.

3.3.3 Phenotyping

The measured traits are: time series crop canopy cover development (referred to as GAI) measured at eight occasions throughout the growing season, canopy temperature (CT) captured around solar noon 19th June 2017 which was notable as one of the hottest days during the entire growing season and plant height at grain filling, multispectral wavelengths reflectance (NDVI) recorded four times since implementing irrigation, flowering time (FT) expressed in thermal degree days, thousand grain weight (TGW), number of grains/ear and

grain yield. Full details of phenotyping the above mentioned traits in addition to quantifying soil water availability, soil moisture deficit (SMD) and soil temperature is described in Chapter 2, except grain yield, which in this experiment was estimated at harvest time by recording plot harvest weight and adjusting for plot length area and grain moisture content on combine. Grain number m^{-2} was calculated by dividing yield (g) per square meter by mean grain weight (g) and canopy temperature depression (CTD) is expressed as the difference between air temperature and canopy temperature (CTD = T.air −T.canopy) (Balota *et al*., 2007). Abbreviations used in this article are listed in Table 3.1.

Time series GAI data was then used to infer secondary traits from a spline curve model that interpolates data between points and extracts potential growth indicators for biomass accumulation, such as the area under the plotted curve (AUC) using R package AUC (Ballings and Van den Poel, 2013).

Table 3.1 Phenotypes abbreviation description

Abbreviation	**Trait**
Grains.E^{-1}	Number of grains per ear
TGW*	Thousand grain weight
Grains.m^{-2}	Number of grains per square meter
FT*	Flowering time
P.ht*	Plant height
GY*	Grain yield
CTD*	Canopy temperature depression
GAI.(213dd)	Green area index (degree days)
GAI.(486dd)	Green area index (degree days)
GAI.(841dd)	Green area index (degree days)
GAI.(1192dd)	Green area index (degree days)
GAI.(1388dd)	Green area index (degree days)
GAI.(1471dd)	Green area index (degree days)
GAI.(1630dd)	Green area index (degree days)
GAI.(1994dd)	Green area index (degree days)
AUC	Area under the spline curve
NDVI.(1409dd)	Normalized difference vegetation index (degree days)
NDVI.(1555dd)	Normalized difference vegetation index (degree days)
NDVI.(1976dd)	Normalized difference vegetation index (degree days)
NDVI.(2553dd)	Normalized difference vegetation index (degree days)

*TGW (g), FT (thermal degree days), P.ht (cm), GY (t/ha) and CTD (^{o}C)

3.3.4 Statistical analysis

R software R 3.3.4 (R development core team, 2017) was used to analyse experimental data. Means and standard errors were calculated using ANOVA to identify differences between treatments. Within the ANOVA the genotypes were defined as a fixed effect as was water stress, while replicates were treated as random. Broad sense heritability of phenotype data was defined as: $H^2=\sigma^2G/\sigma^2P$

where H^2 is the broad sense heritability, σ^2G is the genotypic variance and σ^2P is the total phenotypic variance.

Best Linear Unbiased Predictions "BLUPs" of all phenotypes were calculated to account for variations due to spatial effects of the field prior to genetic analysis using R/Lme4 Package (Bates *et al*., 2015). Correlations between the traits were estimated using the R /corrplot package (Wei and Simko, 2017).

3.3.5 QTL analysis

The MAGIC lines were genotyped using the Illumina Infinium iSelect 90,000 SNP wheat array (Victorian AgriBiosciences Center, Bundoora, VIC 3083, Australia) and a comprehensive genetic linkage map for the population was constructed by Gardner *et al*. (2016). To reduce the computational requirements and simplify the genetic analysis, the full 20,639 full SNP markers set was filtered by eliminating all except one of the perfectly correlated markers, resulting in 3535 unique markers.

The MAGIC linkage map was constructed using R/mpMap (Huang and George, 2011). Founder haplotype probabilities were computed with the mpprob function in mpMap using a Hidden Markov model implemented in R/qtl (Broman *et al*., 2003). Composite interval mapping exploiting the eight founders' identity by-descent haplotype probabilities (IBD-CIM). For interval mapping, a linear model was fit by estimating separated fixed effects for each of the eight founders at each putative QTL position ('Xi-19' was arbitrarily set as the reference haplotype), using the mpIM function in mpMap (program 'qtl'). When determining whether QTL listed independently in the R/mpmap outputs were redundant or collocated (same locus detected independently in treatments or across different traits), initially only QTL confidence intervals sharing a flanking marker were considered redundant/collocated. Further inspection of QTLs for the same trait separated by only a few CM highlighted instances where direction and magnitude of parental effects led us to override the strict positional criterion. In order to detect interactions between loci, a standard two-dimensional whole-genome scan was

performed for each trait using PLINK software (Purcell *et al*., 2007, Chang *et al*., 2015). Redundant interactions between highly correlated SNPs on the same chromosomes were removed and a threshold *P*-value of 1.00^{E-05} was applied to consider a SNP–SNP interaction as significant.

3.4 Results

3.4.1 Level of drought in rainfed conditions

The spring of 2017 was remarkably dry by historical standards of the United Kingdom, where the total rainfall for spring was 40-50%, specifically, April was less than 20% of the 1981-2010 average (Supplementary material, Figure S3. 1). These figures were confirmed by on site rainfall data from the Sonning farm meteorological station from which records are available for 60 years showing that spring months 2017 rainfall was 43% and April monthly rainfall (6.8 mm) was just 13.6 % of the 1981-2010 average of 50 mm. Soil moisture content measured by the access tubes (restricted to 'Kielder' plots to avoid genotypic differences in root absorbance) showed the irrigated plots to have almost double the moisture content of the rainfed plots at all measuring dates across the soil profile (10-100 cm), except for the first measurement in early May that was immediately preceded by a rainfall event (as detailed in chapter 2). Supplementary irrigation lead to a rapid divergence in SMD reaching 30 mm difference between treatments within ten days and reaching 93 mm by mid-June and maintaining a prolonged peak (>100 mm) up to mid-July, causing divergence in crop canopy indices (Supplementary material, Figure S3. 2).

3.4.2 Trait responses

Response to irrigation for all measured traits is displayed in density plots (supplementary material, Figure S3. 3). The mean, S.E., range and broad sense heritability of the measured traits are presented in Table 3.2.

The treatment means of GAI did not show any significant differences until late April (prior to applying irrigation), revealing homogeneity of the replicates designated for both water treatments.

For all traits, Analysis Of Variance (ANOVA) showed highly significant variation among the genotypes and the phenotypic range of the progeny under both treatments greatly exceeded the range of the parental lines indicating transgressive segregation. The main effect of irrigation was highly significant for all traits except the first GAI at 1388(dd) and flowering time were weakly significant ($P \leq 0.07$).

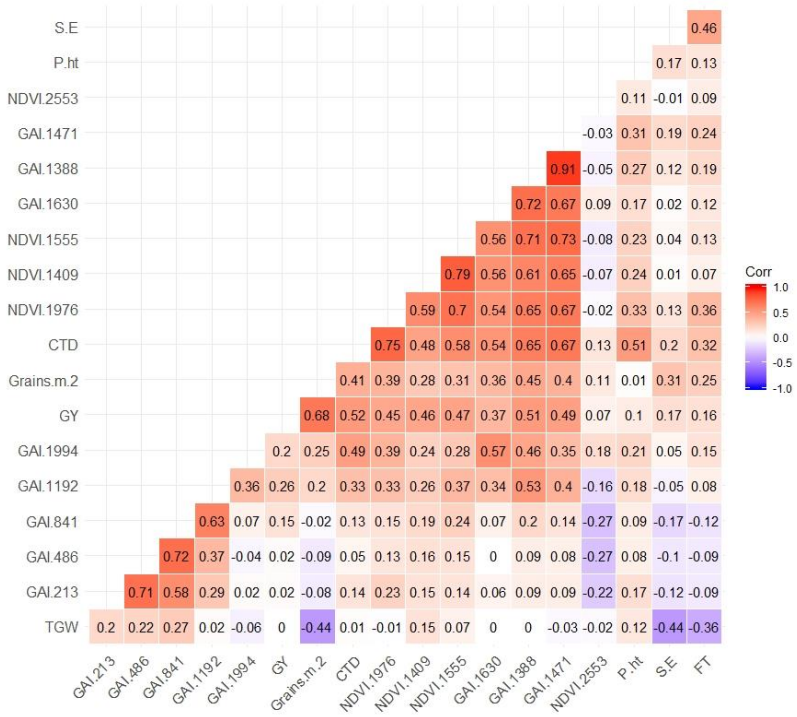
Table 3.2 ANOVA *P*-values, means and heritability of measured phenotypes of the MAGIC RILs and their founder

	Genotype	water stress	Genotype x water stress	irrigated							rainfed						
Trait	*P* value	*P* value	*P* value	SD	Mean	s.e	Min.	Max.	Min. parent	Max. parent	Mean	s.e	Min.	Max.	Min. parent	Max. parent	Heritability
Grains.E-1	ND	ND	ND	13.80	82.80	0.6600	43.00	123.00	67.00	94.00	82.58	0.730	23.00	137.00	52.00	88.00	0.46
TGW	ND	ND	ND	5.41	46.70	0.2600	33.80	63.30	42.00	49.59	43.97	0.270	26.74	59.22	43.15	52.14	0.51
Grains.m-2	ND	ND	ND	5763.40	24564.00	274.493	7946.00	41923.00	24557.18	30182.39	19845.00	251.968	9158.00	39535.00	15918.68	28269.91	0.33
FT	<.001***	0.070	<.001***	48.33	1807.00	1.8000	1631.00	1947.00	1631.00	1883.00	1786.00	1.500	1631.00	1947.00	1631.00	1841.00	0.87
P.ht	<.001***	0.030*	<.001***	8.50	73.00	0.2900	46.00	97.00	64.00	79.00	66.55	0.250	39.00	91.00	57.00	76.00	0.83
GY	<.001***	0.030*	0.003**	2.86	11.40	0.0080	3.93	17.70	9.84	16.11	7.66	0.060	2.33	13.80	5.83	12.75	0.53
CTD	<.001***	0.003**	<.001***	2.68	1.52	0.0200	1.73	3.86	0.64	2.88	-3.40	0.040	-6.91	-0.99	-0.72	-5.49	0.45
GAI.(213dd)	<.001***	0.760	0.831	0.01	0.03	0.0003	0.01	0.06	0.01	0.04	0.03	0.000	0.01	0.06	0.01	0.04	0.46
GAI.(486dd)	<.001***	0.764	0.284	0.03	0.06	0.0010	0.01	0.19	0.01	0.10	0.06	0.001	0.01	0.19	0.01	0.10	0.30
GAI.(841dd)	<.001***	0.708	0.674	0.07	0.20	0.0027	0.04	0.48	0.10	0.36	0.18	0.002	0.05	0.39	0.08	0.30	0.33
GAI.(1192dd)	<.001***	0.191	0.642	0.10	0.42	0.0035	0.11	0.65	0.31	0.60	0.39	0.003	0.16	0.69	0.24	0.60	0.39
GAI.(1388dd)	<.001***	0.070	0.560	0.08	0.38	0.0029	0.15	0.71	0.27	0.50	0.31	0.002	0.11	0.57	0.19	0.52	0.43
GAI.(1471dd)	<.001***	0.040*	0.510	0.10	0.46	0.0034	0.17	0.81	0.32	0.63	0.35	0.003	0.15	0.60	0.22	0.53	0.40
GAI.(1630dd)	<.001***	0.020*	0.500	0.13	0.60	0.0039	0.28	0.89	0.38	0.75	0.42	0.003	0.13	0.69	0.28	0.64	0.41
GAI.(1994dd)	<.001***	0.050*	0.770	0.14	0.61	0.0041	0.21	0.89	0.32	0.81	0.44	0.004	0.12	0.80	0.23	0.62	0.44
AUC	<.001***	0.031*	0.700	113.50	512.63	1.9300	412.75	642.15	457.02	538.97	427.94	1.700	328.18	524.93	395.90	466.90	0.33
NDVI.(1409dd)	<.001***	0.020*	0.935	0.13	0.68	0.0038	0.20	0.95	0.57	0.77	0.50	0.004	0.16	0.76	0.25	0.67	0.30
NDVI.(1555dd)	<.001***	0.008**	0.808	0.17	0.76	0.0032	0.41	0.94	0.60	0.85	0.46	0.004	0.20	0.70	0.30	0.67	0.35
NDVI.(1976dd)	<.001***	0.030*	0.050*	0.13	0.74	0.0022	0.46	0.86	0.64	0.83	0.53	0.004	0.20	0.70	0.41	0.66	0.34
NDVI.(2553dd)	<.001***	0.010**	<.001***	0.06	0.49	0.0019	0.36	0.67	0.43	0.57	0.40	0.001	0.20	0.56	0.35	0.48	0.36

*, **, *** Statistically significant at p≤0.05, 0.01, 0.001, respectively.

ND. Not determined.

(a)



(b)

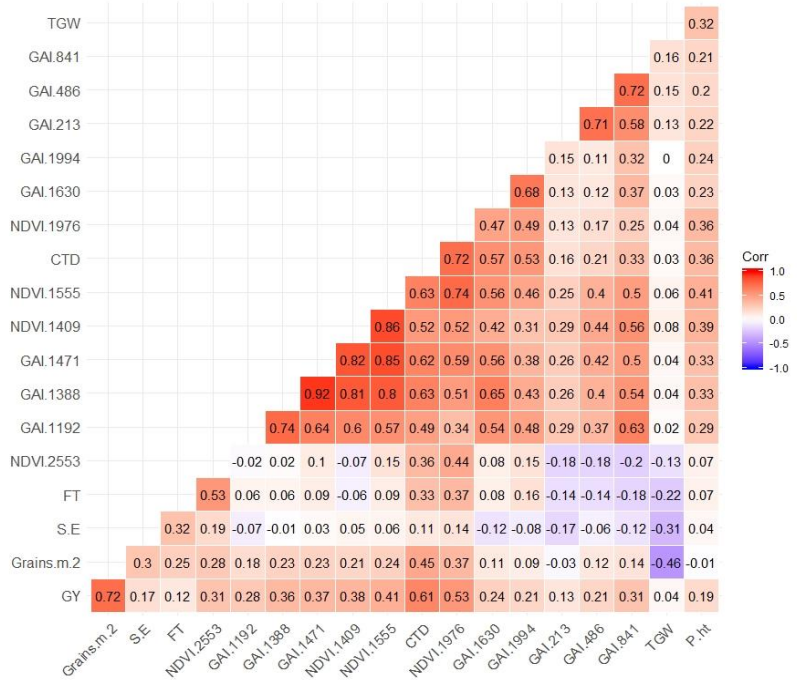


Figure 3.1 Pearson's correlation coefficient for the measured phenotypes of the MAGIC lines. (a) rainfed, (b) irrigated treatment.

Although, TGW, grains/ear and grain number m^{-2} data were collected from one replicate and didn't go through ANOVA, the means under both treatments seem to be close except for grain number m^{-2}. The highly replicated 'Kielder' analysis confirmed no significant effect for water regimes on both traits in this experiment but significant difference in number of ears/unit area.

The interaction between genotypes and water treatment was not significant for the GAI measurements and the first two NDVI, but highly significant for the last two NDVI measurements, flowering time, plant height, canopy temperature depression and grain yield, indicating genetic differential response to treatments as revealed by heritability; which was highest for flowering time, plant height as 0.87 and 0.83 respectively while GAI.(486dd) and NDVI.(1409dd) ranked the least, both with 0.3 heritability, possibly due to strong environmental dependence of these traits.

Correlations (Pearson's Correlation Coefficient - PCC) between traits in both treatments (Figure 3.1) was calculated to set expectations for the degree of overlap in genetic architecture of what were sometimes explicitly inter-dependent traits and to assess the environmental dependence or otherwise of the traits under study. CTD showed high correlation with all traits in both treatments except for TGW, to be the second highest correlated with grain yield (r=0.52) and (r=0.61) following grains.m^{-2} which was (r=0.72) and (r=0.68) for rainfed and irrigation treatments, respectively. All the canopy indices correlated with themselves in both treatments, especially those of consecutive dates. In both treatments, TGW had significant negative correlation with grains/ear and grains.m^{-2} showing the trade-off between grain number and filling potential.

To identify the most significantly contributing traits to grain yield under both treatments, a stepwise regression analysis was computed (Table 3.3). The regression model explained 76% and 65% ($p \leq 0.001$) of the grain yield in the irrigated and rainfed treatments, respectively.

CTD, TGW and grains.m^{-2} appear to have significant contribution in explaining variation of yield in both treatments; the latter had a similar effect in both cases while the two former ones have higher *B* coefficient in irrigated condition.

The significant representation of grains.m^{-2} in the regression model and its correlation with yield might explain the remarkable difference in yield under the two water regimes to be due to the suppression effect of drought on potential tillering, especially as drought stress had minimal effect on the other two components of yield, TGW and grains.E^{-1}.

Late season NDVI.1976 significant effect indicates the high potential of prolonged grain filling on yield in irrigated treatment. GAI.1388 that was measured post prolonged lack of rainfall during April and early May, showed significant contribution to yield in rainfed treatments illustrating how sustaining tillering and canopy development at this stage maintained its effect on yield under stress.

Table 3.3 Stepwise regression showing the relative contribution significant traits in predicting grain yield

Rainfed			**Irrigated**		
Trait	***B* coefficient**	***P*-value**	**Trait**	***B* coefficient**	***P*-value**
Intercept	4.2093	<0.001	Intercept	3.8472	0.088
P.ht	-0.0201	0.004	FT	-0.0035	0.002
GAI.1388	4.2697	0.006	NDVI.1976	4.1574	0.023
CTD	0.2905	<0.001	CTD	0.7112	<0.001
TGW	0.0497	<0.001	TGW	0.1019	<0.001
Grains/m^2	0.0002	<0.001	Grains/m^2	0.0002	<0.001
Final equation	GY=4.209+ 4.26 (GAI.1388)-0.02 (P.ht) +0.29 (CTD)+0.0002 (Grains/m^2)		Final equation	GY=3.847-0.0035 (FT)+4.15(NDVI.1976) + 0.711(CTD)+0.1(TGW)+ 0.0002(Grains/m^2)	

3.4.3 Mapping Quantitative Trait Loci

A total of 309 significant QTL detected for the traits under investigation are listed in Table 3.4. For each trait, in both treatments, between 5 and 13 QTL were discovered, explaining 22.8% and 50.12% of the phenotypic variation, with high correlation (r =0.81, $P \leq 0.001$) between the broad-sense heritability and the percentage of phenotypic variation explained by the detected QTL, suggesting that the main genetic factors driving variability have been discovered in this analysis.

For the dynamic GAI trait over time (eight times during the growing season) in both water regimes, a total of 110 QTL were detected ranging in individual effect size from 1 to 7.14% of variation explained, showing different sets of QTL to control GAI at each growth stage, with occasional collocations detected among consecutive GAI dates and cumulative GAI (AUC) in Figure 3.2.

Five detected QTL appeared to contribute to GAI/AUC under both treatment (shown in yellow circles in Figure 3.2), moreover, remarkable big size effect locus persisting in both cases and over time such as Q.GAI.1192_4B explained 4.35%, Q.GAI.1630_4B explaining

5.7% and 4% and Q.AUC_4B explaining 4.12 and 3.83% in irrigated and rainfed treatments, respectively. These loci (although close but independent of *RhT-B1* locus) showed pleiotropic effect, governing rainfed grain yield Q.GY.(R)_4B (1.7%), Q.NDVI.1976(R)_4B (3.6%) and Q.NDVI.2553(R)_4B (1.52%) and irrigated plant height Q.P.ht(I)_4B.2 (5%).

The traits for which the largest effect size of QTLs detected were flowering time and plant height, Q.FT(I)_2D and Q.FT(R)_2D loci (tagging photoperiod sensitivity gene *Ppd-D1*) was found to explain 13.04% and 12.38%. Q.P.ht(I)_4D and Q.P.ht(R)_4D tagging dwarfing genes *Rht-D1* were detected to explain 15.2% and 12.23% and those tagging *Rht-B1*, Q.P.ht(I)_4B.1 and Q.P.ht(R)_4B explained 7.8% and 11.23 % of phenotypic variation in irrigated and rainfed treatments, respectively.

The QTL tagging *Rht-D1* shows another example of pleiotropy on various traits, such as Q.GAI.841_4D(3.24%), Q.GAI.1630(I)_4D (3.2%), Q.AUC (R)_4D(3.64%), Q.NDVI.1409 (R)_4D (2.4%) and Q.NDVI.1555(R)_4D (1.36%), Q.Grains.E^{-1}.(I)_4D(9.42%), Q.Grains.m^{-2} (I)_4D (7.9%) and Q.GY.(I)_4D(3.42%).

For grain yield, 17 QTL were detected across the two treatments, individually accounting for 1.1% to 4.53% of the phenotypic variation. Only few of them collocated with QTL of other traits which can be explained by the overall low heritability of grain yield, Q.GY.(I)_3A with big size effect (3.7%) matched Q.Grains.m^{-2}.(I)_3A.2 (6.4%) and Q.GY.(I)_6B (2%) coincided with both Q.CTD(I)_6B (1.3%) and Q.GAI.1994.(I)_6B.2 (4.7%), in addition to the pleiotropic case of *Rht-D1* tagged area with multiple phenotypes including grain yield.

Interestingly, one of the biggest effect sizes QTL Q.GY.(I)_4A and Q.GY.(R)_4A appeared to be environmentally independent and explained 2.14% and 3.39% of the phenotypic variation in irrigated and rainfed treatments, respectively. Moreover, chromosome 1B harboured 3 QTL, together explaining 9% of the phenotypic variation of yield.

Table 3.4 QTL table for MAGIC lines measured phenotypes. Trait abbreviations are as shown in Table 3.1, (I) and (R) indicate irrigated and rainfed treatment, respectively.

	QTL name	Chromosome	Left marker	Right marker	Position	Left marker position	Right marker position	*p* value	R^2
1	Q.FT(I)_1A	1A	IAAV3919	BS00026456_51	1.01	1.01	2.53	3.9E-07	3.04
2	Q.NDVI.1976(I)_1A	1A	BS00077350_51	Excalibur_c9196_313	23.76	21.74	23.76	0.0001	1.37
3	Q.GAI.1192_1A	1A	BS00086680_51	BobWhite_c478_1386	71.1	71.10	72.11	4.4E-08	4.14
4	Q.GY.(I)_1A	1A	BS00021864_51	wsnp_Ku_c21316_31053745	85.71	83.17	85.71	0.0113	3.33
5	**Q.GAI.841_1A**	**1A**	**tplb0041a22_935**	**Kukri_c10239_2186**	**96.1**	**96.10**	**100.17**	**6.6E-07**	**1.74**
6	**Q.GAI.213_1A**	**1A**	**Kukri_c10239_2186**	**RAC875_c31419_80**	**101.17**	**100.17**	**101.17**	**0.00211**	**3.6**
7	Q.NDVI.1555(I)_1A	1A	BS00067742_51	BS00088136_51	145.04	143.52	145.04	1.5E-08	2.45
8	**Q.NDVI.2553(I)_1A**	**1A**	**RAC875_c53725_217**	**BS00011521_51**	**211.95**	**210.94**	**211.95**	**0.00671**	**1.64**
9	**Q.Grains.m^{-2}.(I)_1A**	**1A**	**IAAV4238**	**Kukri_c44201_497**	**215.76**	**215.76**	**216.76**	**0.00881**	**2.22**
10	Q.GAI.1471.(R)_1A	1A	IAAV4238	Kukri_c44201_497	216.76	215.76	216.76	3.3E-05	2.93
11	Q.GY.(R)_1A	1A	Kukri_c18608_729	BobWhite_c44947_277	231.14	225.37	231.14	4.6E-05	2.24
12	**Q.GAI.1192_1B**	**1B**	**BS00022180_51**	**BS00093078_51**	**2.51**	**1.51**	**2.51**	**3.4E-06**	**4.95**
13	**Q.AUC(I)_1B**	**1B**	**BS00093078_51**	**BS00050522_51**	**2.51**	**1.51**	**2.51**	**7.5E-07**	**5.89**
14	**Q.AUC(R)_1B**	**1B**	**BS00093078_51**	**BS00050522_51**	**2.51**	**1.51**	**2.51**	**4.1E-07**	**5.16**
15	**Q.GAI.1630(I)_1B**	**1B**	**BS00093078_51**	**BS00050522_51**	**8.9**	**2.51**	**8.90**	**3.5E-08**	**4.49**
16	**Q.NDVI.1409(I)_1B**	**1B**	**BS00050522_51**	**BobWhite_c5793_372**	**13.48**	**8.90**	**13.48**	**0.00202**	**2.93**
17	**Q.GAI.1388.(R)_1B.1**	**1B**	**Kukri_c29655_194**	**RFL_Contig4140_1135**	**21.72**	**21.72**	**22.72**	**5.1E-06**	**4.51**
18	**Q.GAI.841_1B**	**1B**	**Kukri_c29655_194**	**RFL_Contig4140_1135**	**21.72**	**21.72**	**22.72**	**1.7E-05**	**3.91**
19	Q.FT(R)_1B	1B	BS00068182_51	BS00037387_51	26.75	25.75	26.75	0.00074	1.09
20	Q.GAI.1471.(I)_1B	1B	BS00011695_51	CAP7_c3299_316	31.32	30.32	31.32	0.00023	4.24
21	Q.GAI.1471.(R)_1B.1	1B	Tdurum_contig34049_172	BS00106306_51	35.39	35.39	36.39	0.00018	2.47
22	Q.GY.(I)_1B.1	1B	Kukri_c105601_74	IAAV6	56.55	56.55	57.55	0.00017	4.22
23	Q.NDVI.1976(I)_1B	1B	Kukri_rep_c101550_113	Ra_c15153_324	93.08	92.07	93.08	2.5E-07	5.13
24	Q.NDVI.1555(I)_1B	1B	Ra_c15153_324	BS00064406_51	101.92	93.08	101.92	0.0001	3.88
25	Q.GAI.486_1B	1B	BS00108497_51	BS00022324_51	133.11	133.11	134.11	0.0003	1.24
26	Q.GY.(I)_1B.2	1B	Excalibur_c17977_71	Excalibur_c4459_1867	189.63	188.63	189.63	0.0025	3.62
27	Q.Grains.E^{-1}.(R)_1B	1B	RFL_Contig2484_1065	Ku_c1932_1583	229.7	226.87	229.70	1.2E-12	2.13
28	Q.GY.(I)_1B.3	1B	BobWhite_c48071_144	IACX1240	240.3	240.30	241.30	0.00024	1.1
29	Q.GAI.213_1B	1B	BobWhite_c42716_71	BS00022851_51	244.83	244.83	246.34	1.2E-07	2.69
30	Q.CTD.(R)_1B	1B	Tdurum_contig16606_648	Excalibur_c43793_379	265.02	264.01	265.02	0.0119	1.83
31	**Q.GAI.1471.(R)_1B.2**	**1B**	**BS00023173_51**	**BobWhite_c44460_821**	**291.21**	**289.69**	**291.21**	**9.8E-06**	**2.88**
32	**Q.P.ht(R)_1B**	**1B**	**BS00023173_51**	**BobWhite_c44460_821**	**291.21**	**289.69**	**291.21**	**0.00453**	**1.85**
33	**Q.GAI.1388.(R)_1B.2**	**1B**	**BobWhite_c44460_821**	**IACX8074**	**299.25**	**291.21**	**299.25**	**1.4E-05**	**3.73**
34	Q.Grains.E^{-1}.(I)_1B	1B	RAC875_c9770_123	BobWhite_c16824_151	327.18	323.82	327.18	0.00013	2.65
35	Q.NDVI.1555(R)_1B	1B	BobWhite_c20073_382	BobWhite_c4482_73	337.34	337.34	338.34	1.7E-05	3.99
36	Q.Grains.m^{-2}.(R)_1D	1D	BS00022323_51	BS00089270_51	19.25	6.33	19.25	1.24E-08	7.38
37	Q.GY.(I)_1D	1D	D_contig13475_402	wsnp_Ex_c12012_19240904	39.65	39.65	47.46	4.6E-06	2.49
38	Q.CTD.(R)_1D	1D	BS00012936_51	wsnp_CAP12_c633_339740	55	55.00	56.00	8.5E-05	1.02
39	Q.Grains.E^{-1}.(R)_1D	1D	RAC875_rep_c105196_53	IAAV4656	71.23	66.65	71.23	3.7E-13	2.13
40	**Q.GAI.841_1D**	**1D**	**BS00014671_51**	**BS00003816_51**	**89.02**	**89.02**	**99.08**	**0.00071**	**2.27**
41	**Q.GAI.1994.(I)_1D**	**1D**	**BS00014671_51**	**BS00003816_51**	**99.08**	**89.02**	**99.08**	**0.0124**	**1.48**
42	Q.TGW(R)_1D	1D	BobWhite_c1897_1010	Kukri_rep_c69829_307	122.74	122.74	124.25	1.1E-05	4.44
43	Q.Grains.E^{-1}.(R)_2A	2A	BS00022241_51	BS00022377_51	146.83	146.83	151.47	3.6E-05	10.99
44	Q.Grains.m^{-2}.(R)_2A	2A	BS00023214_51	Excalibur_rep_c102052_721	177.36	177.36	184.29	1.2E-08	8.41
45	Q.GAI.486_2A	2A	BS00107804_51	BobWhite_c15867_215	187.33	187.33	191.93	6.3E-06	2.49
46	**Q.CTD(I)_2A**	**2A**	**Kukri_c22047_313**	**RAC875_c104160_61**	**206.04**	**206.04**	**207.05**	**0.00085**	**1.2**
47	**Q.NDVI.1555(I)_2A**	**2A**	**Kukri_c22047_313**	**RAC875_c104160_61**	**206.04**	**206.04**	**207.05**	**0.00479**	**2.44**
48	**Q.GAI.1388(I)_2A.1**	**2A**	**RAC875_c104160_61**	**BobWhite_c25764_348**	**207.05**	**207.05**	**212.27**	**0.00012**	**1.74**
49	**Q.GAI.1388(I)_2A.2**	**2A**	**RAC875_c21013_1187**	**Excalibur_c52319_257**	**230.55**	**230.55**	**232.05**	**0.0125**	**1.57**
50	**Q.GAI.1471.(I)_2A**	**2A**	**RAC875_c21013_1187**	**Excalibur_c52319_257**	**230.55**	**230.55**	**232.05**	**0.00318**	**2.31**
51	**Q.GAI.1192_2A**	**2A**	**RAC875_c259_1339**	**Tdurum_contig8350_350**	**237.63**	**236.63**	**237.63**	**0.0017**	**1.19**
52	Q.AUC(R)_2A	2A	BS00062869_51	wsnp_Ex_rep_c108004_9140264	240.68	240.68	244.25	4.5E-06	3.69
53	**Q.NDVI.1976(R)_2A**	**2A**	**BS00107649_51**	**BS00098312_51**	**250.78**	**249.27**	**250.78**	**2.5E-08**	**3.86**
54	**Q.NDVI.1409(R)_2A**	**2A**	**Ra_c1757_256**	**BS00060596_51**	**252.8**	**251.79**	**252.80**	**1.5E-07**	**2.01**
55	**Q.CTD.(R)_2A**	**2A**	**BS00094172_51**	**BS00064055_51**	**259.39**	**255.31**	**259.39**	**0.00059**	**2.12**
56	**Q.GAI.1630(R)_2A**	**2A**	**BS00094172_51**	**BS00064055_51**	**259.39**	**255.31**	**259.39**	**5.7E-08**	**6.04**
57	**Q.NDVI.1555(R)_2A**	**2A**	**BS00094172_51**	**BS00064055_51**	**259.39**	**255.31**	**259.39**	**2.3E-05**	**4.1**
58	**Q.GAI.1994.(I)_2B**	**2B**	**Kukri_c19266_779**	**BobWhite_c12144_216**	**2.53**	**2.53**	**3.53**	**1.5E-08**	**7.14**
59	**Q.GAI.1630(R)_2B**	**2B**	**Kukri_c19266_779**	**BobWhite_c12144_216**	**3.53**	**2.53**	**3.53**	**8.7E-07**	**1.61**
60	Q.GAI.1994(R)_2B	2B	BS00028028_51	RAC875_c27611_467	10.58	8.56	10.58	9.7E-07	6.29
61	**Q.GAI.1471.(I)_2B**	**2B**	**Excalibur_c14396_1629**	**RAC875_c84991_116**	**27.82**	**27.82**	**29.84**	**0.0001**	**1.88**
62	**Q.GAI.841_2B**	**2B**	**Excalibur_c14396_1629**	**RAC875_c84991_116**	**27.82**	**27.82**	**29.84**	**8.2E-07**	**2.15**

Table 3.4 (continued)

	QTL name	Chromosome	Left marker	Right marker	Position	Left marker position	Right marker position	*p* value	R^2
63	Q.Grains.E^{-1}.(I)_2B	2B	Tdurum_contig64563_491	BobWhite_c2988_2161	72.64	69.57	72.64	9.8E-09	2.98
64	Q.Grains.E^{-1}.(R)_2B.1	2B	Excalibur_c6111_411	BS00022417_51	139.06	136.52	139.06	2.4E-06	1.18
65	Q.P.ht(R)_2B	2B	BS00031143_51	IAAV6994	147.66	146.66	147.66	5.4E-05	1.84
66	Q.FT(R)_2B.1	2B	Excalibur_c19344_137	BobWhite_c892_73	171.86	170.86	171.86	0.00339	1.59
67	Q.Grains.E^{-1}.(R)_2B.2	2B	Tdurum_contig30930_184	wsnp_JD_rep_c67103_42432235	221.23	220.23	221.23	3.3E-05	5.53
68	Q.FT(R)_2B.2	2B	Kukri_c4294_371	Excalibur_c1353_1364	253.98	251.45	253.98	9.4E-05	5.24
69	Q.NDVI.1976(R)_2B	2B	BobWhite_c22728_78	BS00022805_51	271.92	271.92	279.38	0.00027	1.55
70	Q.TGW(I)_2B	2B	CAP11_c2941_210	BS00026432_51	283.93	283.93	285.43	0.00078	1.72
71	Q.GAI.1388.(R)_2B	2B	RAC875_c3259_276	BS00064483_51	375.98	373.94	375.98	0.00577	3.12
72	Q.Grains.m^{-2}.(I)_2D	2D	Tdurum_contig64286_182	BS00063251_51	5.61	5.61	15.33	0.00641	3.82
73	Q.GAI.1994.(I)_2D	2D	BS00010043_51	D_contig17313_245	18.85	18.85	21.94	0.00292	2.83
74	Q.GAI.1630(I)_2D	2D	BS00029208_51	BS00043986_51	42.21	42.21	50.33	4E-06	2.74
75	**Q.NDVI.2553(I)_2D**	**2D**	**BS00022276_51**	**Kukri_c27309_590**	**55.4**	**53.36**	**55.40**	**0.00771**	**1.29**
76	**Q.P.ht(R)_2D**	**2D**	**BS00022276_51**	**Kukri_c27309_590**	**55.4**	**53.36**	**55.40**	**0.00042**	**3.52**
77	**Q.CTD.(R)_2D**	**2D**	**Kukri_c27309_590**	**wsnp_CAP12_c1503_764765**	**62.71**	**55.40**	**62.71**	**3.2E-11**	**6.73**
78	**Q.FT(I)_2D**	**2D**	**Kukri_c27309_590**	**wsnp_CAP12_c1503_764765**	**62.71**	**55.40**	**62.71**	**3.3E-16**	**13.04**
79	**Q.FT(R)_2D**	**2D**	**Kukri_c27309_590**	**wsnp_CAP12_c1503_764765**	**62.71**	**55.40**	**62.71**	**3E-13**	**12.38**
80	**Q.GAI.1388.(R)_2D**	**2D**	**Kukri_c27309_590**	**wsnp_CAP12_c1503_764765**	**62.71**	**55.40**	**62.71**	**0.00268**	**3.87**
81	**Q.GAI.1994(R)_2D**	**2D**	**Kukri_c27309_590**	**wsnp_CAP12_c1503_764765**	**62.71**	**55.40**	**62.71**	**0.00055**	**6.41**
82	**Q.NDVI.1976(R)_2D**	**2D**	**Kukri_c27309_590**	**wsnp_CAP12_c1503_764765**	**62.71**	**55.40**	**62.71**	**1.7E-09**	**8.8**
83	Q.NDVI.2553(R)_2D.1	2D	IACX5935	BS00039211_51	85.59	85.09	85.59	0.00264	3.39
84	Q.GAI.841_2D.1	2D	D_contig77859_59	BS00083504_51	96.26	92.64	96.26	3.3E-06	1.09
85	Q.GAI.486_2D	2D	Kukri_c55028_182	Kukri_rep_c72254_186	106.21	105.71	106.21	0.00788	2.05
86	**Q.GAI.841_2D.2**	**2D**	**BS00011109_51**	**Kukri_c26676_225**	**121.04**	**119.52**	**121.04**	**4.3E-08**	**4.15**
87	**Q.AUC(I)_**	**2D**	**Kukri_c26676_225**	**Kukri_c3344_401**	**121.04**	**121.04**	**122.56**	**6.1E-05**	**4.03**
88	**Q.GAI.1192_2D**	**2D**	**Kukri_c3344_401**	**Ku_c19185_1569**	**128.39**	**122.56**	**128.39**	**4.9E-07**	**2.86**
89	**Q.NDVI.2553(R)_2D.2**	**2D**	**Tdurum_contig12912_308**	**Kukri_c9274_1004**	**171.95**	**171.45**	**171.95**	**0.00935**	**1.6**
90	**Q.TGW(R)_2D**	**2D**	**Tdurum_contig12912_308**	**Kukri_c9274_1004**	**171.95**	**171.45**	**171.95**	**0.00397**	**8.9**
91	Q.GAI.213_2D	2D	Kukri_c9274_1004	RAC875_c50347_258	196.35	171.95	196.35	2.7E-09	4.96
92	Q.GY.(R)_3A	3A	wsnp_Ex_c6833_11782875	RAC875_c36922_829	12.06	10.55	12.06	1.3E-05	4.53
93	Q.Grains.m^{-2}.(R)_3A	3A	RAC875_c787_431	Excalibur_c11505_155	30.01	23.32	30.01	0	3.14
94	**Q.GAI.1471.(I)_3A**	**3A**	**RAC875_c371_251**	**wsnp_Ra_rep_c106523_9027392**	**34.65**	**34.65**	**38.27**	**0.00055**	**3.71**
95	**Q.P.ht(I)_3A**	**3A**	**RAC875_c371_251**	**wsnp_Ra_rep_c106523_9027392**	**34.65**	**34.65**	**38.27**	**0.00442**	**6.34**
96	**Q.NDVI.1409(R)_3A**	**3A**	**wsnp_Ra_rep_c106523_90273922**	**BS00090225_51**	**44.6**	**38.27**	**44.60**	**1E-06**	**6.16**
97	**Q.AUC(R)_**	**3A**	**BS00090225_51**	**BS00057444_51**	**50.32**	**44.60**	**50.32**	**0.00167**	**2.12**
98	**Q.NDVI.1555(R)_3A**	**3A**	**BS00090225_51**	**BS00057444_51**	**50.32**	**44.60**	**50.32**	**0.00162**	**2.98**
99	Q.Grains.m^{-2}.(I)_3A.1	3A	wsnp_Ex_c11085_17973016	BobWhite_c43681_334	128.58	128.58	131.14	1.6E-07	1.41
100	Q.NDVI.1976(R)_3A	3A	BobWhite_c30232_154	IAAV902	136.23	134.19	136.23	0.00107	1.21
101	Q.GAI.1994.(I)_3A.1	3A	Excalibur_rep_c105978_544	BS00047668_51	187.18	187.18	188.69	8.8E-05	3.65
102	**Q.GY.(I)_3A**	**3A**	**BobWhite_c18593_955**	**RAC875_c47550_437**	**204.47**	**198.84**	**204.47**	**4.9E-05**	**3.74**
103	**Q.Grains.m^{-2}.(I)_3A.2**	**3A**	**RAC875_c47550_437**	**BobWhite_c17879_519**	**205.98**	**204.47**	**205.98**	**1.7E-07**	**6.43**
104	Q.GAI.841_3A	3A	RAC875_c52195_324	CAP11_c1022_117	243.45	243.45	247.62	1.4E-05	0.98
105	**Q.GAI.1388.(R)_3A**	**3A**	**CAP7_c3178_52**	**BS00048633_51**	**270.21**	**270.21**	**274.05**	**0.00157**	**3.22**
106	**Q.GAI.1471.(R)_3A**	**3A**	**CAP7_c3178_52**	**BS00048633_51**	**270.21**	**270.21**	**274.05**	**5.5E-07**	**4.09**
107	Q.NDVI.1976(I)_3A	3A	Ku_c26872_269	wsnp_Ex_c361_707953	282.21	281.20	282.21	0.00298	3.2
108	Q.GAI.1994.(I)_3A.2	3A	Kukri_rep_c70441_132	RFL_Contig2394_439	308.3	304.20	308.30	0.00001	1.18
109	**Q.NDVI.1976(R)_3B**	**3B**	**BS00080158_51**	**Kukri_c17082_378**	**1.51**	**0.00**	**1.51**	**0.00613**	**3.27**
110	**Q.TGW(I)_3B**	**3B**	**BS00080158_51**	**Kukri_c17082_378**	**1.51**	**0.00**	**1.51**	**0.00075**	**1.38**
111	Q.GAI.1994.(I)_3B	3B	BS00026471_51	BS00046375_51	39.46	36.17	39.46	0.0302	1.85
112	Q.NDVI.2553(R)_3B	3B	IAAV3924	BobWhite_c12908_381	41.48	41.48	42.74	7.4E-07	3.01
113	**Q.GAI.1630(I)_3B.1**	**3B**	**BS00011596_51**	**BS00044752_51**	**44.77**	**44.77**	**47.31**	**3.2E-13**	**4.16**
114	**Q.Grains.E-1.(I)_3B**	**3B**	**BS00011596_51**	**BS00044752_51**	**47.31**	**44.77**	**47.31**	**4.7E-12**	**3.37**
115	Q.AUC(I)_3B	3B	BS00095638_51	BS00093856_51	52.39	49.87	52.39	0.00203	3.53
116	Q.NDVI.1555(R)_3B.1	3B	BS00022242_51	Excalibur_c35645_587	63.63	63.63	65.14	2.1E-05	4.51
117	Q.GAI.1630(I)_3B.2	3B	BS00066108_51	Ku_c24126_637	85.62	85.62	86.63	0.00128	2.4
118	Q.Grains.m^{-2}.(I)_3B	3B	RAC875_c9095_217	IAAV1079	96.7	96.70	97.70	5.9E-06	2.02
119	Q.TGW(R)_3B.1	3B	BS00076872_51	BobWhite_c13099_755	149.92	147.39	149.92	8.2E-05	7.61
120	Q.GAI.1630(R)_3B.1	3B	BS00073011_51	wsnp_Ku_rep_c72504_72191206	154.44	153.44	154.44	0.0014	1.15
121	Q.TGW(R)_3B.2	3B	Excalibur_c48047_90	Excalibur_c21604_247	179.92	175.11	179.92	6.3E-06	8.46
122	**Q.CTD.(R)_3B**	**3B**	**BS00022611_51**	**Excalibur_c34069_487**	**183.45**	**182.45**	**183.45**	**0.00125**	**1.5**
123	**Q.GAI.1630(R)_3B.2**	**3B**	**BS00022611_51**	**Excalibur_c34069_487**	**183.45**	**182.45**	**183.45**	**3.4E-05**	**2.82**

Table 3.4 (continued)

	QTL name	Chromosome	Left marker	Right marker	Position	Left marker position	Right marker position	*p* value	R^2
124	Q.Grains.m^{-2}.(R)_3B	3B	wsnp_Ex_c13154_20785032	BobWhite_c6015_141	201.56	201.56	205.69	2E-09	2.65
125	Q.NDVI.1555(R)_3B.2	3B	BS00022861_51	BS00024499_51	233.55	233.55	236.59	0.0015	1.15
126	**Q.GAI.1994(R)_3B**	**3B**	**BS00070210_51**	**Excalibur_c72450_483**	**241.15**	**241.15**	**242.67**	**0.0005**	**4.21**
127	**Q.GAI.1192_3B**	**3B**	**RAC875_c7158_687**	**BS00037871_51**	**246.73**	**246.73**	**254.80**	**0.0051**	**1.52**
128	**Q.FT(I)_3D**	**3D**	**Excalibur_c20277_436**	**Excalibur_c83177_99**	**1.01**	**1.01**	**3.53**	**4E-06**	**1.92**
129	**Q.FT(R)_3D**	**3D**	**Excalibur_c20277_436**	**Excalibur_c83177_99**	**1.01**	**1.01**	**3.53**	**7E-06**	**1.18**
130	**Q.P.ht(I)_3D**	**3D**	**IAAV2729**	**wsnp_Ex_c18250_27065775**	**94.19**	**91.10**	**94.19**	**0.0003**	**2.38**
131	**Q.P.ht(R)_3D**	**3D**	**IAAV2729**	**wsnp_Ex_c18250_27065775**	**94.19**	**91.10**	**94.19**	**6E-08**	**1.23**
132	**Q.GAI.1994.(I)_3D**	**3D**	**wsnp_Ex_c7260_12463738**	**Kukri_c43208_335**	**99.87**	**98.36**	**99.87**	**0.0027**	**2.22**
133	**Q.GAI.1630(I)_3D**	**3D**	**Ku_c2845_342**	**Ku_c6080_1667**	**107.27**	**100.88**	**107.27**	**0.0035**	**1.18**
134	Q.GAI.213_3D	3D	Jagger_c3839_60	BS00017789_51	192.5	180.63	192.50	4E-05	4.95
135	**Q.NDVI.1409(I)_4A.1**	**4A**	**BS00021716_51**	**BS00106545_51**	**7.23**	**6.73**	**7.23**	**1E-06**	**3.36**
136	**Q.GAI.1471.(I)_4A**	**4A**	**BS00035307_51**	**wsnp_Ra_c14920_23225219**	**7.74**	**7.74**	**23.34**	**0.0036**	**2.29**
137	**Q.NDVI.2553(R)_4A**	**4A**	**BS00035307_51**	**wsnp_Ra_c14920_23225219**	**23.34**	**7.74**	**23.34**	**6E-09**	**4.78**
138	Q.AUC(I)_4A	4A	BS00065863_51	wsnp_Ex_c28429_37553452	29.17	29.17	33.88	0.0008	1.66
139	**Q.GY.(I)_4A**	**4A**	**Ex_c7626_444**	**RAC875_c28178_889**	**36.98**	**36.98**	**43.14**	**0.0026**	**2.14**
140	**Q.GY.(R)_4A**	**4A**	**Ex_c7626_444**	**RAC875_c28178_889**	**36.98**	**36.98**	**43.14**	**2E-06**	**3.39**
141	Q.Grains.m^{-2}.(R)_4A	4A	CAP12_c2677_138	BS00021752_51	43.64	43.64	44.14	3E-11	4.02
142	Q.NDVI.1976(I)_4A	4A	BS00036493_51	Tdurum_contig11919_360	49.5	49.50	54.62	4E-05	1.03
143	Q.Grains.E^{-1}.(R)_4A	4A	BobWhite_c20514_92	BobWhite_c5633_59	61.2	61.20	63.74	4E-13	7.81
144	Q.GAI.1388(I)_4A	4A	Ex_c66324_1151	Jagger_c4331_105	113.1	110.57	113.10	0.0005	1.94
145	Q.NDVI.1555(I)_4A	4A	IAAV7104	RFL_Contig3679_315	123.55	123.05	123.55	0.0119	1.93
146	Q.Grains.E^{-1}.(I)_4A	4A	IAAV5722	RAC875_c6939_1042	140.85	137.78	140.85	9E-12	3.78
147	Q.GAI.213_4A	4A	BS00064369_51	BS00091752_51	164.01	158.75	164.01	5E-06	1.01
148	Q.FT(R)_4A	4A	BS00039811_51	IAAV2823	178.37	177.87	178.37	0.0024	1.86
149	Q.NDVI.1409(I)_4A.2	4A	Excalibur_c10699_404	IAAV2769	217.29	214.75	217.29	0.0068	1.56
150	**Q.GAI.1630(I)_4B.1**	**4B**	**Excalibur_c7581_1266**	**wsnp_Ex_c30695_39579408**	**18.73**	**14.02**	**18.73**	**2E-07**	**3.25**
151	**Q.GAI.1994.(I)_4B**	**4B**	**wsnp_Ex_c30695_39579408**	**Kukri_c26488_139**	**25.91**	**18.73**	**25.91**	**3E-05**	**3**
152	**Q.P.ht(I)_4B.1**	**4B**	**Tdurum_contig42229_113**	**IAAV585**	**52.17**	**52.17**	**53.17**	**2E-08**	**7.83**
153	**Q.P.ht(R)_4B**	**4B**	**Tdurum_contig42229_113**	**IAAV585**	**52.17**	**52.17**	**53.17**	**0**	**11.23**
154	Q.GY.(I)_4B	4B	BS00029342_51	IACX773	63.34	62.34	63.34	0.0013	1.31
155	Q.TGW(I)_4B	4B	BS00105791_51	BS00062691_51	65.87	65.87	72.57	0.0015	4.73
156	**Q.AUC(R)_4B**	**4B**	**BS00067786_51**	**IACX5989**	**75.09**	**75.09**	**78.18**	**3E-06**	**3.83**
157	**Q.GAI.1192_4B1.1**	**4B**	**BS00067786_51**	**IACX5989**	**75.09**	**75.09**	**78.18**	**2E-08**	**4.35**
158	**Q.GAI.1630(I)_4B.2**	**4B**	**IACX5989**	**Tdurum_contig29989_132**	**78.18**	**78.18**	**79.19**	**6E-06**	**5.67**
159	**Q.GY.(R)_4B**	**4B**	**IACX5989**	**Tdurum_contig29989_132**	**78.18**	**78.18**	**79.19**	**2E-06**	**1.7**
160	**Q.NDVI.1976(R)_4B**	**4B**	**IACX5989**	**Tdurum_contig29989_132**	**78.18**	**78.18**	**79.19**	**0.0012**	**3.6**
161	**Q.NDVI.2553(R)_4B**	**4B**	**IACX5989**	**Tdurum_contig29989_132**	**78.18**	**78.18**	**79.19**	**0.0002**	**1.52**
162	**Q.GAI.1630(R)_4B**	**4B**	**Tdurum_contig29989_132**	**BS00040159_51**	**79.19**	**79.19**	**80.71**	**2E-06**	**4.03**
163	**Q.P.ht(I)_4B.2**	**4B**	**Tdurum_contig29989_132**	**BS00040159_51**	**80.71**	**79.19**	**80.71**	**0.0002**	**4.99**
164	**Q.AUC(I)_4B.1**	**4B**	**wsnp_BE442869B_Ta_1_1**	**BS00011085_51**	**81.72**	**81.72**	**82.72**	**0.0056**	**4.12**
165	**Q.CTD.(R)_4B.1**	**4B**	**BS00064884_51**	**IAAV4595**	**83.73**	**83.73**	**84.73**	**4E-08**	**4.13**
166	**Q.GAI.1388(I)_4B**	**4B**	**BS00064884_51**	**IAAV4595**	**83.73**	**83.73**	**84.73**	**3E-06**	**4.17**
167	Q.CTD(I)_4B	4B	BS00022653_51	Kukri_c11415_1074	111.24	111.24	112.25	4E-05	6.97
168	Q.Grains.E^{-1}.(R)_4B	4B	CAP7_c10839_300	BS00037020_51	143.65	143.65	146.70	2E-08	6.66
169	Q.FT(R)_4B	4B	BobWhite_c27751_206	Ku_c101046_1063	159.51	158.50	159.51	1E-06	4.43
170	Q.NDVI.2553(I)_4B	4B	Ra_c10455_3226	Excalibur_c106884_135	169.15	166.59	169.15	0.0286	1.04
171	**Q.GAI.1471.(I)_4B**	**4B**	**BS00009342_51**	**wsnp_Ex_c16825_25387841**	**178.44**	**173.22**	**178.44**	**0.0002**	**1.55**
172	**Q.CTD.(R)_4B.2**	**4B**	**RAC875_c24515_602**	**wsnp_Ku_c12503_20174234**	**179.45**	**179.45**	**182.54**	**0.0011**	**1.42**
173	**Q.AUC(I)_4B.2**	**4B**	**Tdurum_contig74813_560**	**BS00065222_51**	**226.6**	**226.60**	**230.19**	**0.0005**	**1.47**
174	**Q.GAI.1630(I)_4B.3**	**4B**	**Tdurum_contig74813_560**	**BS00065222_51**	**226.6**	**226.60**	**230.19**	**0.0004**	**1.18**
175	**Q.GAI.1192_4B.2**	**4B**	**Tdurum_contig74813_560**	**BS00065222_51**	**230.19**	**226.60**	**230.19**	**3E-06**	**1.28**
176	**Q.GAI.841_4D**	**4D**	**Kukri_rep_c68594_530**	**Excalibur_c19078_210**	**24.93**	**24.93**	**32.24**	**5E-05**	**3.24**
177	**Q.Grains.E^{-1}.(I)_4D**	**4D**	**Kukri_rep_c68594_530**	**Excalibur_c19078_210**	**24.93**	**24.93**	**32.24**	**4E-07**	**9.42**
178	**Q.NDVI.1555(R)_4D**	**4D**	**Kukri_rep_c68594_530**	**Excalibur_c19078_210**	**24.93**	**24.93**	**32.24**	**0.0007**	**1.36**
179	**Q.AUC(R)_4D**	**4D**	**Excalibur_c19078_211**	**RAC875_rep_c105718_305**	**32.24**	**32.24**	**40.11**	**0.0003**	**3.64**
180	**Q.CTD.(R)_4D**	**4D**	**Excalibur_c19078_210**	**RAC875_rep_c105718_304**	**32.24**	**32.24**	**40.11**	**0.001**	**3.73**
181	**Q.GAI.1630(I)_4D**	**4D**	**Excalibur_c19078_210**	**RAC875_rep_c105718_304**	**32.24**	**32.24**	**40.11**	**4E-07**	**3.2**
182	**Q.Grains.m^{-2}.(I)_4D**	**4D**	**Excalibur_c19078_210**	**RAC875_rep_c105718_304**	**32.24**	**32.24**	**40.11**	**4E-09**	**7.86**
183	**Q.GY.(I)_4D**	**4D**	**Excalibur_c19078_210**	**RAC875_rep_c105718_304**	**32.24**	**32.24**	**40.11**	**3E-07**	**3.42**
184	**Q.NDVI.1409(R)_4D**	**4D**	**Excalibur_c19078_210**	**RAC875_rep_c105718_304**	**32.24**	**32.24**	**40.11**	**5E-06**	**2.44**
185	**Q.P.ht(I)_4D**	**4D**	**Excalibur_c19078_210**	**RAC875_rep_c105718_304**	**32.24**	**32.24**	**40.11**	**0**	**15.18**
186	**Q.P.ht(R)_4D**	**4D**	**Excalibur_c19078_210**	**RAC875_rep_c105718_304**	**32.24**	**32.24**	**40.11**	**0**	**12.23**

Table 3.4 (continued)

	QTL name	Chromosome	Left marker	Right marker	Position	Left marker position	Right marker position	*p* value	R^2
187	Q.TGW(I)_4D.2	4D	Kukri_c20631_614	wsnp_BF473052D_Ta_2_1	62.98	62.98	80.59	6E-06	4.31
188	**Q.GAI.1388(I)_4D**	**4D**	**BobWhite_c20689_427**	**Excalibur_c79009_131**	**99.22**	**99.22**	**106.01**	**2E-06**	**2.68**
189	**Q.NDVI.1409(I)_4D**	**4D**	**BobWhite_c20689_427**	**Excalibur_c79009_131**	**99.22**	**99.22**	**106.01**	**0.004**	**1.18**
190	Q.NDVI.1976(I)_5A	5A	Kukri_c61108_900	wsnp_Ex_c356_698872	51.47	48.38	51.47	1E-04	2.25
191	Q.CTD(I)_5A.1	5A	BobWhite_rep_c49700_452	wsnp_Ex_c7668_13089715	69.43	68.43	69.43	0.002	1.64
192	Q.GY.(I)_5A	5A	BobWhite_c15454_63	Excalibur_c38185_633	83.05	83.05	87.13	9E-06	1.38
193	Q.CTD(I)_5A.2	5A	BS00015653_51	Tdurum_contig69079_300	99.27	98.27	99.27	0.01	2.44
194	Q.GY.(R)_5A	5A	BS00109052_51	RAC875_c232_1895	126.69	118.07	126.69	7E-05	2.68
195	**Q.GAI.1471.(R)_5A**	**5A**	**BobWhite_rep_c61813_322**	**BobWhite_c11512_157**	**128.21**	**128.21**	**137.43**	**0.004**	**2.08**
196	**Q.GAI.1388(I)_5A**	**5A**	**BobWhite_c11512_157**	**wsnp_Ku_c35386_44598937**	**137.43**	**137.43**	**138.95**	**5E-06**	**3.14**
197	Q.NDVI.1555(I)_5A.1	5A	Tdurum_contig43844_1266	Excalibur_c1208_72	139.96	139.96	141.98	5E-04	1.33
198	Q.Grains.m^{-2}.(R)_5A	5A	BS00060445_51	BS00000365_51	166.8	166.80	169.34	1E-10	2.14
199	Q.GAI.486_5A	5A	Excalibur_c9210_168	wsnp_Ex_c18941_27840714	177.07	176.07	177.07	4E-04	3.56
200	Q.GAI.213_5A	5A	wsnp_Ra_c17216_26044790	BS00021955_51	181.64	180.64	181.64	2E-06	3.97
201	**Q.FT(I)_5A.1**	**5A**	**BS00067209_51**	**BS00022644_51**	**183.69**	**183.69**	**189.51**	**3E-09**	**3.27**
202	**Q.Grains.m^{-2}.(I)_5A.1**	**5A**	**BS00067209_51**	**BS00022644_51**	**183.69**	**183.69**	**189.51**	**5E-05**	**1.84**
203	Q.GAI.1994(R)_5A	5A	IAAV9053	IAAV8258	197.58	196.58	197.58	0.002	3.09
204	Q.CTD.(R)_5A	5A	RAC875_rep_c107228_92	BobWhite_c40633_308	206.98	206.98	208.50	7E-05	4.18
205	Q.NDVI.1976(R)_5A.1	5A	BobWhite_c23736_153	Excalibur_c37943_221	211.51	210.51	211.51	0.004	1.5
206	Q.NDVI.1976(R)_5A.2	5A	BobWhite_c16397_524	BobWhite_c15476_88	249.15	245.55	249.15	0.014	1.56
207	**Q.FT(R)_5A**	**5A**	**wsnp_Ex_c16715_25264080**	**Kukri_rep_c102608_599**	**253.77**	**253.77**	**256.86**	**0.004**	**1.12**
208	**Q.Grains.m^{-2}.(I)_5A.2**	**5A**	**wsnp_Ex_c16715_25264080**	**Kukri_rep_c102608_599**	**253.77**	**253.77**	**256.86**	**0.017**	**2.73**
209	Q.NDVI.1409(R)_5A	5A	BS00089076_51	BS00064336_51	278.73	277.73	278.73	3E-05	1.44
210	Q.NDVI.1555(R)_5A	5A	Excalibur_c32414_705	BS00021969_51	300.75	300.75	301.75	5E-06	2.6
211	**Q.FT(I)_5A.2**	**5A**	**BobWhite_c11539_336**	**BobWhite_c8266_227**	**310.04**	**310.04**	**311.55**	**2E-07**	**3.03**
212	**Q.AUC(R)_5A**	**5A**	**BobWhite_c8266_228**	**Excalibur_c46261_343**	**312.56**	**311.55**	**312.56**	**0.003**	**2.27**
213	**Q.GAI.1388.(R)_5A**	**5A**	**BobWhite_c8266_227**	**Excalibur_c46261_342**	**312.56**	**311.55**	**312.56**	**0.003**	**1.5**
214	**Q.NDVI.1555(I)_5A.2**	**5A**	**BobWhite_c8266_227**	**Excalibur_c46261_342**	**312.56**	**311.55**	**312.56**	**0.008**	**1**
215	**Q.AUC(I)_5A**	**5A**	**Excalibur_c46261_342**	**Excalibur_c27357_146**	**313.57**	**312.56**	**313.57**	**4E-04**	**2.58**
216	Q.GAI.213_5B.1	5B	BS00062617_51	wsnp_Ex_c26252_35497729	13.16	12.15	13.16	0.005	1.38
217	Q.NDVI.2553(R)_5B	5B	BobWhite_c47740_85	Kukri_c23070_350	38.92	36.36	38.92	5E-09	3.47
218	**Q.AUC(I)_5B.1**	**5B**	**BS00009311_51**	**IAAV7267**	**81.2**	**77.12**	**81.20**	**81.2**	**2.52**
219	**Q.GAI.1471.(I)_5B.1**	**5B**	**BS00009311_51**	**IAAV7267**	**81.2**	**77.12**	**81.20**	**7E-04**	**2.69**
220	**Q.GAI.1388(I)_5B**	**5B**	**Kukri_c52_225**	**Excalibur_c17055_1451**	**113.4**	**113.40**	**115.44**	**4E-10**	**3.59**
221	**Q.Grains.m^{-2}.(I)_5B**	**5B**	**Kukri_c52_225**	**Excalibur_c17055_1451**	**113.4**	**113.40**	**115.44**	**0.007**	**1.55**
222	Q.CTD(I)_5B.1	5B	Ex_c13277_2025	Kukri_rep_c113115_424	133.41	133.41	134.41	0.005	3.25
223	Q.FT(I)_5B	5B	Kukri_c17396_2448	BS00087043_51	142.56	138.43	142.56	1E-05	1.44
224	Q.AUC(I)_5B.2	5B	BS00012038_51	Ex_c29928_1020	144.57	143.57	144.57	0.03	1.79
225	Q.Grains.E^{-1}.(R)_5B	5B	BS00010311_51	wsnp_Ex_c33675_42124657	152.66	150.12	152.66	2E-08	2.63
226	Q.Grains.m^{-2}.(R)_5B	5B	BS00093522_51	Excalibur_c9391_1016	163.98	163.98	165.50	2E-09	2.85
227	Q.GY.(R)_5B	5B	RAC875_c49291_156	Tdurum_contig57696_133	223.1	223.10	224.10	0.002	2.41
228	Q.GAI.213_5B.2	5B	BS00022991_51	BobWhite_c39214_164	229.16	227.12	229.16	0.001	3.19
229	**Q.CTD(I)_5B.2**	**5B**	**Kukri_c59657_805**	**BS00074721_51**	**281.57**	**281.57**	**282.57**	**0.014**	**2.24**
230	**Q.GAI.1471.(I)_5B.1**	**5B**	**Kukri_c59657_805**	**BS00074721_51**	**281.57**	**281.57**	**282.57**	**5E-05**	**2.92**
231	**Q.GAI.1192_5B**	**5B**	**Kukri_c59657_805**	**BS00074721_51**	**282.57**	**281.57**	**282.57**	**3E-07**	**4.26**
232	**Q.GAI.841_5B**	**5B**	**Kukri_c59657_805**	**BS00074721_51**	**282.57**	**281.57**	**282.57**	**7E-05**	**2.52**
233	**Q.NDVI.1555(I)_5B**	**5B**	**Kukri_c59657_805**	**BS00074721_51**	**282.57**	**281.57**	**282.57**	**2E-04**	**2.52**
234	**Q.NDVI.1976(R)_5B**	**5B**	**BS00024829_51**	**Excalibur_c94390_60**	**289.61**	**289.61**	**291.13**	**0.014**	**2.25**
235	**Q.P.ht(I)_5B**	**5B**	**BS00024829_51**	**Excalibur_c94390_60**	**289.61**	**289.61**	**291.13**	**4E-04**	**4.27**
236	Q.NDVI.1976(I)_5B	5B	Excalibur_c146_170	Excalibur_c71712_180	298.71	298.71	300.72	5E-05	3.54
237	Q.Grains.E^{-1}.(R)_5D	5D	BS00082423_51	Kukri_rep_c110911_477	120.79	120.79	121.79	1E-06	1.18
238	Q.FT(I)_5D	5D	BS00022699_51	CAP7_c3391_238	144.35	144.35	148.86	0.003	1.08
239	Q.Grains.m^{-2}.(R)_5D	5D	wsnp_Ex_rep_c68491_673181	wsnp_Ex_c23618_32855041	150.86	150.86	153.36	3E-12	2.82
240	Q.Grains.E^{-1}.(I)_5D	5D	BS00021991_51	BS00058709_51	171.07	171.07	173.07	0	5.91
241	Q.GAI.1994(R)_5D	5D	Excalibur_c2795_1518	RAC875_c34515_86	198.08	198.08	199.08	8E-04	1.21
242	**Q.GAI.1994.(I)_6A**	**6A**	**Tdurum_contig29823_203**	**BS00066872_51**	**32.88**	**32.88**	**34.40**	**2E-06**	**1.86**
243	**Q.CTD(I)_6A**	**6A**	**BS00022938_51**	**BobWhite_c15802_72**	**37.42**	**36.42**	**37.42**	**0.004**	**2.25**
244	Q.Grains.E^{-1}.(I)_6A	6A	RAC875_c53520_103	BS00022951_51	61.07	58.53	61.07	0.023	3.19
245	**Q.NDVI.2553(I)_6A.1**	**6A**	**CAP7_c2381_149**	**CAP11_c989_113**	**109.46**	**109.46**	**112.02**	**2E-05**	**3.46**
246	**Q.GAI.213_6A**	**6A**	**CAP7_c2381_149**	**CAP11_c989_113**	**112.02**	**109.46**	**112.02**	**0.001**	**1.53**
247	Q.TGW(I)_6A	6A	BS00034886_51	IACX14305	130.43	129.43	130.43	5E-06	7.17

Table 3.4 (continued)

	QTL name	Chromosome	Left marker	Right marker	Position	Left marker position	Right marker position	*p* value	R^2
248	**Q.GAI.1630(I)_6A.1**	**6A**	**BS00010576_51**	**Excalibur_c56264_188**	**146.08**	**146.08**	**148.64**	**5E-05**	**0.92**
249	**Q.NDVI.2553(R)_6A**	**6A**	**BS00010576_51**	**Excalibur_c56264_188**	**148.64**	**146.08**	**148.64**	**5E-08**	**4.32**
250	Q.NDVI.1409(I)_6A	6A	IAAV1652	BS00012028_51	155.74	155.74	157.26	1E-06	3.83
251	**Q.NDVI.1555(I)_6A.1**	**6A**	**wsnp_Ex_c11348_18326787**	**BS00023893_51**	**175.93**	**175.93**	**176.94**	**0.0098**	**1.23**
252	**Q.GAI.486_6A**	**6A**	**BS00023893_51**	**BS00065082_51**	**180.47**	**176.94**	**180.47**	**0.0117**	**1.39**
253	Q.Grains.m^{-2}.(I)_6A	6A	IAAV151	BS00058929_51	191.33	184.00	191.33	5E-05	6.24
254	**Q.NDVI.2553(I)_6A.2**	**6A**	**Kukri_rep_c69627_954**	**Tdurum_contig97355_136**	**203.97**	**192.34**	**203.97**	**0.0082**	**2.41**
255	**Q.GAI.1630(I)_6A.2**	**6A**	**BobWhite_c40051_360**	**RFL_Contig5037_560**	**204.98**	**204.98**	**210.63**	**0.0002**	**2.95**
256	Q.FT(R)_6A	6A	RFL_Contig5037_560	Excalibur_c52196_235	215.25	210.63	215.25	0.0002	1.49
257	Q.NDVI.1555(I)_6A.2	6A	RAC875_c12821_550	BS00011578_51	256.9	256.90	268.25	6E-06	3.6
258	Q.NDVI.1555(R)_6B	6B	CAP7_c10772_156	Ex_c66287_325	10.7	9.70	10.70	0.0192	1
259	Q.NDVI.2553(R)_6B.1	6B	wsnp_Ku_c2119_4098330	BobWhite_c20959_229	15.73	14.47	15.73	1E-06	5.84
260	**Q.GAI.1994.(I)_6B.1**	**6B**	**Excalibur_c39569_79**	**RAC875_c26860_648**	**30.16**	**30.16**	**32.18**	**2E-05**	**1.38**
261	**Q.TGW(R)_6B**	**6B**	**RAC875_c26860_648**	**BS00010403_51**	**35.81**	**32.18**	**35.81**	**5E-05**	**1.46**
262	Q.GAI.1388(I)_6B	6B	BS00027942_51	wsnp_Ex_c4815_8597064	42.6	41.08	42.60	4E-06	1.71
263	**Q.GAI.1630(I)_6B**	**6B**	**BS00011530_51**	**BobWhite_c9330_499**	**56.49**	**56.49**	**57.49**	**0.0003**	**3.19**
264	**Q.GAI.1994(R)_6B**	**6B**	**BS00011530_51**	**BobWhite_c9330_499**	**57.49**	**56.49**	**57.49**	**0.0127**	**2.8**
265	Q.GAI.1630(R)_6B	6B	Ex_c31970_673	CAP8_rep_c9477_231	66.23	66.23	69.86	1E-06	4.25
266	Q.NDVI.1976(R)_6B	6B	BobWhite_c18550_159	wsnp_Ex_c17435_26144201	80.74	79.74	80.74	0.0095	1.32
267	Q.TGW(I)_6B	6B	BS00049942_51	Excalibur_c22998_621	159.18	157.67	159.18	0.0187	2.72
268	**Q.CTD(I)_6B**	**6B**	**Excalibur_rep_c69189_235**	**BobWhite_c33227_82**	**170.78**	**169.78**	**170.78**	**0.0013**	**1.23**
269	**Q.GAI.1994.(I)_6B.2**	**6B**	**RAC875_c6649_642**	**BS00023050_51**	**173.83**	**172.82**	**173.83**	**2E-05**	**4.65**
270	**Q.GY.(I)_6B**	**6B**	**Kukri_c56955_282**	**Tdurum_contig41142_267**	**175.85**	**175.85**	**182.56**	**0.0001**	**1.97**
271	Q.P.ht(I)_6B	6B	RAC875_c23461_428	Excalibur_c76628_251	192.21	189.67	192.21	2E-05	1.08
272	Q.FT(I)_6B.1	6B	Ku_c24158_1468	RAC875_rep_c71463_98	214.55	214.55	221.24	2E-07	1.38
273	Q.NDVI.2553(R)_6B.2	6B	Kukri_c338_109	Ra_c39588_830	235.26	232.24	235.26	0.0047	3.1
274	Q.FT(I)_6B.2	6B	IACX1137	BobWhite_c43263_123	261.89	260.38	261.89	0.0004	2.23
275	**Q.NDVI.1409(R)_6D**	**6D**	**IAAV5171**	**BS00063175_51**	**104.21**	**104.21**	**105.22**	**5E-05**	**1.65**
276	**Q.NDVI.1976(I)_6D**	**6D**	**IAAV5171**	**BS00063175_51**	**104.21**	**104.21**	**105.22**	**0.0005**	**2.19**
277	Q.Grains.E^{-1}.(R)_6D	6D	BS00021881_51	BobWhite_c22280_104	130.85	130.85	190.15	1E-08	4.22
278	Q.GAI.1630(I)_6D	6D	BobWhite_c13435_700	BS00070856_51	215	211.90	215.00	9E-06	0.94
279	**Q.Grains.m^{-2}.(R)_7A.1**	**7A**	**BobWhite_c33300_159**	**Tdurum_contig93663_457**	**33.03**	**33.03**	**35.05**	**4E-10**	**4.28**
280	**Q.GAI.1471.(R)_7A**	**7A**	**BobWhite_c33300_159**	**Tdurum_contig93663_457**	**35.05**	**33.03**	**35.05**	**2E-06**	**3.87**
281	Q.GAI.1388.(R)_7A	7A	RAC875_c6736_336	BS00002974_51	43.65	43.65	50.25	0.0007	1.61
282	Q.GAI.1630(R)_7A.1	7A	Excalibur_c1310_414	Tdurum_contig15285_588	51.26	51.26	52.26	0.0002	1.57
283	Q.FT(R)_7A	7A	BS00111120_51	BS00038787_51	188.57	187.57	188.57	0.0008	1.78
284	Q.NDVI.2553(I)_7A	7A	BS00039561_51	IAAV5154	194.62	193.12	194.62	0.0015	3.57
285	**Q.NDVI.1555(I)_7A**	**7A**	**Kukri_rep_c74538_62**	**RAC875_c37085_317**	**303.2**	**301.69**	**303.20**	**0.0291**	**1.95**
286	**Q.NDVI.1976(I)_7A**	**7A**	**IACX2471**	**BobWhite_c15352_394**	**307.74**	**305.72**	**307.74**	**0.0015**	**3.99**
287	Q.GAI.213_7A	7A	BS00004257_51	Excalibur_c14451_1313	312.27	312.27	313.79	5E-12	3.45
288	Q.GAI.1630(R)_7A.2	7A	wsnp_Ex_c27898_37058842	Excalibur_c60238_183	328.69	327.18	328.69	8E-05	4.22
289	Q.AUC(I)_7A	7A	BS00053365_51	CAP7_rep_c10402_310	342.526	342.53	344.55	0.0118	4.29
290	Q.TGW(R)_7A	7A	CAP7_rep_c10402_310	Excalibur_c1142_724	346.57	344.55	346.57	0.0002	2.25
291	Q.GAI.1994.(I)_7A	7A	wsnp_Ex_c6142_10746442	wsnp_Ex_c53387_56641291	365.23	365.23	366.23	0.0039	3.21
292	**Q.Grains.m^{-2}.(R)_7A.2**	**7A**	**wsnp_BF483039A_Ta_2_1**	**BS00020236_51**	**380.43**	**380.43**	**382.97**	**4E-10**	**4.16**
293	**Q.NDVI.2553(R)_7A**	**7A**	**BS00020236_51**	**BobWhite_c32883_84**	**386.06**	**382.97**	**386.06**	**0.0002**	**1.18**
294	**Q.Grains.E^{-1}.(I)_7B.1**	**7B**	**Excalibur_c15405_808**	**Tdurum_contig5352_556**	**0**	**0.00**	**6.39**	**1E-08**	**3.02**
295	**Q.P.ht(I)_7B**	**7B**	**Excalibur_c15405_808**	**Tdurum_contig5352_556**	**6.39**	**0.00**	**6.39**	**0.0144**	**1.4**
296	Q.TGW(R)_7B	7B	BS00111144_51	BS00022056_51	43.08	41.58	43.08	0.0014	2.02
297	Q.TGW(I)_7B.1	7B	BS00010616_51	BS00064344_51	60.03	57.99	60.03	3E-07	4.8
298	Q.GAI.841_7B.1	7B	CAP11_c106_97	Tdurum_contig92540_1265	90.07	84.25	90.07	7E-05	2.89
299	**Q.Grains.m^{-2}.(I)_7B.1**	**7B**	**Ra_c3470_1551**	**BS00044443_51**	**109.14**	**109.14**	**110.66**	**0.0011**	**3.42**
300	**Q.GAI.1630(R)_7B**	**7B**	**BS00044443_51**	**BS00029286_51**	**110.66**	**110.66**	**121.82**	**1E-06**	**2.57**
301	Q.GAI.841_7B.2	7B	BS00025278_51	Excalibur_c12499_2075	147.07	147.07	148.58	0.0001	6.87
302	Q.Grains.E^{-1}.(I)_7B.1	7B	tplb0058p02_2806	Kukri_rep_c72901_271	194.38	193.38	194.38	9E-12	2.74
303	**Q.NDVI.1409(R)_7B**	**7B**	**Tdurum_contig52096_330**	**CAP7_c3950_160**	**255.55**	**255.55**	**256.55**	**2E-10**	**2.67**
304	**Q.NDVI.1555(R)_7B**	**7B**	**Tdurum_contig52096_330**	**CAP7_c3950_160**	**255.55**	**255.55**	**256.55**	**8E-07**	**1.89**
305	Q.TGW(I)_7B.2	7B	RAC875_c525_106	CAP12_c194_402	274.13	273.13	274.13	6E-05	1.44
306	Q.Grains.m^{-2}.(I)_7B.2	7B	Kukri_c2348_2340	RAC875_c14064_177	287.41	286.40	287.41	0.0019	3.14
307	Q.Grains.m^{-2}.(R)_7D	7D	BS00065628_51	Kukri_c6274_1283	31.95	31.95	32.95	1E-09	2.93
308	Q.Grains.E^{-1}.(I)_7D	7D	wsnp_Ku_c27286_37236472	RAC875_c53629_483	63.26	53.28	63.26	3E-08	1.59
309	Q.CTD(I)_7D	7D	BS00062644_51	BS00070188_51	117.36	117.36	118.37	0.0002	2.13

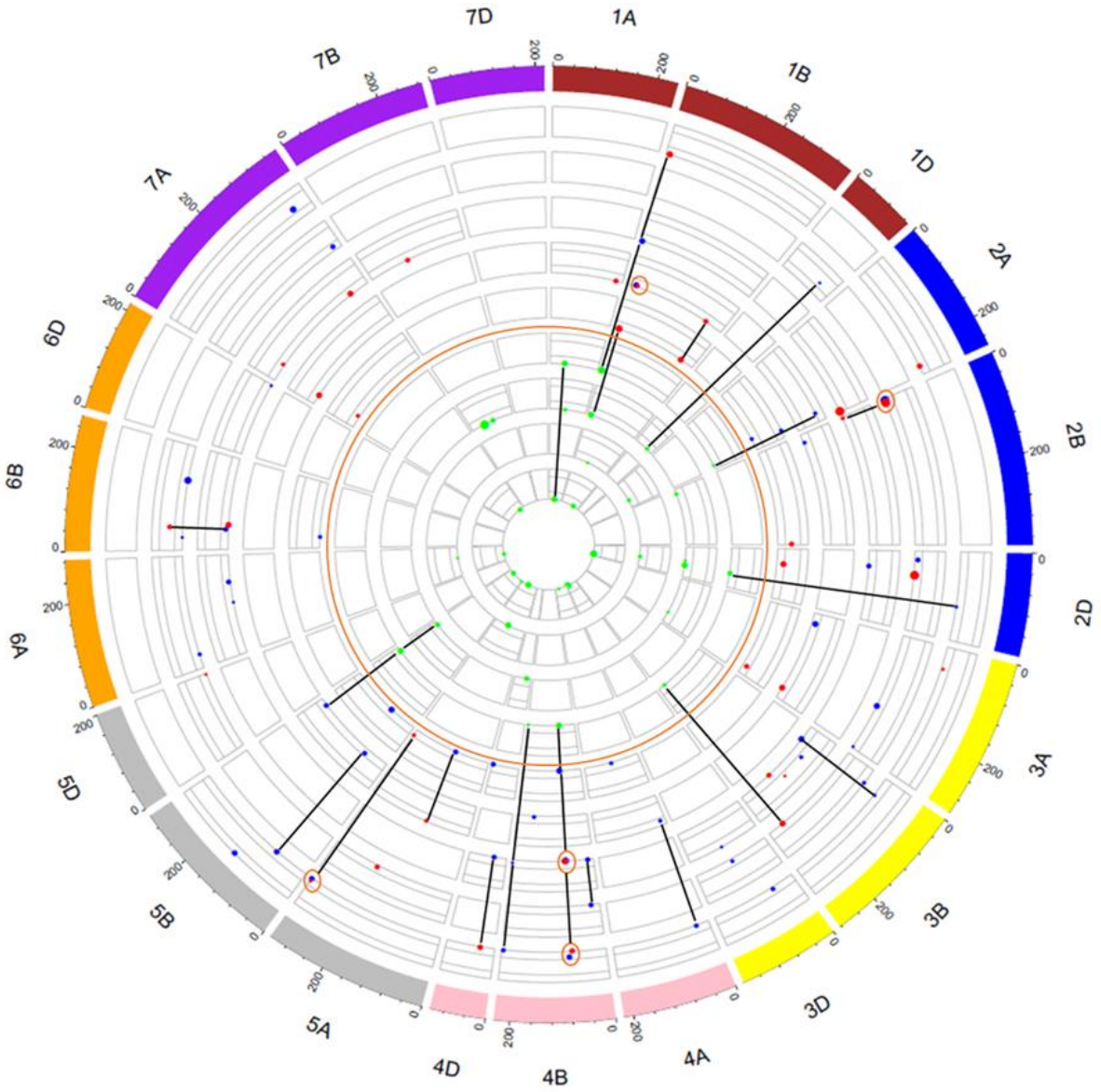


Figure 3.2 Circos plot showing a genetic map of the 21 wheat chromosomes; LOD scores of individual significant SNPs (coloured dots on inner light grey segments- dot size is proportional to the effect size of QTL). LOD 5 and LOD 10 thresholds are marked by concentric circular lines. Chromosomal units in CM. Tracks inner to out illustrate the 8 consecutive GAI dates and the 9th shows AUC. green dots show QTL before applying irrigation in the inner 4 tracks, then red and blue dots mark those of rainfed and irrigated treatments respectively. Black vertical lines link QTL on the same genomic position. Orange circle separates tracks before and after irrigation.

3.4.4 Grain yield bi-locus interactions

An investigation of the two-way epistasis was done by selecting the five top statistically significant interactions of the PLINK output for each treatment, then interrogating them to identify those with big effect size. Six two-way interactions were identified, four of which were in the irrigated and two in the rainfed treatment (Figure 3.3). Among the 12 interacting SNPs, four were coinciding with identified QTL of other traits. The most significant example is one of the two interacting SNPs for rainfed yield (wsnp_CAP12_c1503_764765) on 2D and is tagging *Ppd-D1* photoperiod sensitivity locus with pleiotropic impact on other phenotypes such as GAI.1388, GAI.1994, NDVI.1976 and CTD.

The interacting alleles showed both favourable and unfavourable combinations as presented in Figure 3.4, for example the favourable allele combination in Figure 3.4 a, caused grain yield increase by 1.5 t/ha and this was found in 2.4% of the tested population. On the other hand, the interaction in Figure 3.4.f illustrates an epistasis that suppresses grain yield by more than 2 t/ha represented in 2.8% of the population.

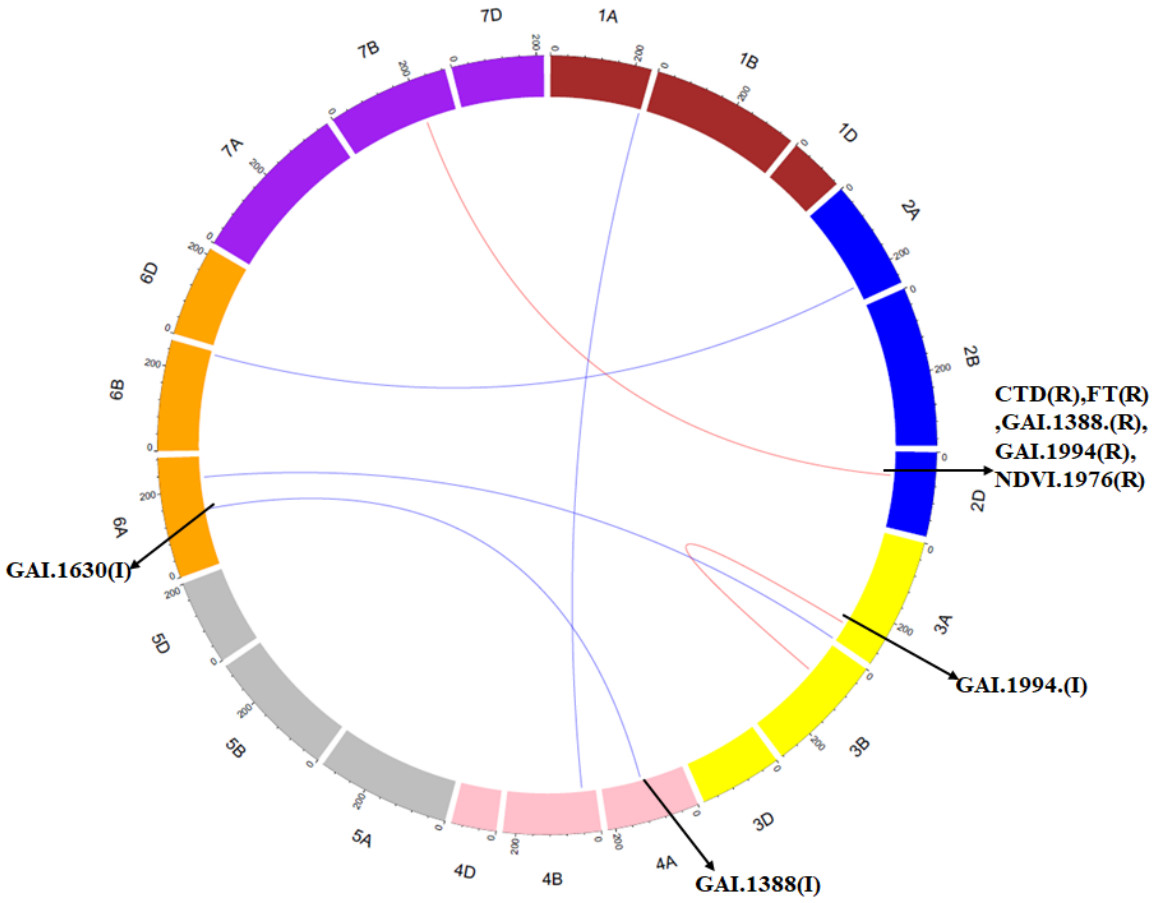


Figure 3.3 Circos plot showing a genetic map of the 21 wheat chromosomes. Chromosomal units in CM. The two-way interacting SNPs are connected with red and blue lines for rainfed and irrigated treatments, respectively. Main QTL of coinciding interactions are pointed with black arrows.

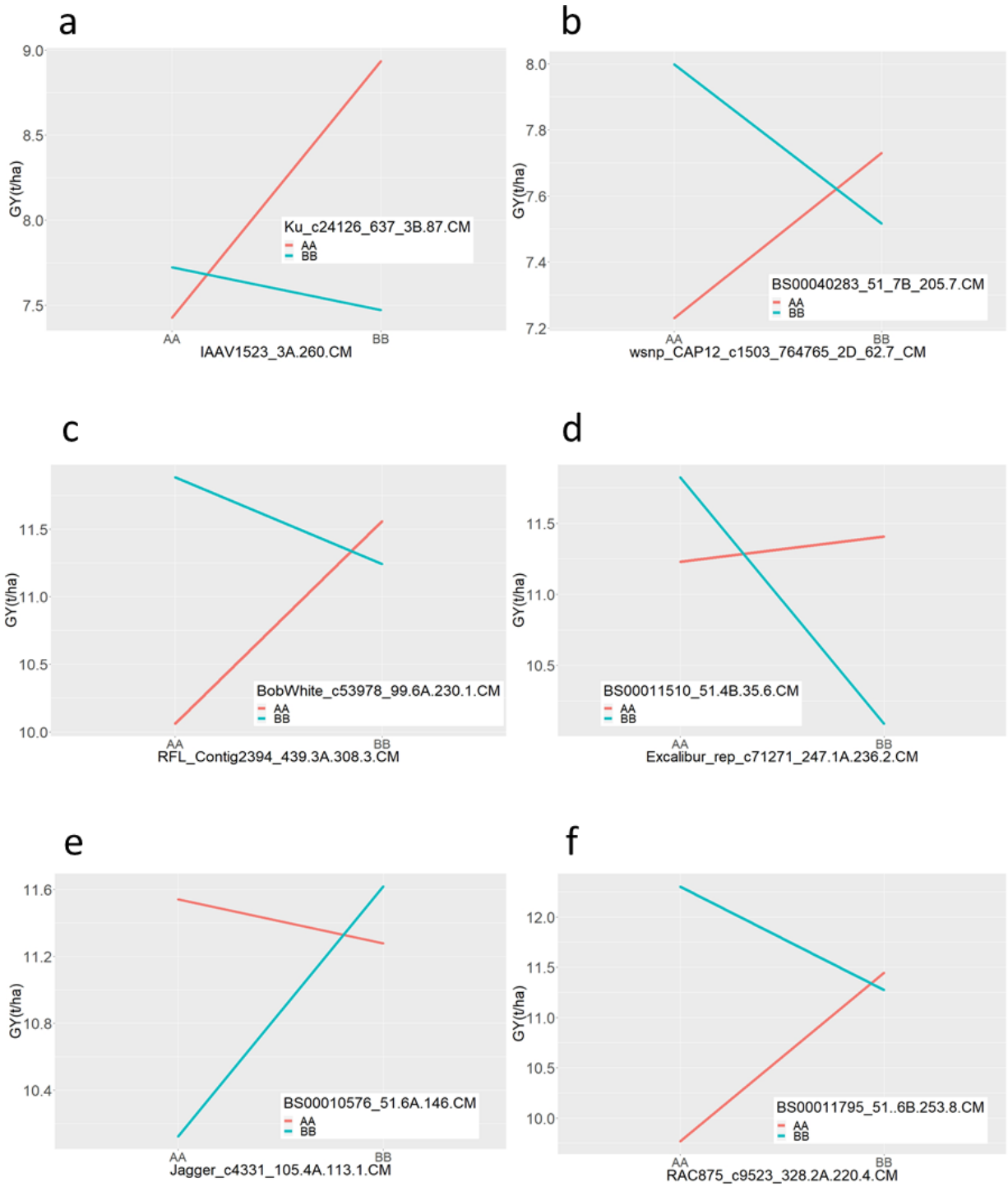


Figure 3.4 Interaction plots of significant epistatic SNP pairs on grain yield. (a, b) in rainfed treatment and (c, d, e and f) in irrigated treatment.

3.5 Discussion

3.5.1 Drought impact on phenology and yield

The limited rainfall caused SMD to exceed 75 mm for prolonged periods of time in the rainfed blocks, which is reported as canopy expansion restriction threshold and consequently limits grain yield in several field trials under UK conditions and might have an amplified impact if coupled with significant high maximum air temperature which was the case in this experiment. The water limitation had a significantly negative impact on crop canopy indices, stem elongation, CTD and grains.m^{-2} (Table 3.2), causing an average yield reduction of 32.8%, which agrees with yield losses reported for winter wheat in the UK in dry years either in commercial cultivars or biparental populations (Foulkes *et al*., 2001, Foulkes *et al*., 2002, Foulkes *et al*., 2007).

Generally, heritability estimates were moderate to low, with considerable variation among traits. FT had the highest heritability value, mainly due to the minimal environmental influence on this trait (even under contrasting water availability regimes in this experiment), that it is largely governed by a limited number of major genes controlling vernalization requirements and photoperiod sensitivity (Griffiths *et al*., 2009) and relatively precise evaluation. While low heritability values for some traits (most of crop canopy indices measurements) indicate that environment had a big role in their expression. Moderate heritability of 0.53 for GY is typical of moderate to low values of grain yield heritability, which is significantly affected by environment, especially under drought stress (Huang *et al*., 2006, Cuthbert *et al*., 2008, El-Feki *et al*., 2018).

All crop canopy indices positively correlated with GY in both treatments, a result agreeing with (Pennacchi *et al*., 2018) who reported significant correlation of yield with canopy cover development with remarkably strong correlation with accumulated green area (r=0.43) and highest cover (r=0.42). Also, (Brian *et al*., 2004, Guan *et al*., 2019) investigated correlation of NDVI at different dates during summer with yield and reported significant correlations between (r=0.48) and (r=0.83). Notably, stepwise regression analysis showed contrast in identifying the key significant date predicting the yield under either treatment. Namely, NDVI.1976 tagging mid-grain filling and maximum crop cover in irrigated blocks and indicates the potential of strong light interception in explaining yield in favourable conditions as agreed by (Pennacchi *et al*., 2018), while in rainfed blocks it was GAI.1388 that coincided with tillering stage, indicating that genotypes with sustained tillering potential could sustain yield under drought commencing early in the season. This interpretation could be confirmed

by the fact that drought in this experiment caused minimal reduction on TGW and grains.ear^{-1} but reduced grains.m^{-2} by 19.2%.

In rainfed treatment, flowering time revealed minor correlation with yield and no significant presence in the prediction model, contradicting the reports of Blum (2011) and Shavrukov *et al*. (2017), where early flowering served as a drought escape and avoidance mechanism, but supported by Foulkes *et al*. (2007) findings of neutral effect of FT in absence of severe terminal drought, such as the drought UK faces in dry years.

In case of soil water limited availability and as air temperature rises during daytime, stomatal conductance tends to decrease and consequently decreases CTD (Urban *et al*., 2007, Thapa *et al*., 2018). In both treatments, CTD measured at mid-grain filling showed strong correlation with grain yield (r=0.52) and (r=0.61) in rainfed and irrigated treatments, respectively, as well as a significant contribution in the stepwise regression model. Lopes and Reynolds (2010) concluded an association of canopy temperature with deep water extraction ability as they found cooler canopy wheat genotypes to develop 40% increase in root mass and outperformed warmer canopy genotypes by a 30% grain yield increase.

Number of grains/unit area is considered the main component of genetic gain in wheat grain yield potential beside grain weight; generally, this gain tended to stabilise TGW or even reduce it in a trade-off that favours number of grains/unit area (Sadras, 2007, Fischer, 2008). Such an important component was the highest phenotype correlated with grain yield in both treatments (r=0.68) and (r=0.72) in rainfed and irrigated treatments, respectively. These high correlation coefficients are broadly typical of GY correlation with grain.m^{-2}, for example, Griffiths *et al*. (2015) investigated this relation in a double haploid population tested in four countries and found significant correlation (r=0.56-0.9) with grain yield. In this experiment, there was minimal reduction in grains.E^{-1} and TGW in response to drought; this indicates grains.m^{-2} to be the main component in yield reduction under stress. This inference could be confirmed by the significant reduction ($p \leq 0.01$) in number of ears/unit area in the highly replicated variety 'Kielder'.

3.5.2 QTL analysis and epistasis

The MAGIC panel has a wide range of morphological and physiological characteristics, in addition to high recombination rate and low population structure, allowing for high resolution QTL analysis to decipher the genetic basis of phenotypic variation of measured traits under both treatments.

QTL analysis for GAI as dynamic individual time points illustrated number of QTL exhibiting contribution over consecutive dates, while most of them were unique to the measuring date. Besides pinpointing significantly associated markers with each phenotype, this gives an insight on when these markers signals are peaking or decaying.

Major effect QTL for plant height were identified on chromosome 4B, investigating parental effect confirmed that *Rht-B1b* is inherited from 'Robigus' and 'Soissons', the two founders harbouring the 4B copy of the dwarfing gene. Also identified major effect QTL for flowering time on chromosome 2D tagging photoperiod sensitivity locus *Ppd-D1*, 'Soissons' was the donor of the insensitive allele *Ppd-D1* and caused early flowering in about ⅛ of the population, which did not appear to contribute to drought resistance in this experiment, contradicting several reports such as Shavrukov *et al*. (2017) and El-Feki *et al*. (2018), mainly as we had mild terminal drought and the biggest stress impact occurred pre-anthesis, so earliness and avoidance were not of much advantage.

The pleiotropy shown by the D-genome copy of reduced height gene (*Rht-D1b*) is supported by previous researchers investigating in the same population used in this study. For example, Camargo *et al*. (2016) found it to explain 24% of phenotypic variation in harvest index, it was reported to explain 24 to 33% of phenotypic variation in above ground plant area at different time points (Camargo *et al*., 2018) and more recently, Jackson (2019) identified various sink traits QTL in the same region, such as grain area, grain width, factor form density (ffd) and TGW.

For grain yield, significant main-effect QTL were found on chromosomes 1A, 1B, 1D, 3A, 4A, 4B, 4D,5A, 5B and 6B, the only treatment independent QTL was identified on 4A. Looking at each treatment *per se*, 10 and 5 unique QTL were identified in the irrigated and rainfed treatment, respectively, emphasising the environmental impact on expression of such complex traits. Using the same population, Jackson (2019) found the same QTL, Q.GY.(R)_4B and Q.GY.(R)_5B to explain 3.32 and 6% of phenotypic variation in a year specific appearance.

Beside main effect QTL, yield epistatic SNP pairs analysis revealed significant combinations of alleles with positive/negative impact on yield, with 0.8 to 2.3 t/ha increase /decrease in the unique combination. A minor percentage of RILs encompassing these combinations ranged from 2.3 to 6.5% of the tested panel. Three of the six significant epistatic SNPs co-located with main QTL found for other traits, mainly crop canopy indices that did not co-locate with

the main effect QTL of grain yield. The only case of non-canopy related phenotype was the QTL tagging *Ppd-D* locus on chromosome 2D, the co-location of major effect QTL for grain yield and *Ppd-D* locus was recently reported by Sehgal *et al*. (2017).

The Multi-Parent Advanced Generation Inter-Crosses (MAGIC) is believed to overcome the drawbacks of both biparental populations and association panels, increasing simultaneously the power, diversity and resolution of detecting genomic regions associated with different traits. The elite MAGIC population used in this experiment has captured > 80% of the genetic diversity of the UK wheat germplasm after comparing the SNP markers to those of the Wheat Association Genetics for Trait Advancement and Improvement of Lineages "WAGTAIL" association panel (520 varieties), giving the chance to identify genotypes with allele combinations that confer higher yield and adaptability to stresses. On the other hand, these allele combinations all came from founder varieties highly adapted to the UK environmental condition with low chances of basis for drought tolerance. For future studies, it might be advised to enrich the MAGIC population genetic base with exotic material providing traits strongly associated with drought tolerance.

3.6 Conclusion

Using the winter wheat elite MAGIC population, this is the first study to investigate this panel's (capturing > 80% of the genetic diversity of the UK wheat germplasm) responses to contrasting water availability regimes in the field and describes the most important phenology predicting grain yield in both treatments, given the environmental parameters such as rainfall, SMD, air temperature and soil temperature and identified drought resistance to occur mainly through sustaining grains.m^{-2} and cooler canopies. In this study, besides previously reported QTL for different phenological traits and grain yield, we identified novel QTL either stable and/or treatment specific and significant epistatic SNP pairs controlling grain yield. These significant QTL provide preliminary genomic architecture that could be used in marker assisted selection for defined drought tolerance, after validation in more field trials.

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3.8 Supplementary materials

Table S3. 1 Founder lines of the eight-parent MAGIC wheat population. Modified from Mackay *et al* (2014).

Variety	Listing Year	Seed Yield (t/ha)	NABIM Quality Group	Trait attributes
Alchemy	2006	9.163	4	Yield, disease resistance, breeding use, soft
Brompton	2005	9.151	4	Hard feed, 1BL/1RS, OWBM-resistant
Claire	1999	8.654	3	Soft biscuit/distilling, slow apical development
Hereward	1991	7.683	1	High-quality benchmark 1 bread-making
Rialto	1994	8.377	2	Moderate bread-making, 1BL/1RS
Robigus	2003	9.053	3	Bread-making quality, early flowering, *Rht-B1*
Soissons	1995	7.553	2	Bread-making quality, early flowering, *Rht-B1*
Xi-19	2002	8.957	1	Bread-making quality, facultative type, breeding use

Table S3. 2 Parental effect (relative to founder Xi-19) for the detected QTL.

	QTL name	Chromosome	Position	Alchemy	Brompton	Claire	Hereward	Rialto	Robigus	Soissons	*p* value	R^2
1	Q.FT(I)_1A	1A	1.01	-58.14	32.55	-40.40	-49.90	-17.40	-28.07	-11.23	4E-07	3.04
2	Q.NDVI.1976(I)_1A	1A	23.76	-0.01	0.03	0.00	-0.01	0.02	-0.01	0.02	0.0001	1.37
3	Q.GAI.1192_1A	1A	71.10	-0.01	-0.01	0.06	-0.14	-0.01	0.04	-0.01	4E-08	4.14
4	Q.GY.(I)_1A	1A	85.71	1.57	0.15	-2.74	-0.82	-0.14	-0.18	-0.62	0.0113	3.33
5	Q.GAI.841_1A	1A	96.10	0.00	0.00	0.00	-0.06	-0.01	0.02	0.01	7E-07	1.74
6	Q.GAI.213_1A	1A	101.17	-0.43	0.02	0.08	-0.38	0.02	0.29	-0.13	0.0021	3.6
7	Q.NDVI.1555(I)_1A	1A	145.04	-0.02	0.03	NA	-0.05	-0.04	-0.02	0.00	1E-08	2.45
8	Q.NDVI.2553(I)_1A	1A	211.95	0.01	-0.01	NA	0.01	0.00	-0.02	-0.01	0.0067	1.64
9	Q.Grains.m^{-2}.(I)_1A	1A	215.76	7944.64	-2501.06	NA	573.05	-1327.48	-1575.55	-1807.77	0.0088	2.22
10	Q.GAI.1471.(R)_1A	1A	216.76	0.00	0.07	NA	0.03	0.02	0.01	0.00	3E-05	2.93
11	Q.GY.(R)_1A	1A	231.14	0.40	0.92	NA	-0.01	0.20	-0.39	-0.10	5E-05	2.24
12	Q.GAI.1192_1B	1B	2.51	0.01	0.04	0.03	0.02	NA	0.01	0.00	3E-06	4.95
13	Q.AUC(I)_1B	1B	8.90	14.09	-71.79	31.85	10.85	114.22	28.16	1.33	7E-07	5.89
14	Q.AUC(R)_1B	1B	8.90	2.31	-161.15	18.88	4.38	186.77	1.74	-19.46	4E-07	5.16
15	Q.GAI.1630(I)_1B	1B	8.90	0.01	-0.05	0.03	0.02	0.11	0.03	-0.01	3E-08	4.49
16	Q.NDVI.1409(I)_1B	1B	13.48	0.02	-0.14	0.01	-0.01	0.15	0.00	-0.01	0.002	2.93
17	Q.GAI.1388.(R)_1B.1	1B	21.72	0.00	-0.01	0.00	0.01	0.02	0.02	-0.02	5E-06	4.51
18	Q.GAI.841_1B	1B	21.72	0.00	-0.03	0.01	0.00	0.06	0.00	0.00	2E-05	3.91
19	Q.FT(R)_1B	1B	26.75	-6.53	-5.45	8.14	-14.09	28.81	3.60	10.60	0.0007	1.09
20	Q.GAI.1471.(I)_1B	1B	31.32	0.01	0.05	-0.02	-0.02	-0.03	0.01	-0.01	0.0002	4.24
21	Q.GAI.1471.(R)_1B.1	1B	291.21	-0.03	0.00	-0.01	-0.03	0.00	-0.03	-0.02	1E-05	2.88
22	Q.GY.(I)_1B.1	1B	56.55	0.68	-2.41	-0.53	0.48	3.61	0.54	0.61	0.0002	4.22
23	Q.NDVI.1976(I)_1B	1B	93.08	0.04	0.01	0.02	0.02	0.05	0.04	0.02	3E-07	5.13
24	Q.NDVI.1555(I)_1B	1B	101.92	0.04	0.02	0.03	0.02	0.03	0.04	0.01	0.0001	3.88
25	Q.GAI.486_1B	1B	133.11	0.00	0.00	0.00	-0.01	0.01	0.00	0.00	0.0003	1.24
26	Q.GY.(I)_1B.2	1B	189.63	0.67	1.37	0.32	0.21	0.24	1.57	0.68	0.0025	3.62
27	Q.Grains.E^{-1}.(R)_1B	1B	229.70	-17.97	1.54	-14.05	-17.03	-14.77	-12.17	-11.33	1E-12	2.13
28	Q.GY.(I)_1B.3	1B	240.30	-0.10	0.20	-0.16	-0.30	-0.13	-1.47	-0.61	0.0002	1.1
29	Q.GAI.213_1B	1B	244.83	0.09	0.09	0.40	0.14	0.02	-0.02	0.02	1E-07	2.69
30	Q.CTD.(R)_1B	1B	265.02	-0.11	0.01	-0.08	0.10	-0.28	0.41	0.15	0.0119	1.83
31	Q.GAI.1471.(R)_1B.2	1B	35.39	0.00	0.00	-0.01	0.01	-0.01	0.00	-0.03	0.0002	2.47
32	Q.P.ht(R)_1B	1B	291.21	0.75	1.33	2.88	-0.41	1.06	-2.19	2.13	0.0045	1.85
33	Q.GAI.1388.(R)_1B.2	1B	299.25	-0.02	0.00	-0.03	-0.03	0.01	-0.01	-0.02	1E-05	3.73
34	Q.Grains.E^{-1}.(I)_1B	1B	327.18	-8.46	-5.05	-10.03	-4.45	-4.04	-0.41	-13.65	0.0001	2.65
35	Q.NDVI.1555(R)_1B	1B	337.34	0.00	0.00	-0.03	-0.02	0.02	0.00	-0.01	2E-05	3.99
36	Q.Grains.m^{-2}.(R)_1D	1D	19.25	-6476.73	-4747.84	-5575.80	-16422.03	-9477.95	-11855.12	-7557.07	1E-08	7.38
37	Q.GY.(I)_1D	1D	39.65	0.45	-1.17	-0.83	-1.53	-0.24	0.39	-0.08	5E-06	2.49
38	Q.CTD.(R)_1D	1D	55.00	1.43	1.20	0.76	1.66	2.71	1.03	1.07	9E-05	1.02
39	Q.Grains.E^{-1}.(R)_1D	1D	71.23	17.15	-10.74	8.62	-2.35	9.93	17.69	7.37	4E-13	2.13
40	Q.GAI.841_1D	1D	89.02	-0.03	-0.01	-0.01	0.00	0.01	0.01	-0.01	0.0007	2.27
41	Q.GAI.1994.(I)_1D	1D	99.08	0.01	-0.02	0.02	0.01	0.00	0.04	0.02	0.0124	1.48
42	Q.TGW(R)_1D	1D	122.74	-2.90	5.61	0.12	5.79	12.11	4.49	8.38	1E-05	4.44
43	Q.Grains.E^{-1}.(R)_2A	2A	146.83	15.86	19.69	7.71	15.13	18.37	13.90	17.20	4E-05	10.99
44	Q.Grains.m^{-2}.(R)_2A	2A	177.36	8983.44	4426.15	4916.88	8787.28	9191.56	3603.20	3519.47	3E-14	8.41
45	Q.GAI.486_2A	2A	187.33	0.01	0.00	0.01	0.00	0.00	0.00	0.00	6E-06	2.49
46	Q.CTD(I)_2A	2A	206.04	-0.01	-0.28	-0.29	-0.19	-0.31	-0.31	-0.04	0.0008	1.2
47	Q.NDVI.1555(I)_2A	2A	206.04	0.02	0.01	-0.01	-0.01	0.01	0.02	-0.02	0.0048	2.44
48	Q.GAI.1388(I)_2A.1	2A	207.05	0.03	0.02	0.00	-0.01	0.03	0.01	-0.01	0.0001	1.74
49	Q.GAI.1388(I)_2A.2	2A	230.55	0.00	0.00	0.03	0.04	0.00	0.01	0.00	0.0125	1.57
50	Q.GAI.1471.(I)_2A	2A	230.55	-0.01	-0.02	0.01	0.01	0.00	0.01	-0.02	0.0032	2.31
51	Q.GAI.1192_2A	2A	237.63	-0.02	-0.02	-0.01	-0.01	-0.02	-0.01	-0.03	0.0017	1.19
52	Q.AUC(R)_2A	2A	240.68	-10.32	-42.23	7.31	-8.72	-22.94	-10.27	-18.67	4E-06	3.69
53	Q.NDVI.1976(R)_2A	2A	250.78	0.00	-0.05	-0.02	-0.02	-0.01	0.03	0.04	2E-08	3.86
54	Q.NDVI.1409(R)_2A	2A	252.80	0.01	-0.03	0.01	0.00	0.02	0.02	0.02	2E-07	2.01
55	Q.CTD.(R)_2A	2A	259.39	-0.01	0.34	-0.05	0.08	0.59	-0.50	-0.65	0.0006	2.12
56	Q.GAI.1630(R)_2A	2A	259.39	0.00	-0.05	0.00	-0.06	-0.02	0.02	0.00	6E-08	6.04
57	Q.NDVI.1555(R)_2A	2A	259.39	-0.01	-0.04	0.02	-0.02	-0.04	0.00	-0.02	2E-05	4.1
58	Q.GAI.1994.(I)_2B	2B	2.53	-0.02	0.03	0.03	0.03	0.02	0.05	0.04	2E-08	7.14
59	Q.GAI.1630(R)_2B	6B	66.23	-0.03	0.02	0.02	0.12	-0.04	0.01	0.01	1E-06	4.25
60	Q.GAI.1994(R)_2B	2B	10.58	-0.02	0.00	0.01	0.01	0.00	0.01	0.03	1E-06	6.29
61	Q.GAI.1471.(I)_2B	2B	27.82	-0.03	0.00	0.00	0.00	-0.02	-0.01	-0.01	0.0001	1.88
62	Q.GAI.841_2B	2B	27.82	-0.01	0.00	0.01	0.01	-0.01	0.00	0.00	8E-07	2.15

Table S3. 3 (continued)

	QTL name	Chromosome	Position	Alchemy	Brompton	Claire	Hereward	Rialto	Robigus	Soissons	*p* value	R^2
63	Q.Grains.E^{-1}.(I)_2B	2B	72.64	-14.48	-8.05	4.21	-8.26	-10.11	-4.61	-1.17	1E-08	2.98
64	Q.Grains.E^{-1}.(R)_2B.1	2B	139.06	-13.77	-109.10	-16.79	-7.89	76.50	-10.94	-15.11	2E-06	1.18
65	Q.P.ht(R)_2B	2B	147.66	-2.98	-45.69	-12.92	-3.89	11.30	-11.62	-12.50	5E-05	1.84
66	Q.FT(R)_2B.1	2B	171.86	-95.16	-246.23	-84.81	-55.67	-68.85	-98.24	-111.46	0.0034	1.59
67	Q.Grains.E^{-1}.(R)_2B.2	2B	221.23	19.08	68.08	-26.74	14.85	16.38	16.00	27.28	3E-05	5.53
68	Q.FT(R)_2B.2	2B	253.98	125.24	196.73	224.57	11.72	154.23	104.91	110.72	9E-05	5.24
69	Q.NDVI.1976(R)_2B	2B	271.92	0.06	-0.02	0.18	0.22	-0.01	0.08	0.08	0.0003	1.55
70	Q.TGW(I)_2B	2B	283.93	-11.07	-6.02	-25.98	-5.45	-9.67	-11.54	-10.42	0.0008	1.72
71	Q.GAI.1388.(R)_2B	2B	375.98	-0.01	0.02	0.01	0.00	-0.01	0.02	0.00	0.0058	3.12
72	Q.Grains.m^{-2}.(I)_2D	2D	5.61	-6430.64	572.91	-514.64	6913.28	-3038.06	-452.22	-5633.53	0.0064	3.82
73	Q.GAI.1994.(I)_2D	2D	18.85	-0.04	-0.03	-0.01	0.04	-0.02	0.01	0.00	0.0029	2.83
74	Q.GAI.1630(I)_2D	2D	42.21	-0.02	-0.01	0.06	-0.03	-0.05	0.05	0.00	4E-06	2.74
75	Q.NDVI.2553(I)_2D	2D	55.40	-0.04	-0.01	-0.02	-0.01	-0.01	-0.03	-0.03	0.0077	1.29
76	Q.P.ht(R)_2D	2D	55.40	0.33	0.40	-0.48	2.61	-0.41	-1.01	-3.91	0.0004	3.52
77	Q.CTD.(R)_2D	2D	62.71	-0.05	0.10	0.10	-0.33	0.38	0.11	1.33	3E-11	6.73
78	Q.FT(I)_2D	2D	62.71	-44.43	-20.10	-0.55	14.95	-11.07	-12.30	-64.75	3E-16	13.04
79	Q.FT(R)_2D	2D	62.71	-12.00	-5.80	11.95	4.07	-8.47	-4.87	-54.81	3E-13	12.38
80	Q.GAI.1388.(R)_2D	2D	62.71	-0.02	-0.01	-0.01	0.00	-0.02	0.00	-0.03	0.0027	3.87
81	Q.GAI.1994(R)_2D	2D	62.71	0.00	-0.01	0.03	0.00	-0.01	0.03	-0.02	0.0005	6.41
82	Q.NDVI.1976(R)_2D	2D	62.71	0.00	-0.03	0.02	-0.02	-0.01	-0.03	-0.07	2E-09	8.8
83	Q.NDVI.2553(R)_2D.1	2D	85.59	0.00	0.00	0.01	0.00	-0.01	-0.01	0.00	0.0026	3.39
84	Q.GAI.841_2D.1	2D	121.04	-0.01	0.06	0.05	0.04	0.04	0.04	0.03	4E-08	4.15
85	Q.GAI.486_2D	2D	106.21	0.02	0.01	-0.01	0.01	0.00	0.00	0.00	0.0079	2.05
86	Q.GAI.841_2D.2	2D	96.26	0.01	-0.02	-0.03	-0.03	-0.04	-0.02	0.00	3E-06	1.09
87	Q.AUC(I)_2D	2D	122.56	8.84	50.10	5.01	35.72	27.87	32.33	35.97	6E-05	4.03
88	Q.GAI.1192_2D	2D	128.39	0.01	0.07	0.02	0.05	0.03	0.04	0.05	5E-07	2.86
89	Q.NDVI.2553(R)_2D.2	2D	171.95	0.00	-0.01	-0.01	-0.01	-0.01	0.00	0.00	0.0094	1.6
90	Q.TGW(R)_2D	2D	171.95	-2.26	-2.79	-1.69	4.34	13.39	-3.27	1.87	0.004	8.9
91	Q.GAI.213_2D	2D	196.35	2.01	1.88	1.96	1.30	3.19	2.66	2.59	3E-09	4.96
92	Q.GY.(R)_3A	3A	12.06	0.65	-0.60	-1.07	0.18	-0.47	0.00	-0.28	1E-05	4.53
93	Q.Grains.m^{-2}.(R)_3A	3A	30.01	6020.94	-2139.10	1850.11	2700.49	-2008.31	696.36	-6077.33	0	3.14
94	Q.GAI.1471.(I)_3A	3A	34.65	0.01	-0.01	-0.01	0.01	0.02	-0.03	0.01	0.0005	3.71
95	Q.P.ht(I)_3A	3A	34.65	1.28	-1.67	-4.15	0.62	-1.32	-2.90	2.37	0.0044	6.34
96	Q.NDVI.1409(R)_3A	3A	44.60	0.04	-0.02	0.00	-0.01	-0.01	0.01	-0.02	1E-06	6.16
97	Q.AUC(R)_3A	3A	50.32	-5.54	-31.30	14.55	-13.41	-27.38	-9.88	-9.93	0.0017	2.12
98	Q.NDVI.1555(R)_3A	3A	50.32	0.04	-0.02	-0.01	0.01	-0.01	0.00	0.00	0.0016	2.98
99	Q.Grains.m^{-2}.(I)_3A.1	3A	128.58	-4885.78	-1075.10	-3611.46	-2992.52	1112.92	3001.36	-4240.35	2E-07	1.41
100	Q.NDVI.1976(R)_3A	3A	136.23	0.01	-0.03	-0.03	0.00	-0.01	0.00	-0.02	0.0011	1.21
101	Q.GAI.1994.(I)_3A.1	3A	308.30	0.01	0.00	0.02	0.01	0.05	0.04	0.05	1E-05	1.18
102	Q.GY.(I)_3A	3A	204.47	1.10	-0.02	1.01	-0.15	-0.27	0.05	0.08	5E-05	3.74
103	Q.Grains.m^{-2}.(I)_3A.2	3A	205.98	3094.98	-1955.78	3799.69	1962.20	-4860.64	-5093.58	22.33	2E-07	6.43
104	Q.GAI.841_3A	3A	243.45	-0.02	-0.02	-0.01	0.00	-0.02	0.00	-0.01	1E-05	0.98
105	Q.GAI.1388.(R)_3A	3A	270.21	0.02	0.01	-0.03	0.02	0.02	0.02	0.03	0.0016	3.22
106	Q.GAI.1471.(R)_3A	3A	270.21	0.03	0.02	-0.03	0.02	0.01	0.03	0.03	6E-07	4.09
107	Q.NDVI.1976(I)_3A	3A	282.21	-0.02	0.00	-0.03	0.00	-0.01	-0.01	0.01	0.003	3.2
108	Q.GAI.1994.(I)_3A.2	3A	187.18	0.03	0.01	-0.02	0.02	0.03	0.01	0.01	9E-05	3.65
109	Q.NDVI.1976(R)_3B	3B	1.51	0.06	0.03	NA	0.03	0.04	0.01	0.03	0.0061	3.27
110	Q.TGW(I)_3B	3B	1.51	2.91	-0.08	NA	-2.23	0.14	3.25	1.73	0.0008	1.38
111	Q.GAI.1994.(I)_3B	3B	39.46	0.03	-0.02	-0.02	-0.01	0.01	0.01	0.01	0.0302	1.85
112	Q.NDVI.2553(R)_3B	3B	41.48	-0.01	0.01	0.01	0.00	0.00	0.00	0.00	7E-07	3.01
113	Q.GAI.1630(I)_3B.1	3B	44.77	0.12	0.00	-0.09	-0.03	0.04	0.01	-0.03	3E-13	4.16
114	Q.Grains.E^{-1}.(I)_3B	3B	47.31	2.45	-16.21	-16.64	-5.09	3.21	-0.81	-0.41	5E-12	3.37
115	Q.AUC(I)_3B	3B	44.77	3.43	-7.16	15.30	-0.01	18.09	22.92	-8.67	0.0007	2.83
116	Q.NDVI.1555(R)_3B.1	3B	63.63	-0.03	-0.01	0.06	0.02	0.03	0.02	0.03	2E-05	4.51
117	Q.GAI.1630(I)_3B.2	3B	85.62	0.01	0.04	0.10	0.10	0.01	0.04	0.05	0.0013	2.4
118	Q.Grains.m^{-2}.(I)_3B	3B	96.70	-1025.22	-535.01	-2268.38	2663.05	-2239.16	4281.22	76.16	6E-06	2.02
119	Q.TGW(R)_3B.1	3B	149.92	30.22	33.37	29.67	33.07	67.13	33.41	25.38	8E-05	7.61
120	Q.GAI.1630(R)_3B.1	7A	328.69	-0.02	0.01	-0.01	-0.03	0.00	0.00	0.02	8E-05	4.22
121	Q.TGW(R)_3B.2	3B	179.92	-59.43	-65.41	-57.41	-62.74	-122.34	-67.19	-61.04	6E-06	8.46
122	Q.CTD.(R)_3B	3B	183.45	-4.33	-3.48	-3.91	-3.70	-7.25	-3.79	-3.40	0.0013	1.5
123	Q.GAI.1630(R)_3B.2	4B	79.19	-0.03	-0.02	0.04	0.02	0.01	0.03	-0.02	2E-06	4.03

Table S3. 4 (continued)

	QTL name	Chromosome	Position	Alchemy	Brompton	Claire	Hereward	Rialto	Robigus	Soissons	*p* valu	R^2
124	Q.Grains.m^{-2}.(R)_3B	3B	201.56	-6672.12	658.12	-1186.32	224.68	-6962.44	-843.75	NA	2E-09	2.65
125	Q.NDVI.1555(R)_3B.2	3B	233.55	0.00	-0.02	0.04	0.01	0.04	0.03	NA	0.002	1.15
126	Q.GAI.1994(R)_3B	3B	241.15	0.02	-0.03	0.03	0.00	0.03	0.06	NA	5E-04	4.21
127	Q.GAI.1192_3B	3B	246.73	0.01	-0.02	0.00	0.00	0.02	0.04	NA	0.005	1.52
128	Q.FT(I)_3D	3D	1.01	-2943.30	2871.15	NA	-11.91	NA	-28.20	15.93	4E-06	1.92
129	Q.FT(R)_3D	3D	1.01	-1029.65	965.46	NA	-14.30	NA	-25.62	28.83	7E-06	1.18
130	Q.P.ht(I)_3D	3D	94.19	0.75	3.36	-0.17	0.60	-6.39	2.28	2.65	3E-04	2.38
131	Q.P.ht(R)_3D	3D	94.19	-3.17	-0.29	-1.17	3.01	-10.85	0.05	2.28	6E-08	1.23
132	Q.GAI.1994.(I)_3D	3D	99.87	0.00	0.01	0.01	0.04	-0.06	-0.01	0.00	0.003	2.22
133	Q.GAI.1630(I)_3D	3D	107.27	0.04	0.06	0.07	0.08	0.06	0.05	0.08	0.003	1.18
134	Q.GAI.213_3D	3D	192.5	-0.76	0.43	-0.31	-0.30	NA	-0.32	NA	4E-05	4.95
135	Q.NDVI.1409(I)_4A.1	4A	7.23	-0.03	0.15	-0.03	-0.03	-0.13	-0.02	0.00	1E-06	3.36
136	Q.GAI.1471.(I)_4A	4A	7.74	0.01	0.09	0.01	0.00	-0.04	-0.01	0.01	0.004	2.29
137	Q.NDVI.2553(R)_4A	4A	23.34	0.01	-0.03	0.00	0.00	0.03	0.00	-0.01	6E-09	4.78
138	Q.AUC(I)_4A	4A	29.17	0.27	116.53	-0.75	-7.93	-75.34	0.89	12.53	8E-04	1.66
139	Q.GY.(I)_4A	4A	36.98	-0.37	2.27	0.71	-0.98	-1.16	-0.40	0.27	0.003	2.14
140	Q.GY.(R)_4A	4A	36.98	-0.19	-0.93	0.81	-0.42	0.08	0.16	0.19	2E-06	3.39
141	Q.Grains.m^{-2}.(R)_4A	4A	43.64	2880.92	-5140.45	259.01	362.15	-3145.10	1695.27	-2455.75	3E-11	4.02
142	Q.NDVI.1976(I)_4A	4A	49.5	0.02	0.07	0.04	-0.01	-0.02	-0.02	0.01	4E-05	1.03
143	Q.Grains.E^{-1}.(R)_4A	4A	61.2	2.52	-77.28	3.56	11.02	-8.87	0.02	11.94	4E-13	7.81
144	Q.GAI.1388(I)_4A	4A	113.1	-0.01	0.02	0.00	0.03	0.00	0.01	-0.01	5E-04	1.94
145	Q.NDVI.1555(I)_4A	4A	123.55	-0.03	-0.03	-0.01	0.00	0.00	-0.01	-0.02	0.012	1.93
146	Q.Grains.E^{-1}.(I)_4A	4A	140.85	16.92	9.02	13.42	21.84	18.40	17.43	15.08	9E-12	3.78
147	Q.GAI.213_4A	4A	164.01	-0.01	-0.54	-0.38	-0.04	-0.16	-0.25	-0.02	5E-06	1.01
148	Q.FT(R)_4A	4A	178.37	66.46	45.78	-17.24	17.75	18.21	17.46	4.49	0.002	1.86
149	Q.NDVI.1409(I)_4A.2	4A	217.29	0.10	0.03	-0.03	0.02	-0.01	-0.05	-0.18	0.007	1.56
150	Q.GAI.1630(I)_4B.1	4B	78.18	-0.01	-0.02	0.00	0.02	0.01	0.03	-0.02	6E-06	5.67
151	Q.GAI.1994.(I)_4B	4B	25.91	-0.04	0.00	-0.07	-0.04	-0.04	-0.04	-0.04	3E-05	3
152	Q.P.ht(I)_4B.1	4B	52.17	2.67	-1.30	-0.16	-4.20	-3.06	-8.56	-6.80	2E-08	7.83
153	Q.P.ht(R)_4B	4B	52.17	-0.75	1.22	2.64	-1.31	0.22	-7.22	-8.27	0	11.23
154	Q.GY.(I)_4B	4B	63.34	-1.04	-0.74	-0.67	-0.18	-0.37	0.08	-1.06	0.001	1.31
155	Q.TGW(I)_4B	4B	65.87	4.42	-1.25	-1.03	1.68	1.15	0.27	-3.09	0.001	4.73
156	Q.AUC(R)_4B	4B	75.09	-22.99	-17.20	21.99	15.47	-2.47	18.30	-13.36	3E-06	3.83
157	Q.GAI.1192_4B1.1	4B	75.09	-0.01	-0.03	-0.01	0.02	0.01	0.00	-0.01	2E-08	4.35
158	Q.GAI.1630(I)_4B.2	4B	18.73	0.01	0.05	-0.10	0.01	-0.02	-0.03	0.00	2E-07	3.25
159	Q.GY.(R)_4B	4B	78.18	-0.33	-0.70	-0.50	0.31	-0.29	0.44	-0.13	2E-06	1.7
160	Q.NDVI.1976(R)_4B	4B	78.18	-0.05	-0.01	0.04	0.01	0.02	0.01	-0.01	0.001	3.6
161	Q.NDVI.2553(R)_4B	4B	78.18	0.00	-0.01	-0.01	0.00	-0.01	0.00	-0.01	2E-04	1.52
162	Q.GAI.1630(R)_4B	3B	183.45	0.17	0.15	0.13	0.21	0.40	0.21	0.18	3E-05	2.82
163	Q.P.ht(I)_4B.2	4B	80.71	-5.98	1.01	5.20	8.03	3.40	4.57	0.72	2E-04	4.99
164	Q.AUC(I)_4B.1	4B	81.72	1.22	-8.27	-14.45	20.34	3.78	14.00	1.74	0.006	4.12
165	Q.CTD.(R)_4B.1	4B	179.45	0.47	-0.45	-0.39	0.10	0.37	-0.01	-0.47	0.001	1.42
166	Q.GAI.1388(I)_4B	4B	83.73	-0.01	-0.03	-0.03	0.01	0.00	0.00	-0.02	3E-06	4.17
167	Q.CTD(I)_4B	4B	111.24	-0.04	0.12	0.58	-0.06	0.02	-0.07	0.16	4E-05	6.97
168	Q.Grains.E^{-1}.(R)_4B	4B	143.65	-14.37	9.28	12.91	5.23	7.35	7.66	-2.57	2E-08	6.66
169	Q.FT(R)_4B	4B	159.51	-32.82	2.20	29.52	13.53	3.35	24.99	-6.77	1E-06	4.43
170	Q.NDVI.2553(I)_4B	4B	169.15	-0.04	-0.02	0.00	-0.01	-0.01	-0.01	0.00	0.029	1.04
171	Q.GAI.1471.(I)_4B	4B	178.44	-0.04	0.02	-0.01	-0.01	-0.03	-0.02	-0.02	2E-04	1.55
172	Q.CTD.(R)_4B.2	4B	83.73	1.40	0.67	-0.45	-0.54	0.03	0.01	0.43	4E-08	4.13
173	Q.AUC(I)_4B.2	4B	226.6	-34.19	37.28	-18.53	-31.78	-31.40	-5.10	-24.72	5E-04	1.47
174	Q.GAI.1630(I)_4B.3	4B	226.6	-0.05	-0.01	-0.04	0.01	-0.05	-0.04	-0.04	4E-04	1.18
175	Q.GAI.1192_4B.2	4B	230.19	-0.02	0.05	-0.04	-0.06	-0.01	0.00	-0.02	3E-06	1.28
176	Q.GAI.841_4D	4D	24.93	-0.04	0.00	NA	0.02	-0.03	0.00	0.00	5E-05	3.24
177	Q.Grains.E^{-1}.(I)_4D	4D	24.93	26.93	15.79	NA	8.76	9.84	6.21	-0.75	4E-07	9.42
178	Q.NDVI.1555(R)_4D	4D	24.93	0.04	0.01	NA	-0.02	-0.02	-0.01	0.02	7E-04	1.36
179	Q.AUC(R)_4D	4D	32.24	-26.72	25.12	NA	-34.99	-31.78	-19.44	-6.91	3E-04	3.64
180	Q.CTD.(R)_4D	4D	32.24	0.49	-0.10	NA	1.23	0.56	0.52	0.15	1E-03	3.73
181	Q.GAI.1630(I)_4D	4D	32.24	0.08	-0.02	NA	-0.02	0.00	-0.03	0.00	4E-07	3.2
182	Q.Grains.m^{-2}.(I)_4D	4D	32.24	3574.90	3930.89	NA	19.23	-3741.80	-4425.27	-3142.67	4E-09	7.86
183	Q.GY.(I)_4D	4D	32.24	-0.49	0.43	NA	-0.35	-0.47	-1.34	-0.83	3E-07	3.42
184	Q.NDVI.1409(R)_4D	4D	32.24	0.00	0.02	NA	-0.02	-0.03	-0.01	0.01	5E-06	2.44
185	Q.P.ht(I)_4D	4D	32.24	-4.58	0.71	NA	-3.17	-1.74	5.84	4.51	0	15.18
186	Q.P.ht(R)_4D	4D	32.24	-0.46	-0.98	NA	-4.51	-2.02	4.04	5.06	0	12.23

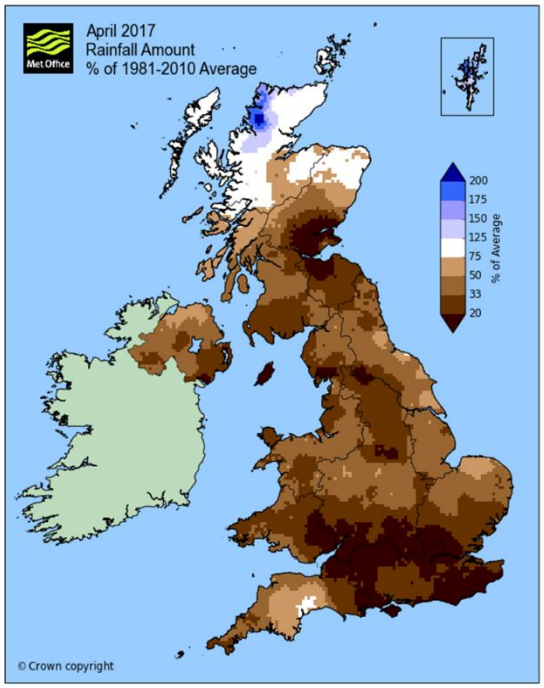
Table S3. 5 (continued)

	QTL name	Chromosome	Position	Alchemy	Brompton	Claire	Hereward	Rialto	Robigus	Soissons	*p* value	R^2
187	Q.TGW(I)_4D.2	4D	62.98	5.27	3.11	NA	3.31	-3.80	6.44	-4.57	6E-06	4.31
188	Q.GAI.1388(I)_4D	4D	99.22	-0.02	0.04	NA	-0.03	-0.03	-0.02	-0.03	2E-06	2.68
189	Q.NDVI.1409(I)_4D	4D	99.22	0.01	0.04	NA	-0.02	-0.01	0.01	0.01	0.004	1.18
190	Q.NDVI.1976(I)_5A	5A	51.47	0.00	-0.05	0.03	0.00	-0.01	-0.01	-0.03	0.0001	2.25
191	Q.CTD(I)_5A.1	5A	99.27	-0.15	-0.06	-0.29	-0.14	-0.25	0.16	-0.10	0.0104	2.44
192	Q.GY.(I)_5A	5A	83.05	-0.61	-0.92	0.15	0.88	0.22	-1.13	-0.17	9E-06	1.38
193	Q.CTD(I)_5A.2	5A	69.43	0.16	0.28	0.37	-0.06	0.38	0.12	0.09	0.0023	1.64
194	Q.GY.(R)_5A	5A	126.69	0.37	-0.01	-0.02	1.09	0.30	0.23	-0.22	7E-05	2.68
195	Q.GAI.1471.(R)_5A	5A	128.21	0.03	-0.03	-0.01	0.00	-0.01	0.01	-0.01	0.0042	2.08
196	Q.GAI.1388(I)_5A	5A	137.43	0.01	0.00	0.04	0.00	-0.03	-0.02	0.02	5E-06	3.14
197	Q.NDVI.1555(I)_5A.1	5A	312.56	0.02	-0.02	0.01	-0.04	-0.03	-0.01	-0.01	0.0078	1
198	Q.Grains.m^{-2}.(R)_5A	5A	166.8	-3787.01	-5163.65	-7062.84	-1761.08	-9374.33	-6299.46	-6319.52	1E-10	2.14
199	Q.GAI.486_5A	5A	177.07	-0.01	-0.01	0.00	-0.01	0.00	-0.01	0.00	0.0004	3.56
200	Q.GAI.213_5A	5A	181.64	-0.18	-0.01	-0.04	-0.11	-0.10	-0.17	0.19	2E-06	3.97
201	Q.FT(I)_5A.1	5A	183.69	1.11	-44.16	-57.46	-3.67	-4.26	-12.21	-30.39	3E-09	3.27
202	Q.Grains.m^{-2}.(I)_5A.1	5A	183.69	1343.76	2789.05	-2728.05	-6329.76	-1051.93	2647.07	-1440.01	5E-05	1.84
203	Q.GAI.1994(R)_5A	5A	197.58	0.00	0.00	-0.02	-0.01	-0.03	-0.02	0.00	0.0022	3.09
204	Q.CTD.(R)_5A	5A	206.98	0.66	0.39	0.59	-0.14	0.41	0.02	0.16	7E-05	4.18
205	Q.NDVI.1976(R)_5A.1	5A	249.15	-0.02	0.03	-0.02	-0.01	-0.02	0.02	0.00	0.0138	1.56
206	Q.NDVI.1976(R)_5A.2	5A	211.51	-0.02	-0.05	-0.02	-0.02	-0.03	-0.02	-0.01	0.0043	1.5
207	Q.FT(R)_5A	5A	253.77	-11.25	3.39	-30.46	0.34	-17.48	-17.68	-11.85	0.0044	1.12
208	Q.Grains.m^{-2}.(I)_5A.2	5A	256.86	-1777.32	910.99	4375.45	598.97	-1006.60	3474.63	-3181.03	0.0165	2.73
209	Q.NDVI.1409(R)_5A	5A	278.73	-0.04	0.00	0.00	0.00	0.00	-0.02	0.00	3E-05	1.44
210	Q.NDVI.1555(R)_5A	5A	300.75	-0.03	-0.01	0.02	0.00	-0.03	-0.03	-0.02	5E-06	2.6
211	Q.FT(I)_5A.2	5A	310.04	-13.75	-1.25	36.36	51.69	3.21	1.34	-6.52	2E-07	3.03
212	Q.AUC(R)_5A	5A	312.56	-28.87	-22.84	-32.54	1.17	-30.73	-13.68	-20.47	0.0033	2.27
213	Q.GAI.1388.(R)_5A	5A	312.56	-0.03	-0.01	0.01	0.03	-0.02	0.00	-0.01	0.0025	1.5
214	Q.NDVI.1555(I)_5A.2	5A	139.96	0.02	0.02	0.07	0.00	-0.01	0.00	0.02	0.0005	1.33
215	Q.AUC(I)_5A	5A	313.57	-15.70	-7.29	11.48	18.18	-23.49	9.51	-13.63	0.0004	2.58
216	Q.GAI.213_5B.1	5B	13.16	0.03	0.21	0.02	0.05	-0.02	-0.06	0.17	0.0047	1.38
217	Q.NDVI.2553(R)_5B	5B	38.92	0.01	-0.01	-0.01	0.00	0.00	0.00	0.00	5E-09	3.47
218	Q.AUC(I)_5B.1	5B	81.2	9.58	-37.61	46.22	-45.38	-41.73	-13.82	-33.11	0.0011	2.52
219	Q.GAI.1471.(I)_5B.1	5B	81.2	-0.02	-0.04	0.06	-0.06	-0.04	-0.02	-0.02	0.0007	2.69
219	Q.GAI.1471.(I)_5B.1	5B	281.57	-0.15	0.13	-0.05	-0.04	-0.01	-0.03	-0.02	5E-05	2.92
220	Q.GAI.1388(I)_5B	5B	113.4	-0.06	-0.04	0.03	-0.17	0.10	-0.06	-0.03	4E-10	3.59
221	Q.Grains.m^{-2}.(I)_5B	5B	113.4	-5657.30	-1474.53	-1936.60	-18536.82	15972.94	-253.96	-1251.77	0.007	1.55
222	Q.CTD(I)_5B.1	5B	281.57	0.26	-0.98	1.23	0.24	0.08	0.13	0.21	0.0136	2.24
223	Q.FT(I)_5B	5B	142.56	2.97	41.50	5.16	-82.99	76.85	9.30	21.14	1E-05	1.44
224	Q.AUC(I)_5B.2	5B	144.57	-27.59	3.19	-2.45	-49.07	46.19	-13.99	6.75	0.0299	1.79
225	Q.Grains.E^{-1}.(R)_5B	5B	152.66	-12.51	-1.23	7.44	3.62	2.19	0.12	1.45	2E-08	2.63
226	Q.Grains.m^{-2}.(R)_5B	5B	163.98	-1880.33	576.50	1370.02	15324.17	-15405.93	357.14	1961.36	2E-09	2.85
227	Q.GY.(R)_5B	5B	223.1	-0.96	-0.20	-0.51	-0.01	-0.33	-0.71	-0.28	0.002	2.41
228	Q.GAI.213_5B.2	5B	229.16	-0.06	-0.28	-0.23	-0.26	-0.08	-0.33	-0.18	0.0012	3.19
229	Q.CTD(I)_5B.2	5B	133.41	0.24	0.12	-0.30	1.00	-0.28	0.15	-0.06	0.0045	3.25
231	Q.GAI.1192_5B	5B	282.57	-0.08	0.03	-0.04	-0.04	-0.02	-0.01	-0.04	3E-07	4.26
232	Q.GAI.841_5B	5B	282.57	-0.04	0.05	-0.06	-0.01	-0.01	0.00	-0.02	7E-05	2.52
233	Q.NDVI.1555(I)_5B	5B	282.57	-0.10	0.08	-0.06	-0.04	-0.02	-0.02	-0.05	0.0002	2.52
234	Q.NDVI.1976(R)_5B	5B	289.61	0.04	-0.01	-0.02	-0.01	0.00	-0.03	-0.03	0.0137	2.25
235	Q.P.ht(I)_5B	5B	289.61	-6.05	11.12	-9.06	-2.94	0.05	2.22	-1.35	0.0004	4.27
236	Q.NDVI.1976(I)_5B	5B	298.71	-0.10	0.08	-0.06	-0.02	0.00	-0.03	-0.02	5E-05	3.54
237	Q.Grains.E^{-1}.(R)_5D	5D	120.79	16.45	15.39	5.62	-19.28	8.05	-7.80	16.98	1E-06	1.18
238	Q.FT(I)_5D	5D	144.35	-34.98	-14.29	-19.92	17.48	-34.44	-34.60	-33.98	0.0029	1.08
239	Q.Grains.m^{-2}.(R)_5D	5D	150.86	-8842.46	-1042.88	331.52	-1791.56	2655.31	-4993.37	-4263.01	3E-12	2.82
240	Q.Grains.E^{-1}.(I)_5D	5D	171.07	-8.29	21.95	10.08	16.70	19.48	23.28	24.08	0	5.91
241	Q.GAI.1994(R)_5D	5D	198.08	0.00	0.03	0.02	0.01	0.05	0.00	0.02	0.0008	1.21
242	Q.GAI.1994.(I)_6A	6A	32.88	0.01	0.04	-0.01	-0.01	0.01	0.03	0.00	2E-06	1.86
243	Q.CTD(I)_6A	6A	37.42	0.15	-0.15	-0.02	0.13	-0.09	-0.24	-0.05	0.0042	2.25
244	Q.Grains.E^{-1}.(I)_6A	6A	61.07	-3.51	-9.79	-3.76	-3.85	-7.92	-5.90	-10.05	0.0225	3.19
245	Q.NDVI.2553(I)_6A.1	6A	203.97	-0.01	0.01	0.04	0.03	0.01	0.02	0.03	0.0082	2.41
246	Q.GAI.213_6A	6A	112.02	0.00	0.03	0.21	-0.09	0.03	-0.17	0.04	0.0011	1.53
247	Q.TGW(I)_6A	6A	130.43	0.65	0.56	-2.25	0.18	3.21	6.82	2.16	5E-06	7.17

Table S3. 6 (continued)

	QTL name	Chromosome	Position	Alchemy	Brompton	Claire	Hereward	Rialto	Robigus	Soissons	*p* value	R^2
248	Q.GAI.1630(I)_6A.1	6A	204.98	-0.01	-0.02	-0.02	-0.02	-0.02	-0.02	0.02	0.0002	2.95
249	Q.NDVI.2553(R)_6A	6A	148.64	0.01	0.00	0.00	-0.01	0.01	0.01	0.00	5E-08	4.32
250	Q.NDVI.1409(I)_6A	6A	155.74	0.03	-0.02	0.05	0.01	0.04	0.05	0.02	1E-06	3.83
251	Q.NDVI.1555(I)_6A.1	6A	175.93	0.04	0.01	0.04	0.02	0.02	0.02	0.03	0.0098	1.23
252	Q.GAI.486_6A	6A	180.47	0.00	-0.01	0.01	0.01	0.00	0.00	0.00	0.0117	1.39
253	Q.Grains.m^{-2}.(I)_6A	6A	191.33	-4853.37	-7206.50	-377.41	-988.12	5088.19	1951.96	-14.66	5E-05	6.24
254	Q.NDVI.2553(I)_6A.2	6A	109.46	0.00	0.01	-0.01	-0.01	0.01	0.03	0.01	2E-05	3.46
255	Q.GAI.1630(I)_6A.2	6A	146.08	0.00	-0.02	0.02	-0.01	0.02	0.00	-0.06	5E-05	0.92
256	Q.FT(R)_6A	6A	215.25	-27.80	-14.13	20.14	13.27	-39.65	-1.81	-8.41	0.0002	1.49
257	Q.NDVI.1555(I)_6A.2	6A	256.90	-0.03	-0.05	0.02	0.00	0.01	0.05	0.00	6E-06	3.6
258	Q.NDVI.1555(R)_6B	6B	10.70	-0.05	0.00	-0.01	0.02	-0.01	0.00	0.01	0.0192	1
259	Q.NDVI.2553(R)_6B.1	6B	15.73	0.00	-0.01	-0.01	0.00	-0.01	-0.01	-0.01	1E-06	5.84
260	Q.GAI.1994.(I)_6B.1	6B	30.16	-0.01	-0.01	0.03	0.03	0.03	0.01	0.01	2E-05	1.38
261	Q.TGW(R)_6B	6B	35.81	-2.43	-1.71	-7.91	-3.16	-3.14	-4.27	-0.92	5E-05	1.46
262	Q.GAI.1388(I)_6B	6B	42.60	0.02	0.01	0.02	0.02	0.06	0.01	0.02	4E-06	1.71
263	Q.GAI.1630(I)_6B	6B	56.49	-0.05	-0.04	-0.02	-0.03	-0.01	-0.02	-0.02	0.0003	3.19
264	Q.GAI.1994(R)_6B	6B	57.49	-0.04	-0.01	-0.01	0.02	-0.03	0.00	0.00	0.0127	2.8
265	Q.GAI.1630(R)_6B	7B	110.66	0.04	0.01	0.09	0.03	-0.03	0.01	0.00	1E-06	2.57
266	Q.NDVI.1976(R)_6B	6B	80.74	-0.08	0.01	-0.01	0.08	0.01	0.02	0.00	0.0095	1.32
267	Q.TGW(I)_6B	6B	159.18	9.47	1.52	-11.55	6.16	-7.43	-1.98	0.87	0.0187	2.72
268	Q.CTD(I)_6B	6B	170.78	0.47	0.21	-0.05	0.16	0.84	0.36	0.47	0.0013	1.23
269	Q.GAI.1994.(I)_6B.2	6B	173.83	-0.10	-0.03	0.04	0.03	-0.12	-0.04	-0.03	2E-05	4.65
270	Q.GY.(I)_6B	6B	175.85	-2.93	-1.40	1.49	0.87	-0.74	-1.15	-0.63	0.0001	1.97
271	Q.P.ht(I)_6B	6B	192.21	-8.57	-0.72	4.67	-0.60	5.18	-3.49	-0.74	2E-05	1.08
272	Q.FT(I)_6B.1	6B	214.55	67.35	10.84	15.47	-13.20	7.42	71.04	27.51	2E-07	1.38
273	Q.NDVI.2553(R)_6B.2	6B	235.26	0.00	-0.01	0.00	0.00	0.00	0.00	0.00	0.0047	3.1
274	Q.FT(I)_6B.2	6B	261.89	46.08	26.09	3.39	44.15	26.71	-66.52	-42.08	0.0004	2.23
275	Q.NDVI.1409(R)_6D	6D	104.21	-0.12	-0.48	NA	0.38	NA	-0.04	-0.01	5E-05	1.65
276	Q.NDVI.1976(I)_6D	6D	104.21	-0.19	0.47	NA	-0.61	NA	0.03	-0.03	0.0005	2.19
277	Q.Grains.E^{-1}.(R)_6D	6D	130.85	13.35	-40.39	NA	39.02	NA	11.29	-6.30	1E-08	4.22
278	Q.GAI.1630(I)_6D	6D	215.00	0.69	-0.01	-0.63	NA	NA	-0.01	0.05	9E-06	0.94
279	Q.Grains.m^{-2}.(R)_7A.1	7A	33.03	3086.38	-1504.09	NA	-4602.55	3854.80	268.16	-291.39	1E-04	4.28
280	Q.GAI.1471.(R)_7A	7A	35.05	0.01	0.03	NA	-0.01	0.01	0.03	-0.01	2E-06	3.87
281	Q.GAI.1388.(R)_7A	7A	43.65	0.01	0.01	NA	0.01	0.03	0.03	-0.01	0.0007	1.61
282	Q.GAI.1630(R)_7A.1	2B	3.53	-0.03	0.04	0.02	-0.02	0.01	0.04	0.04	9E-07	1.61
283	Q.FT(R)_7A	7A	188.57	52.96	7.71	NA	-3.98	41.63	-21.41	11.29	0.0008	1.78
284	Q.NDVI.2553(I)_7A	7A	194.62	0.04	0.01	NA	0.02	0.04	0.03	0.03	0.0015	3.57
285	Q.NDVI.1555(I)_7A	7A	303.20	-0.02	0.00	0.01	-0.01	0.00	-0.01	-0.03	0.0291	1.95
286	Q.NDVI.1976(I)_7A	7A	307.74	-0.03	0.02	0.03	-0.01	-0.01	0.00	-0.02	0.0015	3.99
287	Q.GAI.213_7A	7A	312.27	0.32	0.01	-0.57	-0.26	0.11	-0.33	-0.21	5E-12	3.45
288	Q.GAI.1630(R)_7A.2	7A	51.26	0.01	0.08	NA	0.03	-0.02	0.02	-0.01	0.0002	1.57
289	Q.AUC(I)_7A	7A	344.55	-1.37	-19.95	-29.49	-13.38	-1.08	-15.42	-20.05	0.0118	4.29
290	Q.TGW(R)_7A	7A	346.57	3.20	-0.50	-1.93	1.90	-2.29	-5.04	-0.57	0.0002	2.25
291	Q.GAI.1994.(I)_7A	7A	365.23	-0.03	-0.01	-0.02	-0.01	0.02	-0.02	0.00	0.0039	3.21
292	Q.Grains.m^{-2}.(R)_7A.2	7A	380.43	6907.46	-617.28	925.83	-1059.00	7657.81	4789.18	2214.78	4E-10	4.16
293	Q.NDVI.2553(R)_7A	7A	386.06	0.00	0.00	0.00	-0.01	-0.01	0.00	-0.01	0.0002	1.18
294	Q.Grains.E^{-1}.(I)_7B.1	7B	0.00	-2.53	25.10	-17.23	13.86	NA	-6.09	-1.63	1E-08	3.02
294	Q.Grains.E^{-1}.(I)_7B.1	7B	194.38	8.60	8.79	-8.01	-2.83	-23.51	-5.34	-6.48	9E-12	2.74
295	Q.P.ht(I)_7B	7B	6.39	4.03	4.49	3.24	6.36	NA	6.95	2.22	0.0144	1.4
296	Q.TGW(R)_7B	7B	43.08	0.22	22.30	-2.70	-0.73	-24.87	-1.01	1.80	0.0014	2.02
297	Q.TGW(I)_7B.1	7B	60.03	4.71	-16.85	-5.24	-1.80	17.86	9.29	0.96	3E-07	4.8
298	Q.GAI.841_7B.1	7B	147.07	-0.02	0.00	0.00	-0.02	-0.01	-0.01	-0.02	0.0001	6.87
299	Q.Grains.m^{-2}.(I)_7B.1	7B	109.14	1394.64	-1599.07	-2237.67	-3007.09	4271.40	-8434.36	-368.24	0.0011	3.42
300	Q.GAI.1630(R)_7B	3B	154.44	0.15	0.15	0.19	0.12	0.20	0.12	0.13	0.0014	1.15
301	Q.GAI.841_7B.2	7B	90.07	0.06	0.03	0.06	0.03	0.06	-0.02	0.02	7E-05	2.89
303	Q.NDVI.1409(R)_7B	7B	255.55	-0.01	0.02	-0.02	0.02	NA	-0.02	0.01	2E-10	2.67
304	Q.NDVI.1555(R)_7B	7B	255.55	0.04	-0.15	-0.11	-0.06	NA	-0.10	-0.08	8E-07	1.89
305	Q.TGW(I)_7B.2	7B	274.13	-3.02	1.59	-0.38	-0.22	NA	4.89	1.19	6E-05	1.44
306	Q.Grains.m^{-2}.(I)_7B.2	7B	287.41	5008.97	1588.83	-1670.76	4134.22	NA	1183.35	-735.50	0.0019	3.14
307	Q.Grains.m^{-2}.(R)_7D	7D	31.95	-6764.21	14851.88	4065.09	459.69	-17356.66	-1554.77	NA	1E-09	2.93
308	Q.Grains.E^{-1}.(I)_7D	7D	63.26	1.22	-5.39	8.52	-30.74	-2.33	6.12	NA	3E-08	1.59
309	Q.CTD(I)_7D	7D	117.36	0.00	-0.15	-0.23	-0.17	-0.68	-0.56	NA	0.0002	2.13

(a)



(b)

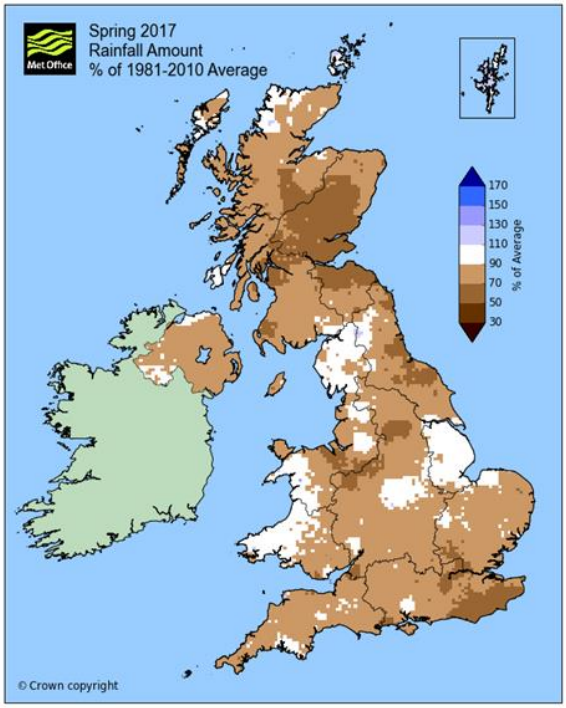


Figure S3. 1 UK rainfall anomaly map for (a) April 2017, (b) spring 2017 (Met office 2017)

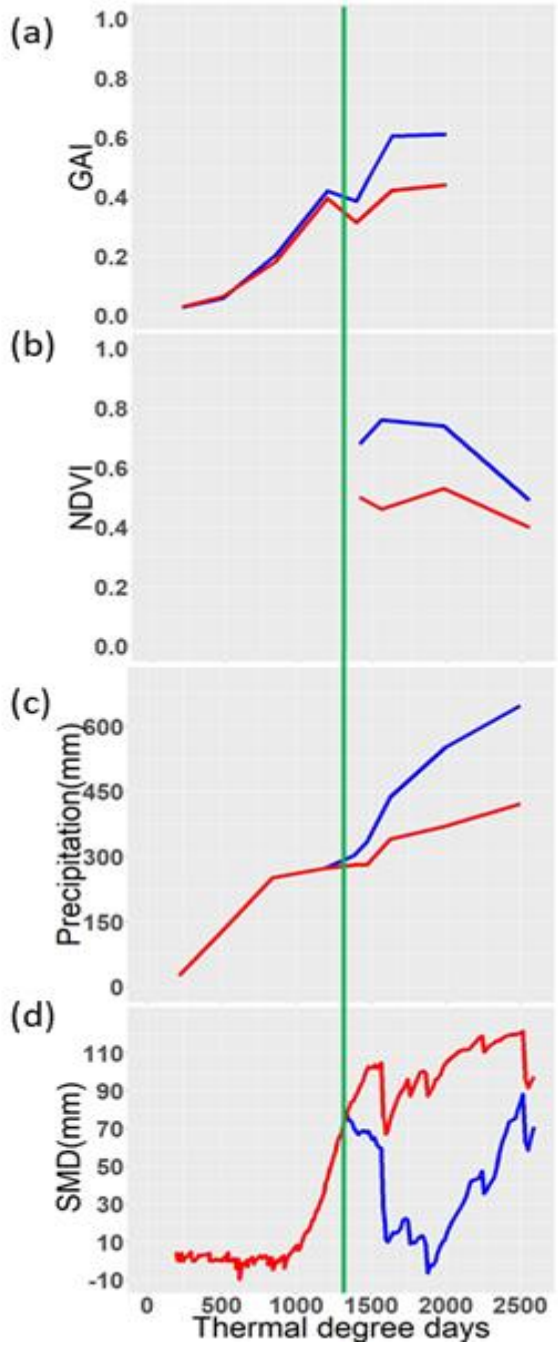


Figure S3. 2 Average Changes of the MAGIC population in (a) GAI; (b) NDVI, (c) cumulative precipitation and (d) soil moisture deficit, from germination to late grain filling over thermal time (expressed in degree days). Blue and red lines show irrigated and rainfed treatments respectively. The green vertical line indicates the time of onset of supplementary irrigation.

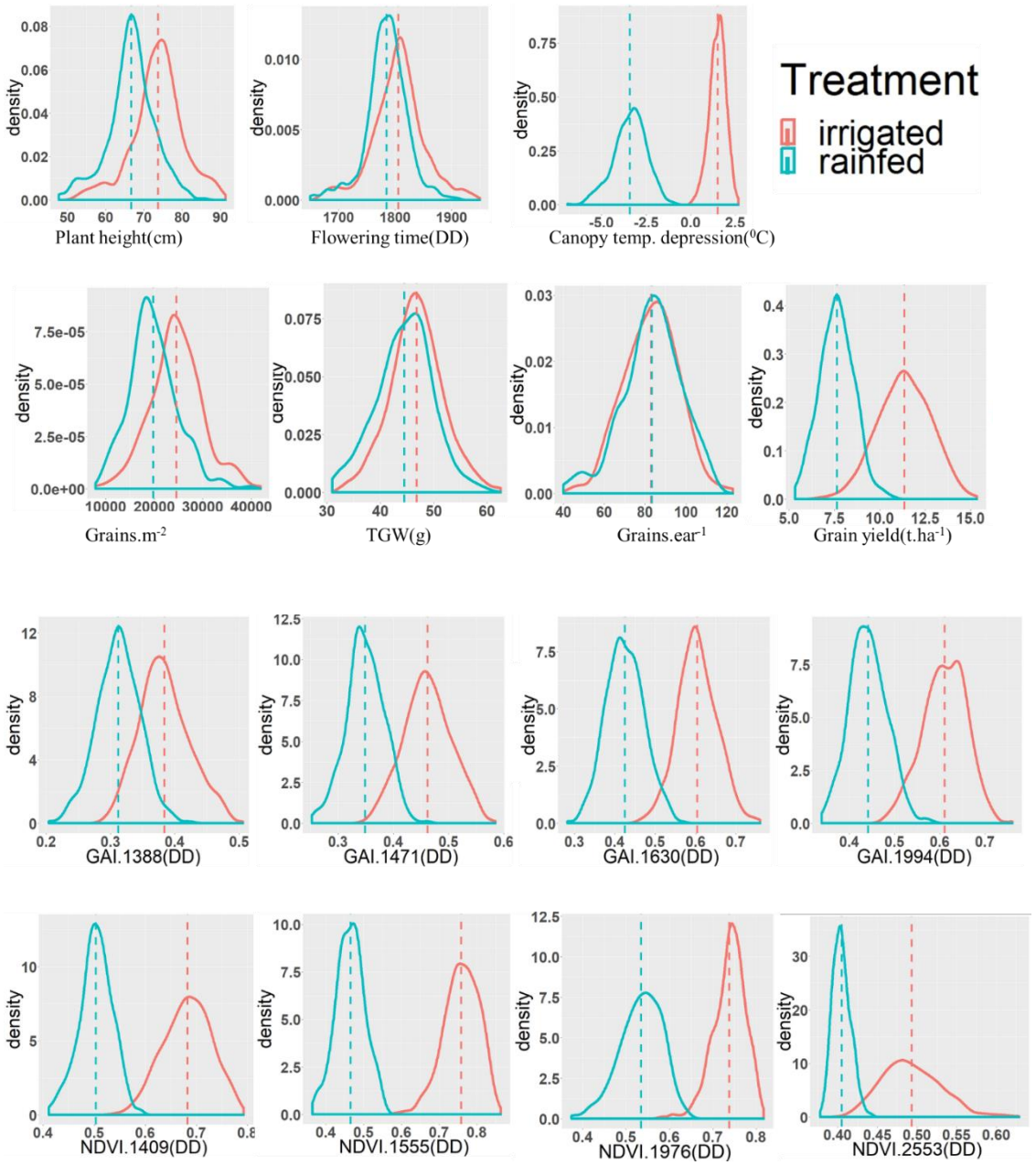


Figure S3. 3 Density plots of phenotypes data for MAGIC population response to drought.

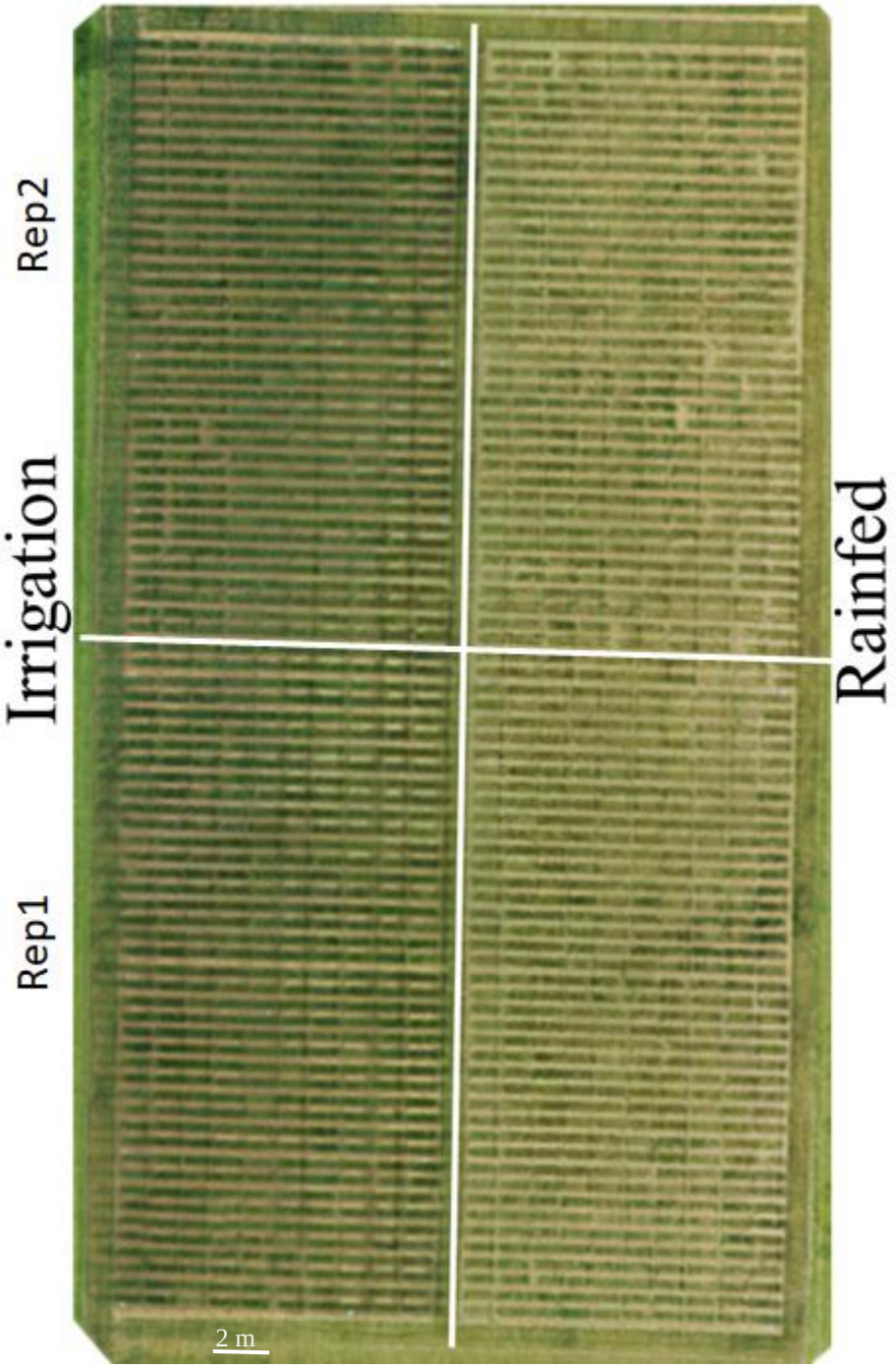


Figure S3. 4 Aerial (RGB) field view at mid-May

NDVI.(1409dd) NDVI.(1555dd) NDVI.(1976dd) NDVI.(2553dd

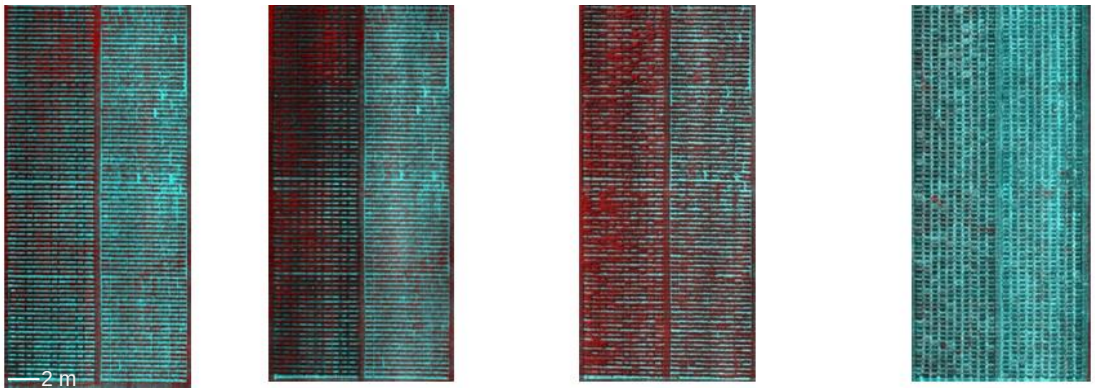


Figure S3. 5 Aerial field view for time series NDVI.

4 Chapter 4: Genomic regions associated with response of MAGIC wheat to *Piriformospora indica* symbiosis under drought

4.1 Abstract

Globally, wheat growing areas are experiencing significantly increased incidents of drought, negatively impacting wheat growth and development and resulting in reduced grain yield. *Piriformospora indica* (*Serendipita indica*), a root-colonizing endophyte of Sebacinales is known to improve drought stress tolerance in wheat and other field crops, but quantitative trait loci (QTL) underpinning drought-resistance traits influenced by endophytic growth in wheat were not reported before. A representative subset of the elite winter wheat MAGIC population consisting of 200 RILs was phenotyped for the presence/absence of fungal inoculation under both well-watered and drought stress conditions. Overall, *P.indica* conferred an increase in plant height and grain yield, however, for most traits, there was significant difference in genotypes responses to colonization under both water availability regimes. Several QTL unique to colonized plants were detected on most chromosomes and linked to measured traits under drought. For grain yield two associated QTL were detected on chromosomes 5A and 7B accounting for 2.95% and 12.3% of the variation in yield, respectively, moreover, *P. indica* colonization masked the detection of QTL tagging known genes, such as reduced height locus *Rht-B1* on chromosome 4B and photoperiod sensitivity locus *Ppd-D1* on chromosome 2D. We conclude that the genotypic specific variation in response can be used in breeding for highly responsive lines and improved wheat performance under drought and after validating these findings with more replicated trials, the identified QTL can be investigated for candidate genes governing the symbiosis between wheat and *P. indica*.

Keywords: wheat, *Piriformospora indica*, drought stress tolerance, MAGIC population, QTL

4.2 Introduction

Yield improvement through conventional breeding either under standard management or stress conditions is always slow and impaired by its complex polygenic nature, suggesting the promising idea to utilize the genotypic differences in response to endophyte inoculation to improve plant performance and breed for better response to inoculation (Galván *et al*., 2011, Fester and Sawers, 2011).

Increased attention is being paid to the growth enhancing effect of endophytes on plants driven by relatively recent reports indicating fungal endophytes effectively increase crop productivity by means of boosting the plant tolerance to abiotic stresses such as heat (Hubbard *et al*., 2012), drought (Hubbard *et al*., 2012, Hubbard *et al.*, 2014) high salinity (Ghaffari *et al*., 2016) and heavy metals (Dourado *et al*., 2015). Fungal endophytes are known to be capable of increasing resistance to biotic stresses as well (Rabiey *et al*., 2015, Rabiey and Shaw, 2016).

Symbiosis between endophytes and plants started and evolved around 400 million years ago, mediating water and nutrients to terrestrial plants (Taylor *et al*., 1995). Currently, mycorrhizal symbiosis is found in many land plants, using the symbiotic interface to transfer nutrients to the host plant and acquire photosynthetic assimilates (Smith and Read, 2008, Pellegrino *et al*., 2015). Plant performance enhancement by symbiosis is well known in favourable conditions, but more imminent under stress or resource limited conditions. Nevertheless, the host plant response is largely dependent on plant species, genotype x endophyte interaction and environmental conditions (Johnson *et al*., 1997, Johnson *et al*., 2015). Mycorrhizal endophytes were extensively studied in the previous decades regarding their interaction with host plants and ecology. However, recent research designed to investigate endophytic Basidio and Ascomycota interaction with different plant tissues, illustrated the potential increase in growth parameters and yield in presence or absence of stress (Redman *et al*., 2002, Sun *et al*., 2010, Hubbard *et al*., 2012, Rabiey and Shaw, 2016).

The root endophytic fungus *Piriformospora indica* is the archetype for the mutualistic symbioses between fungi and Angiosperms with a broad range of compatible hosts (that were tested in the last years under field and/or controlled environment conditions) exceeding 50 different species which encompass monocots, dicots, perennial and annual plants (Kost and Rexer, 2013); most of them share the responses of increased yield, promoting plant growth and resistance to biotic and abiotic stresses (Gill *et al*., 2016). In durum wheat, inoculation

with *P. indica* showed about 40% increase in unstressed plants and up to 2.2-fold increase in total seed weight for drought conditions relative to controls (Hubbard *et al*., 2014). Spring wheat inoculated with *P.indica* showed improved vegetative growth under various moisture levels and significantly suppressed levels of hydrogen peroxide and lipid peroxidation rate under drought compared to control (Yaghoubian *et al*., 2014). This enhancement interaction was reported in other crops under drought, such as Arabidopsis (Sherameti *et al*., 2008), Chinese cabbage (Sun *et al*., 2010), quinoa (Hussin *et al*., 2017), maize (Xu *et al*., 2017, Zhang *et al*., 2018) and barley (Ghabooli *et* al., 2013, Ghaffari *et al*., 2016). In these cases, drought effect mitigation is mainly obtained through upregulating drought associated genes expression, such as DREB2A, CBL1, ANAC072 and RD29A and a boost in the activity of antioxidant enzymes and modulating ROS system. In stress-free experiments, *P. indica* produced IAA and mediated nutrient transfer to host plants resulting in enhanced plant growth (Archana *et al*., 2000, Sherameti *et al*., 2005, Sirrenberg *et al*., 2007, Schäfer *et al*., 2009).

Genotypic differences in mapping populations or association panels are used to identify quantitative trait loci (QTL) linked to drought associated traits, which can then be used for marker assisted selection or for further investigation to identify candidate genes. For example, QTL associated with response to mycorrhizal inoculation were identified in *Allium* species and maize (Kaeppler *et al*., 2000, Galván *et al*., 2011), but so far there have been no reports regarding the genetic underpinnings of response to *P.indica* inoculation under drought.

Therefore, a representative subset of the highly recombined winter wheat MAGIC population was used to address the following aims: (a) investigating wheat response to *P.indica* inoculation under contrasting water availability regimes and (b) identifying QTL involved in governing these responses under drought.

4.3 Materials and methods

4.3.1 Plant Material

The eight founder varieties of winter wheat elite eight-founder MAGIC population (Mackay *et al*., 2014) were used for the pilot experiment, while 200 high yielding recombinant inbred lines (RILs) of the population alongside the eight founders were used in the main trial.

4.3.2 Fungal inoculation

The *P.indica* isolate (Mycobank#812127) was obtained from Warwick University, UK. It was then multiplied by sub-culturing on Potato dextrose agar (PDA) and incubated for 7 days at 22 ^{0}C. The inoculum was produced by adding five plugs (approximately 5mm each) of the

fungus culture to 250 ml flasks of CM medium (Pham *et al*., 2008) and incubated at room temperature for 14 days on an orbital shaker (Stuart SLL1, Bibby Scientific Ltd, UK) at 140 rpm.

Seeds were placed in plastic trays containing compost (John Innes Composts, BHGS Ltd, UK) and spent 9 weeks of vernalizing at 2 ^{0}C post-sowing, then transplanted to 3 L Pots (270g) filled with 1:1, sand: compost (John Innes Composts, BHGS Ltd, UK) mixed with Osmocote slow-release granules (2 kg/m^{3}) containing a ratio of 15:11:13:2 of N:P2O5:K2O:MgO.

Inoculation was performed the next day to transplanting by mixing 4 ml of liquid CM medium containing unquantified amount of *P. indica* mycelia and chlamydospores into the surface layer of the soil and a control CM medium was added to the control pots, A week later random root samples per pot were collected for microscopic inspection following Vierheilig *et al*. (1998), by soaking for 1 hour in 10% KOH(w/v) at 80^{o}C, then for 30 minutes in 2% HCl(v/v), before covering roots with 50g L^{-1} black ink for 30 minutes and examined microscopically confirming the success of inoculation (Figure 4.1)

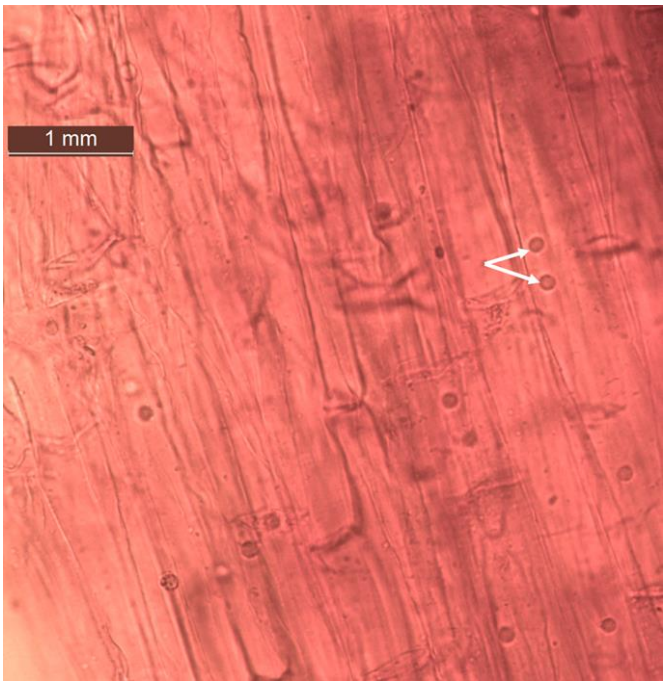


Figure 4.1 *Piriformospora indica* chlamydospores in wheat roots (seven days post inoculation) indicated by arrows.

4.3.3 Pilot experiment

This was started November 2016 in a glass house compartment in the crop environment laboratory facility, University of Reading. The eight founder varieties were tested in a split-split plot experiment, where the whole plots were meant for the drought/well-watered, the subplots for genotypes and the sub-subplots for the +*P.indica*/-*P.indica*, with 4 seedlings/pot, each treatment was replicated 3 times. Irrigation was applied to maintain soil moisture at 75% of field capacity for all treatments, then reduced for the drought treated pots to 50% field capacity around growth stage GS14-16 (Zadoks stages) before undergoing further reduction of 25% field capacity after two weeks. Plant height, TGW and grain yield/plant were measured.

4.3.4 Main trial

Seeds were sown in November 2017 to complete vernalisation requirements in growth chamber before being transplanted into 3L pots with 3 seedlings/pot, in an open sided polyethylene tunnel. Well-watered pots were maintained at 75% field capacity until two weeks before harvesting, drought stress treatment pots started receiving 50% field capacity at early tillering (GS 21) and were reduced to 25 % field capacity two weeks later.

The whole set of genotypes was replicated twice in the drought treatment, while only 40 of them were represented in the replicated well irrigated pots to investigate the differential impact of *P. indica* in contrasting water availability regimes.

4.3.5 Phenotyping

The measured traits were estimated as the average of plants/pot : Plant height (cm) was measured at grain filling stage from the soil surface to the top of the ears (excluding awns); flowering time (FT) was recorded as number of days from 1st of May to Zadok's growth stage (GS67) where about 75% of anthers are extruded in more than 50% of the plants in the pot and expressed in thermal degree days; number of ears/plant, thousand grain weight (TGW), number of grains/ear and grain yield /plant(g). Responsiveness in yield and growth parameters was calculated for each genotype following the equation, relative response = $[(T_p - T_n)/T_n]$ *100, where T_p is the genotype mean of *P.indica* inoculated plants and T_n is the genotype mean of non-inoculated plants.

4.3.6 Statistical analysis

R software R3.3.4 (R development core team, 2017) was used to analyse experimental data. Means and standard errors were calculated using ANOVA to identify differences between

treatments, Within the ANOVA the Genotypes were defined as a fixed effect so was water stress, while replicates were treated as random. Broad sense heritability of phenotype data (Supplementary material, Table S4. 1) was defined as: $H^2=\sigma^2G/\sigma^2P$.

where H^2 is the broad sense heritability, σ^2G is the genotypic variance and σ^2P is the total phenotypic variance.

Table 4.1 Phenotypes abbreviation description

Abbreviation	**Trait**
Grains.E^{-1}	Number of grains per ear
TGW*	Thousand grain weight
FT*	Flowering time
P.ht*	Plant height
GY*	Grain yield
Ears.p^{-1}	Ears per plant

*TGW (g), FT (thermal degree days), P.ht (cm), GY (g/plant)

4.3.7 QTL analysis

The MAGIC linkage map was constructed using R/ mpMap (Huang and George, 2011) based on 3535 unique SNP markers. Founder haplotype probabilities were computed with the mpprob function in mpMap using a Hidden Markov model implemented in R/qtl (Broman *et al*., 2003). Composite interval mapping exploiting the eight founders' identity by-descent haplotype probabilities (IBD-CIM). For interval mapping, a linear model was fit by estimating separated fixed effects for each of the eight founders at each putative QTL position ('Xi-19' was arbitrarily set as the reference haplotype), using the mpIM function in mpMap (program 'qtl'). When determining whether QTL listed independently in the R/mpmap outputs were redundant or co-located (same locus detected independently in different inoculation treatments across different traits), initially only QTL confidence intervals sharing a flanking marker were considered redundant/co-located. Further inspection of QTLs for the same trait

separated by only a few CM highlighted instances where direction and magnitude of parental effects led us to override the strict positional criterion.

4.4 Results

4.4.1 Pilot experiment

Table 4.2 ANOVA P-values of all factors combinations for measured traits of pilot experiment

Trait	Genotype	Stress	*P.indica*	Stress x *P.indica*	Stress x Genotype	Genotype x *P.indica*	Genotype x Stress x *P.indica*
P.ht	0.041*	0.003**	0.012*	0.001***	0.574	0.032*	0.042*
GY	0.035*	<.001***	<.001***	0.985	0.978	0.028*	0.487
TGW	0.038*	0.031*	0.183	0.476	0.467	0.398	0.728

*, **, *** Statistically significant at $p \leq 0.05$, 0.01, 0.001, respectively.

Drought caused about 81% drop in yield, which was amplified due to uncontrolled heat rising in the compartment during above average hot summer (peaking to 38ºC inside glass house) and a 15% reduction in height. *P.indica* inoculation reduced the drought response to 75 % drop, not all genotypes responded in the same way to colonization (Table 4.2) as ANOVA finds *P.indica* x genotype as significant and *P.indica* x water stress interaction was highly significant. Plant height appears to be affected by all main effects and interactions except water stress x genotype. Total seed yield showed similar trend to plant height except the lack of significance of the 3-way interaction. TGW was significantly affected only by the main effect of the genotype and water treatment, where it may be accepted that the severe heat wave that took place in the grain filling stage (raising the temperature inside the glass house up to 35ºC for more than a week) has masked a great proportion of the interactions. This pilot experiment indicated the potential of *P.indica* to induce increase in yield and growth parameters under both well-watered and drought conditions.

4.4.2 Main experiment

Based on 40 MAGIC RILs that were tested under all combinations of fungal inoculation and water stress treatments, there was a significant main effect of both genotype and drought stress in all measured traits, while inoculation with *P.indica* had a positive significant main effect

only on P.ht and GY (Table 4.3). The *P.indica* effect appeared conditioned by water availability in GY (Figure 4.2.e) as it did not change significantly under drought, but was significantly higher in the well-watered treatment, mainly caused by an increase (although not significant *per se*) in ears.p^{-1}, TGW and grains.E^{-1}. The interaction of the RILs set with *P.indica* inoculation was significant for all measured traits except ears.p^{-1} indicating a genetic basis of these responses. This is reflected in the moderate to high heritability of all traits but grains.E^{-1} which had the second lowest heritability after GY (Supplementary table S4.1). As expected from the genetic diversity within the RILs, significant interaction was found between genotypes and irrigation treatments except for FT, TGW and ears.p^{-1}.

Table 4.3 ANOVA *P*-values of all factors combination for measured traits of main trial.

Trait	Genotype	Stress	*P.indica*	Stress x *P.indica*	Genotype x *P.indica*	Stress x Genotype	Genotype x stress x *P.indica*
FT	< .001***	< .001***	0.554	0.087	0.042*	0.119	0.328
P.ht	< .001***	0.012*	0.010**	0.884	0.024*	< .001***	0.432
Ears.p^{-1}	< .001***	< .001***	0.855	0.115	0.349	0.082	0.031*
TGW	< .001***	< .001***	0.539	0.070	0.050*	0.073	0.487
GY	< .001***	0.010**	0.037*	<.001***	0.020*	0.010**	0.033*
Grains.E^{-1}	<.001***	< .001***	0.554	0.219	0.010**	0.010**	0.091

*, **, *** Statistically significant at $p \leq 0.05$, 0.01, 0.001, respectively.

The three way interaction showing genotypes responding differently to *P.indica* inoculation depending on irrigation treatment was significant for ears.p^{-1} and GY. Table 4.4 illustrates the ranges for traits under treatment combination and shows the phenotypic range of the progeny under both treatments greatly exceeded the range of the parental lines indicating transgressive segregation in the MAGIC panel for response to *P.indica* under both water availability regimes.

Table 4.4 Means, minimum, maximum and standard error for measured traits of main trial.

		Trait	**FT**	**P.ht**	**Ears.p^{-1}**	**TGW**	**GY**	**Grains.E^{-1}**
Irrigated	(+) *P.indica*	Mean	1321.00	70.40	3.20	42.10	7.31	175.10
		s.e	7.96	1.22	0.09	2.69	0.28	5.92
		Min.	1191.00	49.00	2.00	33.47	3.21	114.00
		Max.	1435.00	96.00	5.00	55.44	11.54	311.00
		Min. parent	1210.90	59.00	2.00	41.84	4.93	120.00
		Max. parent	1402.21	78.00	4.50	47.52	11.00	311.00
	(-) *P.indica*	Mean	1329.00	69.09	3.11	41.41	6.44	164.30
		s.e	8.25	1.41	0.08	0.86	0.26	5.04
		Min.	1211.00	48.50	1.50	23.94	3.42	115.30
		Max.	1453.00	94.50	4.00	55.81	7.52	269.00
		Min. parent	1340.00	69.00	2.33	44.67	4.10	117.30
		Max. parent	1402.00	71.00	4.00	44.89	4.94	253.00
Drought	*(+) P.indica*	Mean	1254.00	54.79	2.23	35.98	2.63	78.00
		s.e	6.58	1.03	0.08	0.97	0.10	2.69
		Min.	1104.00	36.00	1.33	22.30	1.04	33.67
		Max.	1354.00	69.00	3.50	56.75	4.71	108.5
		Min. parent	1104.00	42.75	1.66	32.40	2.11	50.67
		Max. parent	1323.00	67.00	3.00	48.26	3.99	106
	(-) P.indica	Mean	1248.00	52.85	2.26	35.06	2.81	73.07
		s.e	5.76	0.74	0.10	0.91	0.11	2.97
		Min.	1104.00	36.00	1.00	24.01	1.22	35.67
		Max.	1340.00	64.00	4.00	54.66	4.44	113.67
		Min. parent	1104.00	45.50	1.00	24.01	2.13	44
		Max. parent	1323.00	62.00	4.00	54.66	4.19	111

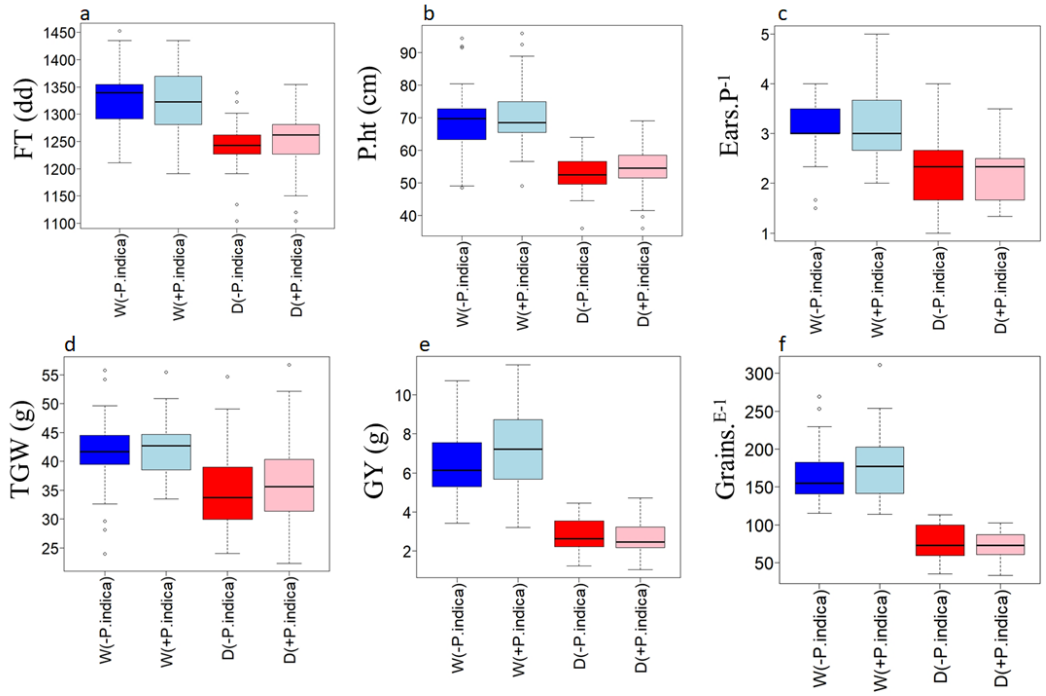


Figure 4.2 Boxplots of genotype means for the 40 RILs under combination of drought and fungal inoculation treatments for (a) flowering time, (b) plant height, (c) number of ears/plant, (d) thousand grain weight, (e) grain yield/plant, (f) number of grains/ear. (W) and (D) refer to the well-watered and drought treatments, respectively.

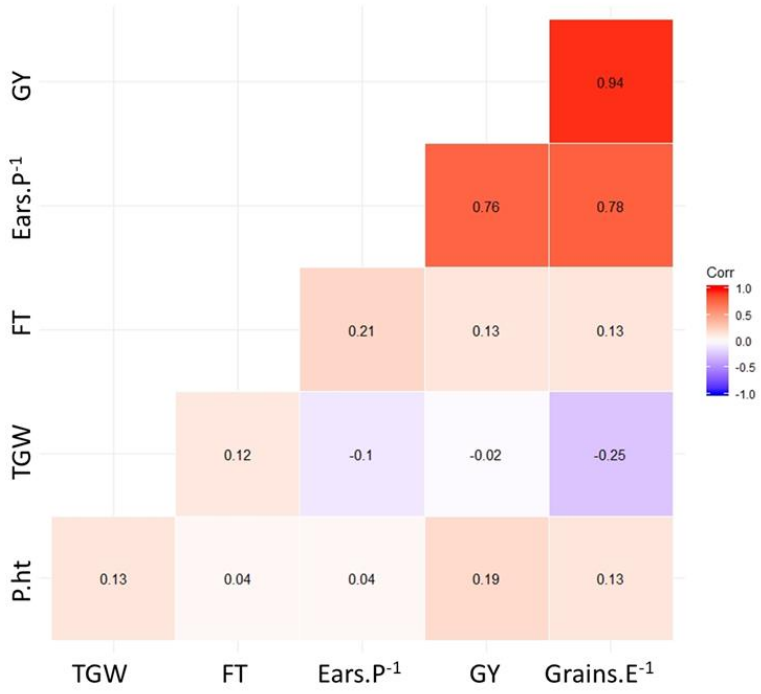


Figure 4.3 Pearson's correlation coefficient for the measured phenotypes' response to inoculation of the 200 MAGIC lines under drought.

Correlation among trait responses to *P.indica* inoculation ranged from weak to highly significant (Figure 4.3). For example, P.ht response was significantly correlated only to GY response (r=0.19), TGW response negatively correlated to grains.E^{-1} which follows the expected trade-off between number of grains and grain weight. The positive correlation between FT and ears.p^{-1} might suggest that delaying flowering offered more time for tillering and bearing more ears/plant. The strong correlation between GY response and responses of ears.p^{-1} and grains.E^{-1} was (r=0.76) and (r=0.94), respectively, depicting these two phenotypes as responsive phenotypes with biggest contribution to GY response.

4.4.2.1 Genetic analysis

A total of 89 significant non-redundant QTL were found for the measured traits under drought treatment in presence/absence of *P.indica* inoculation (Table 4.5), 40 of them were unique to the *P.indica* colonized treatment. Most QTLs were found on chromosomes 2B and 7A with 12 QTL each and the least on 5D having only one QTL, while none were detected on chromosomes 3D and 7D. The few major endophyte-independent QTL are FT associated QTL on 2B, grains.E^{-1} revealing two separate loci on 4A and P.ht and TGW on 4D. Although these loci were detected in both inoculated and control treatments, the effect size of phenotypic variation was significantly altered for two of them, Q.Grains.E^{-1}.(-p)_4A.2 and Q.Grains.E^{-1}.(+p)_4A.2 explained 1.14% and 10.48% of the phenotypic variation and Q.P.ht(-p)_4D and Q.P.ht(+p)_4D explained 11.43 and 4% of the phenotypic variation, respectively.

Nine QTL regions on chromosomes 2A, 2B, 3B, 6A, 6B, 6D and 7A were associated with Ears.p^{-1} inoculated with *P.indica*, explaining a sum of 45.06 % of variation, where Q.Ears.p^{-1}(+p)_6A alone accounts for 9.8% and 10 QTLs on chromosomes 1B, 2B, 3A, 3B, 4A, 4B, 4D, 6A and 7A accounting for 39.4 % in fungus free treatment with two big size effect loci, Q.Ears.p^{-1}(-p)_3B and Q.Ears.p^{-1}(-p)_7A explaining 9.2 and 9.4% of the variation, respectively.

Nine chromosomal regions with QTL associated with FT inoculated with *P.indica* were detected on chromosomes 1D, 2B, 5A, 5D and 7A explaining 23.4% and seven significantly associated loci to FT in *P.indica*-free plants on chromosomes 1A, 2B, 2D, 3A, 5A, 6A and 7A explaining 23.6%, 6.65% came from Q.FT(-p)_2D which tags photoperiod sensitivity locus *Ppd-D1*.

Chromosomes 2B, 4A, 5B, 6A and 7A had six QTL for grains.E^{-1} in the presence of *P.indica*, summing 36.9% of phenotypic variation, with the biggest impact of 10.48% contributed by Q.Grains.E^{-1}.(+p)_4A, while in the absence of *P.indica* eight QTL were identified on chromosomes 1A, 3B, 4A, 6A, 6B and 7A accounting for a total phenotypic variation of 30.28%, Q.Grains.E^{-1}.(-p)_6B had the biggest effect size of 7.73%.

For GY, only two QTL were detected in the presence of *P.indica* on chromosomes 5A and 7B explaining 15.27% where Q.GY.(+p)_7B accounted for 12.32%, in the absence of *P.indica*, six QTL were found on chromosomes 2B, 3B, 4A and 6B summing 28.2% of the variation as Q.GY.(-p)_3B explained 7.13%.

P.ht in the presence of *P.indica* had five QTL explaining 27.4% on chromosomes 1D, 2B, 4B, 4D and 7B, Q.P.ht(+p)_2B contributed the biggest effect size of 12.9%. Six QTL associated with P.ht in the fungus-free treatment explaining 29.12% on chromosomes 1A, 1B, 1D, 4B, 4D and 5A.

TGW had the largest number of individual QTL, 14 in the *P.indica* inoculated treatment accounting for 51.7% of phenotypic variation, Q.TGW(+p)_4D which had the biggest effect of 8.23% is co-locating the pleiotropic genomic region tagging the reduced height locus *Rht-D1*. 1o genomic regions associated with TGW in *P.indica* free treatment, explaining 48.4%.

4.5 Discussion

The drought stress negative impact on wheat performance and yield and advancing flowering was previously reported in field and controlled environment experiments (Dodig *et al*., 2012, Edae *et al*., 2014, Liu *et al*., 2017, Elfeki *et al*., 2018, Lehnert *et al*., 2018, Yadav *et al*., 2019). We obtained similar results for the set of 40 RILs investigated under both water regimes, as all measured traits were significantly reduced in various degrees leading to average GY reduction of 62% which could be attributed to reduction in ears.p^{-1}, TGW and grains.E^{-1} by 31.5, 13.9 and 57.3%, respectively. This indicates grains.E^{-1} as the main cause of yield loss while TGW ranked least, this can be confirmed as the strongest correlation of GY response to *P.indica* was detected with grains.E^{-1} (r=0.94). This high influence of number of grains per ear and lower impact of TGW on yield agrees with Dolferus *et al*. (2011) and Dodig *et al*. (2012), specially as extended drought stress during flowering and grain filling severely affects meiosis and reduces fertility, resulting in reduced number of grains per ear (Onyemaobi *et al*., 2017). However, we found a decrease in TGW, other researchers reported significant increase

in TGW under early induced drought, where assimilates are translocated to a smaller number of grains (Lehnert *et al*., 2018).

In this study, inoculation with *P.indica* mitigated the drought effect on GY in a genotype dependent scheme, as well as increasing GY in well-watered treatment, where the effect size was stronger in well-watered treatment. This result contradicts Vahabi *et al*. (2015) who found the mutualistic interaction of *P.indica* with Arabidopsis is promoted under stress and Hubbard *et al*. (2014) who found *P.indica* yield increase response is significantly higher under drought.

The positive effects, alleviating drought stress effects induced by *P.indica* are proposed in different crop plants to be associated with enhancing the electron transfer chain and photosystem activity (Ghaffari *et al*., 2019), improved water balance by increasing leaf water potential (Hussin *et al*., 2017), increased water absorbance potential reflected by increase in root mass (Ghabooli and Mondani, 2016) and upregulating activities of catalases and superoxide dismutases and drought related genes such as ANAC072, RD29A, DREB2A and CBL1 (Xu *et al*., 2017). In wheat, it increased F_v/F_m values (Hubbard *et al*., 2014) and significantly decreased hydrogen peroxide and lipid peroxidation rate (Yaghoubian *et al*., 2014).

In the main trial, for most measured traits there was significant interaction between *P.indica* and the genotypes. However, all genotypes exhibited successful inoculation, the colonisation rate was not investigated in this experiment, which might have had a significant role in the differential response of the genotypes as observed in barley where cultivars varied in their colonization rate of *P.indica* (Gravouil, 2012) and perhaps needs to be addressed in future experiments.

Combined heritability estimates were moderate to high, which is typical of controlled environment experiments, with lowest heritability detected for GY, as it is known by its genetic complexity and significant response to environment. Heritability estimates were lower under *P.indica* interaction compared to control, since part of the variation is deducted from the genotypic effect and attributed to the fungal interference.

To our knowledge, no information is available about QTL associated with *P.indica* mutualistic interaction with wheat; here we report our findings of QTL governing wheat response to *P.indica* under drought. QTL analysis in both cases of presence/absence of *P.indica* showed the previously reported pleiotropy of *Rht-D1* Locus on chromosome 4D to be associated with P.ht and TGW in this study as it did for both traits in Jackson (2019).

Interestingly, the effect size of *Rht-D1* locus dropped from 11.43% to 4% upon inoculation, while Q.P.ht(-p)_4B tagging *Rht-B1* on chromosome 4B did not show any detectable effect in the *P.indica* inoculated plants. The strong interference with reduced height genes might be explained by the reported upregulation of GA in several plants, such as maize and tomato (Liu *et al*., 2019) and reduction in expression of some members of the DELLA gene family, such as RGA1 in Arabidopsis as found by Pan *et al*. (2017). Q.FT(-p)_2D which tags the photoperiod sensitivity locus *Ppd-D1* was detected only in fungus-free treatment and was masked by *P.indica* inoculation, indicating profound interference with *Ppd-D1* locus mode of action.

Upon investigating co-location of the detected loci with those previously reported, it was noticed that those found in *P.indica* inoculated treatment coincided with QTL detected in growing seasons receiving above average rainfall, while the fungus-free ones co-located those found in dry year, for example Q.TGW(+p)_2D was found co-locating with grain weight QTL in 2016 rainy year and missed in 2015 dry year and Q.FT(+p)_5A coincided with the locus associated with FT in 2016 rainy year (Jackson, 2019). Also Q.GY.(-p)_3B which is missed in the inoculated plants was co-located with a previously detected QTL for number of grains/m^2 in drought stressed field in 2017 (Chapter 3) as shown (Figure 4.4).

Interestingly, Q.Grains.E^{-1}._4A that showed a minimal effect in the absence of *P.indica* (1.14%) and revealed amplified effect by the fungal interaction up to 10.48% (Figure 4.4). This was the same QTL identified for Grains.E^{-1} in well-irrigated field but missed in the drought stressed treatment in 2017 field trial (Chapter3), which supports the proposed *P.indica* mitigation effect of drought effect.

Q.GY.(+p)_5A, one of the two loci identified associated with GY under inoculated conditions is coinciding with a QTL associated with canopy temperature depression (CTD) in the well irrigated field treatment, this might suggest the potential of *P.indica* potential to reduce plant temperature, where cooler canopies are known to correlate to higher yield (Tattaris *et al*., 2016).

The presence of 40 QTL (for different traits) unique to the colonized plants and the fact that the common QTL did not exceed five loci with major effect, suggest a significant effect of the *P. indica* on gene expression of phenotypes of growth development and yield.

Table 4.5 QTL analysis QTL table for MAGIC lines measured phenotypes. Trait abbreviations are as shown in (Table 4.1).

	QTL name	Chromosome	Position	Left marker	Right marker	Left marker position	Rightmarker position	*p*- value	R^2
1	Q.FT(-p)_1A	1A	40.51	wsnp_Ex_c64327_63176640	BS00078982_51	40.51	41.51	0.0105	2.28
2	Q.Grains.E^{-1}.(-p)_1A	1A	106.72	IACX3496	RFL_Contig3203_1971	105.21	106.72	0.0027	3.18
3	Q.P.ht(-p)_1A	1A	161.25	BS00073413_51	Excalibur_c97157_65	161.25	162.25	2.00E-13	1.54
4	Q.TGW(+p)_1A.1	1A	178.52	Excalibur_c66_147	JD_c13024_360	178.52	179.53	7.00E-07	2.3
5	Q.TGW(+p)_1A.2	1A	220.84	BS00022514_51	Excalibur_c12932_2102	220.84	221.84	3.00E-08	7.64
6	Q.Ears.p^{-1}(-p)_1B	1B	21.72	Kukri_c29655_194	RFL_Contig4140_1135	21.72	22.72	1.00E-08	2.71
7	Q.P.ht(-p)_1B	1B	87.54	BS00108806_51	Kukri_c18052_356	87.54	89.04	5.00E-05	3.54
8	Q.TGW(+p)_1B	1B	321.3	RAC875_c9082_267	wsnp_CAP11_c2596_1325540	316.63	321.30	9.00E-13	6.53
9	Q.FT(+p)_1D	1D	0	D_GDS7LZN02IXNP1_255	BS00022323_51	0.00	6.33	4.00E-05	2.11
10	**Q.TGW(-p)_1D**	**1D**	**71.23**	**RAC875_rep_c105196_532**	**IAAV4656**	**66.65**	**71.23**	**4E-13**	**4.54**
11	**Q.P.ht(-p)_1D**	**1D**	**75.67**	**IAAV4656**	**BS00038418_51**	**71.23**	**75.67**	**4E-08**	**4.75**
12	Q.P.ht(+p)_1D	1D	84.41	Ra_c3045_1739	Excalibur_c3596_144	83.40	84.41	0.0016	6.18
13	**Q.TGW(+p)_2A.1**	**2A**	**76.42**	**BS00022332_51**	**BS00049937_51**	**76.42**	**77.93**	**0.0001**	**2.49**
14	**Q.Ears.p^{-1}(+p)_2A**	**2A**	**78.93**	**BS00049937_51**	**IAAV7468**	**77.93**	**78.93**	**1E-09**	**1.51**
15	Q.TGW(+p)_2A.2	2A	162.06	wsnp_BE445431A_Td_2_1	wsnp_Ra_c32271_41304469	162.06	163.06	0.0091	2.76
16	Q.TGW(+p)_2A.3	2A	240.68	BS00062869_51	wsnp_Ex_rep_c108004_91402649	240.68	244.25	5.00E-11	4.45
17	Q.GY.(-p)_2B.1	2B	57.75	BobWhite_c30520_323	Kukri_c40764_367	51.42	57.75	6.00E-06	5.25
18	**Q.TGW(+p)_2B**	**2B**	**76.16**	**wsnp_Ex_rep_c72527_70882805**	**wsnp_Ex_c1962_3696265**	**74.65**	**76.16**	**2E-08**	**2.34**
19	**Q.P.ht(+p)_2B**	**2B**	**81.2**	**wsnp_Ex_c14711_22788263**	**BS00071995_51**	**81.20**	**82.20**	**0.0015**	**12.9**
20	Q.GY.(-p)_2B.2	2B	100.99	Kukri_c16479_765	BS00067962_51	99.47	100.99	7.00E-08	5.09
21	**Q.FT(-p)_2B**	**2B**	**165.81**	**BS00092273_51**	**BobWhite_c7786_376**	**165.81**	**167.33**	**0.0141**	**3.92**
23	**Q.FT(+p)_2B.1**	**2B**	**171.86**	**Excalibur_c19344_137**	**BobWhite_c892_73**	**170.86**	**171.86**	**1E-08**	**2.66**
24	Q.Ears.p^{-1}(-p)_2B	2B	208.43	wsnp_Ex_c4218_7618252	BS00066545_51	208.43	212.56	1.00E-10	2.34
25	**Q.Grains.E^{-1}.(+p)_2B**	**2B**	**248.88**	**BobWhite_c38001_528**	**BobWhite_c3146_128**	**243.11**	**248.88**	**0.0018**	**6.46**
26	**Q.FT(+p)_2B.2**	**2B**	**248.88**	**BobWhite_c38001_528**	**BobWhite_c3146_128**	**243.11**	**248.88**	**0.00001**	**2.08**
27	Q.Ears.p^{-1}(+p)_2B	2B	285.43	CAP11_c2941_210	BS00026432_51	283.93	285.43	3.00E-12	3.35
28	Q.FT(+p)_2B.3	2B	351.13	BS00083998_51	Excalibur_c48871_625	351.13	352.63	0.0002	3.12
29	Q.TGW(-p)_2B	2B	375.98	RAC875_c3259_276	BS00064483_51	373.94	375.98	6.00E-11	3.42
30	Q.FT(-p)_2D	2D	62.71	Kukri_c27309_590	wsnp_CAP12_c1503_764765	55.40	62.71	0.0006	6.65
31	Q.TGW(+p)_2D	2D	113.91	wsnp_Ex_rep_c68555_67394261	Kukri_c54059_654	113.91	117.99	0	2.37
32	Q.FT(-p)_3A	3A	76.83	Tdurum_contig91865_242	Kukri_c64268_101	76.83	78.85	2.00E-05	2.32
33	Q.TGW(-p)_3A.1	3A	174.7	BS00070870_51	BS00056089_51	163.97	174.70	5.00E-10	1.99
34	Q.Ears.p^{-1}(-p)_3A	3A	224.76	BS00022459_51	BS00022368_51	221.19	224.76	1.00E-06	3.61
35	Q.TGW(+p)_3A	3A	237.62	BS00026396_51	Excalibur_c24354_465	232.95	237.62	2.00E-07	2.07
36	Q.TGW(-p)_3A.2	3A	274.05	CAP7_c3178_52	BS00048633_51	270.21	274.05	2.00E-07	1.7
37	Q.GY.(-p)_3B.1	3B	68.17	wsnp_Ku_c17659_26797674	RAC875_c46194_201	68.17	69.68	2.00E-05	7.13
38	Q.TGW(+p)_3B	3B	109.12	wsnp_JD_c17082_16025440	BobWhite_c634_420	108.12	109.12	2.00E-11	1.81
39	Q.Ears.p^{-1}(-p)_3B	3B	112.15	Kukri_c26490_145	Jagger_c2707_152	112.15	113.15	3.00E-09	9.17
40	Q.Ears.p^{-1}(+p)_3B	3B	153.44	BS00073011_51	wsnp_Ku_rep_c72504_72191206	153.44	154.44	2.00E-10	3.19
41	Q.GY.(-p)_3B.2	3B	205.69	BobWhite_c6015_141	Excalibur_c33274_498	205.69	207.73	8.00E-05	2.22
42	Q.Grains.E^{-1}.(-p)_3B	3B	269.25	BS00044942_51	Excalibur_c3556_1758	269.25	270.25	0.0012	1.81
43	**Q.Grains.E^{-1}.(-p)_4A.1**	**4A**	**6.73**	**Ku_c2478_227**	**BS00021716_51**	**6.23**	**6.73**	**0.0003**	**1.92**
44	**Q.Grains.E^{-1}.(+p)_4A.1**	**4A**	**6.73**	**Ku_c2478_227**	**BS00021716_51**	**6.23**	**6.73**	**0.00001**	**1.99**
45	Q.Ears.p^{-1}(-p)_4A	4A	54.62	Tdurum_contig11919_360	Kukri_c10501_313	54.62	55.12	0.0041	2.61
46	Q.TGW(-p)_4A	4A	110.57	Ex_c66324_1151	Jagger_c4331_105	110.57	113.10	0.0014	2.53
47	**Q.Grains.E^{-1}.(-p)_4A.2**	**4A**	**133.75**	**wsnp_Ex_c41074_47987860**	**BS00039641_51**	**133.75**	**135.26**	**0.00001**	**1.14**
48	**Q.Grains.E-1.(+p)_4A.2**	**4A**	**137.78**	**wsnp_Ex_c3988_7221220**	**IAAV5722**	**137.28**	**137.78**	**0.0087**	**10.5**

Table 4.5 (continued)

	QTL name	Chromosome	Position	Left marker	Right marker	Left marker position	Rightmarker position	*p*-value	R^2
49	Q.GY.(-p)_4A	4A	182.89	RAC875_c33109_72	Excalibur_c6050_323	180.88	182.89	2.00E-06	2.88
50	Q.Ears.p-^{1}(-p)_4B.1	4B	7.88	Tdurum_contig5427_314	BS00106142_51	7.88	9.92	0.0001	3.47
51	Q.TGW(-p)_4B.1	4B	14.02	Excalibur_c7581_1266	wsnp_Ex_c30695_39579408	14.02	18.73	4.00E-05	4.39
52	**Q.TGW(-p)_4B.2**	**4B**	**51.16**	**BS00084070_51**	**BS00033614_51**	**49.66**	**51.16**	**0.0008**	**6.49**
53	**Q.P.ht(-p)_4B**	**4B**	**53.17**	**Tdurum_contig42229_113**	**IAAV585**	**52.17**	**53.17**	**2E-10**	**4.74**
54	**Q.Ears.p^{-1}(-p)_4B.2**	**4B**	**53.17**	**Tdurum_contig42229_113**	**IAAV585**	**52.17**	**53.17**	**0.00004**	**1.33**
55	Q.P.ht(+p)_4B	4B	93.39	Ex_c32540_659	Excalibur_c42450_727	92.39	93.39	0.0077	3
56	**Q.P.ht(-p)_4D**	**4D**	**24.93**	**Kukri_rep_c68594_530**	**Excalibur_c19078_210**	**24.93**	**32.24**	**2E-06**	**11.4**
57	**Q.TGW(-p)_4D.1**	**4D**	**24.93**	**Kukri_rep_c68594_530**	**Excalibur_c19078_210**	**24.93**	**32.24**	**0**	**13.1**
58	**Q.P.ht(+p)_4D**	**4D**	**32.24**	**Excalibur_c19078_210**	**RAC875_rep_c105718_304**	**32.24**	**40.11**	**6E-09**	**4**
59	**Q.TGW(+p)_4D**	**4D**	**32.24**	**Excalibur_c19078_210**	**RAC875_rep_c105718_304**	**32.24**	**40.11**	**0**	**8.23**
60	**Q.Ears.p^{-1}(-p)_4D**	**4D**	**32.24**	**Excalibur_c19078_210**	**RAC875_rep_c105718_304**	**32.24**	**40.11**	**2E-07**	**1.17**
61	Q.TGW(-p)_4D.2	4D	106.01	Excalibur_c79009_131	IAAV5607	106.01	110.72	2.00E-12	4.01
62	Q.FT(+p)_5A	5A	56.05	wsnp_Ex_c1981_3728899	BS00021660_51	56.05	58.07	0.0007	2.01
63	Q.GY.(+p)_5A	5A	99.27	BS00015653_51	Tdurum_contig69079_300	98.27	99.27	5.00E-05	2.95
64	Q.P.ht(-p)_5A	5A	177.07	Excalibur_c9210_168	wsnp_Ex_c18941_27840714	176.07	177.07	0.0054	3.12
65	Q.FT(-p)_5A	5A	216.05	Excalibur_c37943_221	wsnp_Ex_c37943_45584325	211.51	216.05	0.0063	2.12
66	Q.TGW(+p)_5B	5B	14.17	wsnp_Ex_c26252_35497729	BobWhite_c5887_1277	13.16	14.17	3.00E-12	4.27
67	Q.Grains.E^{-1}.(+p)_5B	5B	258.41	BS00039874_51	Kukri_c87328_116	257.41	258.41	5.00E-05	6.39
68	Q.FT(+p)_5D	5D	11.57	BS00065296_51	BS00003975_51	11.57	12.57	0.0036	4.4
69	Q.FT(-p)_6A	6A	81.29	IAAV7418	IAAV7349	81.29	84.65	0.0014	2.09
70	**Q.Ears.p^{-1}(+p)_6A.1**	**6A**	**92.56**	**BS00023627_51**	**BS00063296_51**	**92.56**	**96.41**	**6E-11**	**9.83**
71	**Q.Grains.E^{-1}.(+p)_6A**	**6A**	**103.35**	**wsnp_Ku_c26784_36748247**	**Tdurum_contig50698_601**	**102.35**	**103.35**	**2E-06**	**7.88**
72	Q.Ears.p^{-1}(-p)_6A	6A	215.25	RFL_Contig5037_560	Excalibur_c52196_235	210.63	215.25	3.00E-07	3.56
73	**Q.Grains.E^{-1}.(-p)_6A**	**6A**	**223.42**	**RAC875_c103443_475**	**BS00000750_51**	**223.42**	**227.59**	**7E-10**	**6.02**
74	**Q.Ears.p^{-1}(+p)_6A.2**	**6A**	**223.42**	**RAC875_c103443_475**	**BS00000750_51**	**223.42**	**227.59**	**0.0002**	**5.29**
75	Q.TGW(+p)_6B	6B	15.73	wsnp_Ku_c2119_4098330	BobWhite_c20959_229	14.47	15.73	0.0019	3.04
76	**Q.Grains.E^{-1}.(-p)_6B**	**6B**	**69.86**	**CAP8_rep_c9477_231**	**Excalibur_c63243_434**	**69.86**	**72.95**	**0.0054**	**7.73**
77	**Q.GY.(-p)_6B**	**6B**	**76.72**	**Excalibur_c48499_250**	**BS00074041_51**	**75.72**	**76.72**	**2E-08**	**5.62**
78	Q.TGW(-p)_6B	6B	185.6	BobWhite_c3392_749	BS00109036_51	185.60	186.60	1.00E-08	5.02
79	Q.Ears.p^{-1}(+p)_6B	6B	201.81	RAC875_c12907_515	BS00023080_51	199.77	201.81	0.0003	2.35
80	Q.Ears.p^{-1}(+p)_6D	6D	192.69	BobWhite_c22280_104	wsnp_Ex_c10718_17457870	190.15	192.69	1.00E-10	3.74
81	Q.FT(-p)_7A	7A	65.48	Kukri_c79627_494	BS00110940_51	65.48	74.73	0.0002	4.25
82	Q.TGW(+p)_7A	7A	100.88	RAC875_c52560_123	BS00061012_51	100.88	104.47	4.00E-09	1.49
83	Q.Grains.E^{-1}.(-p)_7A.1	7A	105.98	BS00061012_51	Ex_c9615_1202	104.47	105.98	7.00E-13	6.71
84	Q.Ears.p^{-1}(-p)_7A	7A	110.06	BobWhite_rep_c58252_112	BS00007429_51	110.06	112.62	9.00E-05	9.37
85	Q.FT(+p)_7A.1	7A	161.15	BS00040657_51	Excalibur_c20062_195	161.15	162.15	0.001	1.13
86	Q.Grains.E^{-1}.(-p)_7A.2	7A	202.68	BS00030028_51	BS00030940_51	202.68	203.68	1.00E-07	1.77
87	Q.FT(+p)_7A.2	7A	216.28	tplb0024l08_1395	BobWhite_c44628_61	216.28	217.28	0.0091	2.61
88	Q.TGW(-p)_7A	7A	234.92	BS00064351_51	BS00110561_51	232.90	234.92	9.00E-08	1.27
89	**Q.Ears.p^{-1}(+p)_7A**	**7A**	**296.59**	**wsnp_Ex_c23102_32328851**	**Kukri_c19696_60**	**296.59**	**298.60**	**0.0015**	**7.57**
90	**Q.FT(+p)_7A.3**	**7A**	**304.72**	**JD_c149_1700**	**IACX2471**	**304.72**	**305.72**	**5E-06**	**3.27**
91	Q.Ears.p^{-1}(+p)_7A	7A	361.69	RAC875_c2532_64	Tdurum_contig97505_172	360.69	361.69	5.00E-09	8.23
92	Q.Grains.E^{-1}.(+p)_7A	7A	365.23	wsnp_Ex_c6142_10746442	wsnp_Ex_c53387_56641291	365.23	366.23	0.0022	3.72
93	Q.P.ht(+p)_7B	7B	168.07	Kukri_c15912_1189	BS00047083_51	168.07	171.16	0.003	1.33
94	Q.GY.(+p)_7B	7B	187.3	BS00066479_51	BS00023166_51	187.30	188.82	3.00E-06	12.3

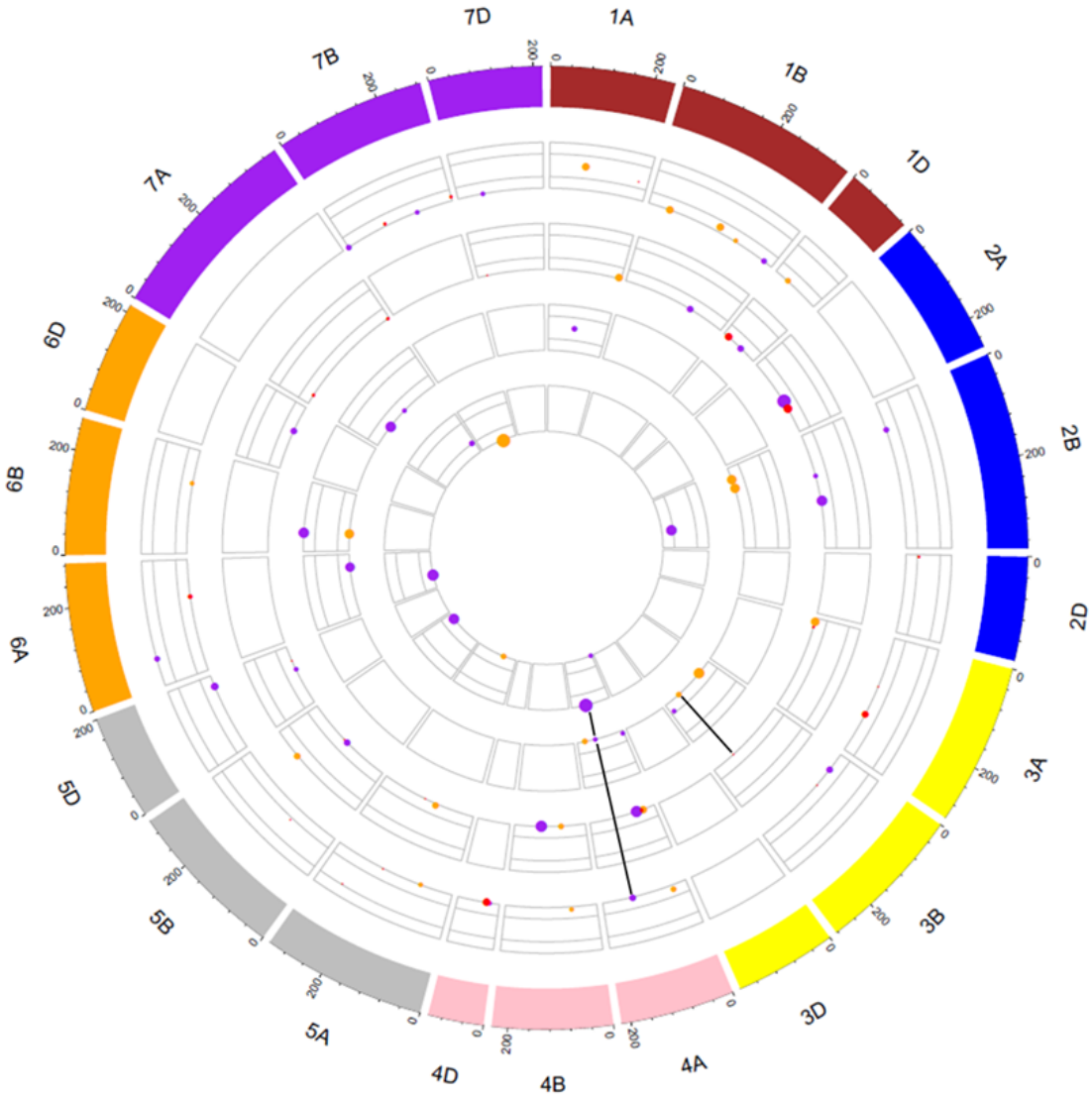


Figure 4.4 Circos plot showing a genetic map of the 21 wheat chromosomes; LOD scores of individual significant SNPs (coloured dots on inner light grey segments- dot size is proportional to the effect size of QTL). LOD 5 and LOD 10 thresholds are marked by concentric circular lines. Chromosomal units in CM. Tracks inner to outer illustrate + *P.indica*, -*P. indica*, rainfed field and irrigated field treatments, respectively. Orange, purple and red dots indicate GY, Grains.E^{-1} and grains.m^{-2}, respectively. Black vertical lines link QTL on the same genomic position.

4.6 Conclusion

In this study, it has been shown that wheat inoculation with *P.indica* increased grain yield under both well water and drought stress conditions and that the response of GY, that most of the measured trait responses are genotype specific and that GY response is highly correlated to response of ears.p^{-1} and grains.E^{-1}. The moderate to high heritability in the presence /absence *P.indica* interaction allowed us to detect a few major and several minor effect QTL for grain yield and all measured traits. The absence of some major QTL under *P.indica* interaction confirms the interference of the fungus with gene action and metabolic pathways. Given the detection of loci specific to *P.indica* interaction in a subset of the MAGIC lines, the genotypic specific variation in response can be proposed for breeding to select highly responsive lines and improve wheat performance under drought. This study might be considered the first report identifying QTL that can be used to investigate for candidate genes associated with wheat response to endophyte fungal inoculation and propose informative SNP markers for wheat breeding under drought.

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4.8 Supplementary materials

Figure S4. 1 Open-sided polytunnel of the main trial.

Table S4. 1 Broad sense heritability.

Trait	H^2 combined	H^2(-) *P.indica*	H^2(+) *P.indica*
P.ht	0.84	0.72	0.70
FT	0.82	0.65	0.62
Ears.p^{-1}	0.63	0.45	0.42
TGW	0.75	0.59	0.57
GY	0.54	0.42	0.26
Grains.E^{-1}	0.59	0.54	0.36

Table S4. 2 Means, minimum, maximum and standard error for measured traits of pilot experiment

	irrigated								drought							
	(+) *P.indica*				(-) *P.indica*				(+) *P.indica*				(-) *P.indica*			
Trait	Mean	s.e	Min.	Max.	Mean	s.e	Min.	Max.	Mean	s.e	Min.	Max.	Mean	s.e	Min.	Max.
P.ht	44.36	1.48	40.25	51.60	41.97	1.44	35.25	49.00	39.11	0.54	37.00	41.00	36.78	0.93	33.36	40.20
GY	9.85	1.07	6.00	13.82	8.01	0.54	5.70	10.08	2.03	0.26	1.02	3.19	1.15	0.17	0.30	1.73
TGW	27.30	1.33	21.40	32.40	23.78	1.38	20.00	30.90	20.80	1.42	15.66	28.15	18.32	0.89	14.6	21.21

Table S4. 3 Parental effect (relative to founder Xi-19) for the detected QTL.

	QTL name	Chromosome	Position	Alchemy	Brompton	Claire	Hereward	Rialto	Robigus	Soissons	*p* value	R^2
1	Q.FT(-p)_1A	1A	40.51	-0.81	11.06	38.06	-19.87	0.63	-13.97	-13.39	0.0185	2.28
2	Q.Grains.E^{-1}.(-p)_1A	1A	106.72	-5.62	17.60	106.01	-71.39	-1.75	-18.84	19.90	0.0027	3.18
3	Q.P.ht(-p)_1A	1A	161.25	18.20	8.07	NA	4.31	8.77	2.73	13.19	2E-13	1.54
4	Q.TGW(+p)_1A.1	1A	178.52	-11.14	-12.28	10.86	-1.55	-2.63	2.80	-0.18	7E-07	2.3
5	Q.TGW(+p)_1A.2	1A	220.84	-9.73	11.51	NA	0.95	3.22	5.14	-0.07	3E-08	7.64
6	Q.Ears.p^{-1}(-p)_1B	1B	21.72	-0.89	0.35	-0.59	-0.73	-2.54	-0.47	-0.37	1E-08	2.71
7	Q.P.ht(-p)_1B	1B	87.54	-5.17	-1.92	-4.73	-0.74	-5.04	-4.22	-5.47	5E-05	3.54
8	Q.TGW(+p)_1B	1B	321.30	-2.67	-2.38	-0.82	-3.69	-1.98	-3.87	0.37	9E-13	6.53
9	Q.FT(+p)_1D	1D	0.00	-159.55	-166.15	-163.31	-380.10	-125.58	-153.71	-166.17	4E-05	2.11
10	Q.TGW(-p)_1D	1D	71.23	0.85	-4.66	1.72	-4.50	-0.53	0.89	-0.84	4E-13	4.54
11	Q.P.ht(-p)_1D	1D	75.67	-18.50	-19.32	-8.44	-15.19	-34.14	-10.47	-17.49	4E-08	4.75
12	Q.P.ht(+p)_1D	1D	84.41	-12.40	-8.35	-2.44	-12.85	-18.73	-7.81	-10.70	0.0016	6.18
13	Q.TGW(+p)_2A.1	2A	76.42	1.05	2.68	4.49	3.29	4.80	3.28	1.22	0.0001	-2.49
14	Q.Ears.p^{-1}(+p)_2A	2A	78.93	-0.43	-0.31	-0.73	-0.40	-0.52	0.31	0.42	1E-09	-1.51
15	Q.TGW(+p)_2A.2	2A	162.06	1.72	1.17	0.65	-0.66	1.57	0.33	2.26	0.0091	-2.76
16	Q.TGW(+p)_2A.3	2A	240.68	3.05	-1.80	3.68	3.28	0.92	2.81	1.40	5E-11	4.45
17	Q.GY.(-p)_2B.1	2B	57.75	0.76	-0.71	-0.22	1.67	-0.13	-0.30	0.34	6E-06	5.25
18	Q.TGW(+p)_2B	2B	76.16	-0.76	-4.61	0.72	-1.89	4.14	1.57	-2.88	2E-08	2.34
19	Q.P.ht(+p)_2B	2B	81.20	2.74	-1.89	2.56	1.19	8.31	2.85	-3.50	0.0015	12.92
20	Q.GY.(-p)_2B.2	2B	100.99	1.07	1.93	1.69	-0.12	4.64	1.77	0.88	7E-08	5.09
21	Q.FT(-p)_2B	2B	165.81	-99.90	-318.69	-108.11	-0.35	-93.01	-98.08	-105.90	0.0141	3.92
23	Q.FT(+p)_2B.1	2B	171.86	10.80	-167.05	13.66	179.98	-46.45	27.46	-4.68	1E-08	2.66
24	Q.Ears.p^{-1}(-p)_2B	2B	208.43	-1.22	7.65	7.29	-3.09	-12.57	0.21	0.96	1E-10	2.34
25	Q.Grains.E^{-1}.(+p)_2B	2B	248.88	-134.69	109.52	-41.11	-272.05	-78.99	-59.76	-70.66	0.0018	6.46
26	Q.FT(+p)_2B.2	2B	248.88	134.76	182.01	221.24	147.01	152.50	108.78	125.69	1E-05	2.08
27	Q.Ears.p^{-1}(+p)_2B	2B	285.43	-1.02	3.56	-3.73	0.55	-1.86	-0.11	0.01	3E-12	3.35
28	Q.FT(+p)_2B.3	2B	351.13	15.75	12.10	-17.11	-37.29	-4.99	6.41	4.69	0.0002	3.12
29	Q.TGW(-p)_2B	2B	375.98	-9.74	-2.12	-3.47	1.47	-2.46	-4.33	-5.67	6E-11	3.42
30	Q.FT(-p)_2D	2D	62.71	-21.78	-3.28	-19.10	9.67	-7.86	-13.70	-36.47	0.0006	6.65
31	Q.TGW(+p)_2D	2D	113.91	-4.18	-4.36	5.41	-3.93	0.48	2.44	-2.35	0	2.37
32	Q.FT(-p)_3A	3A	76.83	-1.37	-31.47	-20.24	-27.89	-24.98	-13.96	-11.87	2E-05	2.32
33	Q.TGW(-p)_3A.1	3A	174.70	-0.09	-1.71	-2.29	0.16	-5.80	-1.09	-0.38	5E-10	-1.99
34	Q.Ears.p^{-1}(-p)_3A	3A	224.76	0.35	0.61	-0.04	0.22	0.90	0.81	0.11	1E-06	3.61
35	Q.TGW(+p)_3A	3A	237.62	0.97	0.76	0.63	0.43	-4.45	0.61	1.49	2E-07	2.07
36	Q.TGW(-p)_3A.2	3A	274.05	1.48	-0.38	-4.42	1.16	1.42	0.33	1.99	2E-07	1.7
37	Q.GY.(-p)_3B.1	3B	68.17	3.50	-0.12	-3.12	-0.54	-0.50	-0.73	-0.83	2E-05	7.13
38	Q.TGW(+p)_3B	3B	109.12	2.56	-2.39	1.43	1.13	-0.90	-1.86	-2.73	2E-11	-1.81
39	Q.Ears.p^{-1}(-p)_3B	3B	112.15	-1.41	-2.53	-2.17	-2.06	-3.11	-2.38	-2.17	3E-09	9.17
40	Q.Ears.p^{-1}(+p)_3B	3B	153.44	4.44	5.04	3.08	5.40	9.87	3.94	4.52	2E-10	3.19
41	Q.GY.(-p)_3B.2	3B	205.69	0.05	-0.78	-2.06	-1.55	-0.20	-0.20	NA	8E-05	2.22
42	Q.Grains.E^{-1}.(-p)_3B	3B	269.25	6.69	31.25	-6.87	-20.17	-7.90	-22.27	NA	0.0012	-1.81
43	Q.Grains.E^{-1}.(-p)_4A.1	4A	6.73	-36.20	-118.65	-27.64	-5.26	29.84	-36.14	-26.20	0.0003	-1.92
44	Q.Grains.E^{-1}.(+p)_4A.1	4A	6.73	82.20	-54.48	72.08	75.16	159.95	105.71	69.69	1E-05	1.99
45	Q.Ears.p^{-1}(-p)_4A	4A	54.62	0.40	0.96	-0.03	-0.98	1.28	-0.38	-0.07	0.0041	2.61
46	Q.TGW(-p)_4A	4A	110.57	0.79	0.76	1.12	2.44	0.15	-0.50	2.08	0.0014	2.53
47	Q.Grains.E^{-1}.(-p)_4A.2	4A	133.75	-0.34	5.80	21.80	25.32	17.08	58.36	-17.24	1E-05	-1.14
48	Q.Grains.E^{-1}.(+p)_4A.2	4A	137.78	-66.32	-36.77	-9.32	-73.26	-34.94	25.43	-51.91	0.0087	10.48

Table S4.3(continued)

	QTL name	Chromosome	Position	Alchemy	Brompton	Claire	Hereward	Rialto	Robigus	Soissons	*p* value	R^2
49	Q.GY.(-p)_4A	4A	182.89	-4.21	-0.76	2.00	-0.72	-1.26	-0.59	-2.72	2E-06	2.88
50	Q.Ears.p^{-1}(-p)_4B.1	4B	7.88	-0.05	0.10	-0.08	-0.20	0.69	-0.15	0.16	0.0001	3.47
51	Q.TGW(-p)_4B.1	4B	14.02	-1.32	2.00	-0.60	-1.88	1.53	0.00	-1.10	4E-05	4.39
52	Q.TGW(-p)_4B.2	4B	51.16	0.81	0.23	2.00	0.32	0.36	2.49	-1.66	0.0008	6.49
53	Q.P.ht(-p)_4B	4B	53.17	-2.72	-2.62	-1.79	-3.50	-3.42	-5.62	-11.76	2E-10	4.74
54	Q.Ears.p^{-1}(-p)_4B.2	4B	53.17	0.82	0.45	0.19	-0.02	0.29	-0.52	0.13	4E-05	-1.33
55	Q.P.ht(+p)_4B	4B	93.39	2.11	3.43	3.31	-1.38	-0.52	-2.90	-0.79	0.0077	3
56	Q.P.ht(-p)_4D	4D	24.93	-0.86	0.50	NA	1.33	10.34	5.01	6.48	2E-06	11.43
57	Q.TGW(-p)_4D.1	4D	24.93	-5.25	-3.62	NA	1.79	3.95	-1.01	4.30	0	13.06
58	Q.P.ht(+p)_4D	4D	32.24	6.07	4.46	NA	-5.04	5.11	4.62	7.59	6E-09	4
59	Q.TGW(+p)_4D	4D	32.24	3.11	0.95	NA	-2.83	5.09	3.59	4.24	0	8.23
60	Q.Ears.p^{-1}(-p)_4D	4D	32.24	-1.08	0.35	NA	-1.21	0.02	0.13	-0.63	2E-07	-1.17
61	Q.TGW(-p)_4D.2	4D	106.01	2.97	8.20	NA	-1.61	1.68	2.29	-4.44	2E-12	4.01
62	Q.FT(+p)_5A	5A	56.05	7.43	-29.92	18.32	15.70	12.90	-5.40	4.89	0.0007	2.01
63	Q.GY.(+p)_5A	5A	99.27	-1.30	1.12	-0.98	-1.67	-1.65	-2.11	-1.25	5E-05	2.95
64	Q.P.ht(-p)_5A	5A	177.07	4.32	4.21	7.27	3.63	6.68	4.32	0.40	0.0054	3.12
65	Q.FT(-p)_5A	5A	216.05	-2.37	-20.88	-5.06	6.50	-10.31	-17.72	3.19	0.0063	2.12
66	Q.TGW(+p)_5B	5B	14.17	-9.24	0.80	4.20	-1.30	2.26	-1.15	3.18	3E-12	4.27
67	Q.Grains.E^{-1}.(+p)_5B	5B	258.41	-88.64	156.14	-7.56	-4.51	-29.80	-30.99	11.52	5E-05	6.39
68	Q.FT(+p)_5D	5D	11.57	32.72	-1.86	8.79	0.32	21.61	4.29	NA	0.0036	4.4
69	Q.FT(-p)_6A	6A	81.29	-14.26	-2.99	-3.87	-1.94	8.87	-7.02	-18.88	0.0014	2.09
70	Q.Ears.p^{-1}(+p)_6A.1	6A	92.56	-0.81	-1.09	-1.26	-0.74	-0.38	0.14	-0.89	6E-11	9.83
71	Q.Grains.E^{-1}.(+p)_6A	6A	103.35	-17.54	-85.40	-12.54	16.02	0.30	32.16	-19.87	2E-06	7.88
72	Q.Ears.p^{-1}(-p)_6A	6A	215.25	0.31	-0.19	-0.48	0.73	-1.16	0.41	-0.04	3E-07	3.56
73	Q.Grains.E^{-1}.(-p)_6A	6A	223.42	31.30	2.51	-23.90	56.46	-23.63	41.95	-8.14	7E-10	6.02
74	Q.Ears.p^{-1}(+p)_6A.2	6A	223.42	-0.36	-0.46	-0.12	0.44	0.01	-0.36	-0.24	0.0002	5.29
75	Q.TGW(+p)_6B	6B	15.73	3.12	-2.90	-1.70	-0.86	-3.44	-1.19	-0.68	0.0019	3.04
76	Q.Grains.E^{-1}.(-p)_6B	6B	69.86	23.33	21.83	-7.53	-55.61	76.79	10.37	27.24	0.0054	7.73
77	Q.GY.(-p)_6B	6B	76.72	2.24	0.84	-0.87	-3.28	3.48	1.80	0.49	2E-08	5.62
78	Q.TGW(-p)_6B	6B	185.60	-0.90	1.77	3.29	-3.15	-2.28	1.26	1.31	1E-08	5.02
79	Q.Ears.p^{-1}(+p)_6B	6B	201.81	0.59	0.38	0.03	-0.48	0.16	-0.72	0.21	0.0003	2.35
80	Q.Ears.p^{-1}(+p)_6D	6D	192.69	7.48	-0.06	NA	-4.54	NA	-0.37	1.02	1E-10	3.74
81	Q.FT(-p)_7A	7A	65.48	19.76	-13.31	-31.47	47.07	33.36	9.88	4.12	0.0002	4.25
82	Q.TGW(+p)_7A	7A	100.88	-2.42	3.66	NA	0.04	-1.24	1.79	2.08	4E-09	1.49
83	Q.Grains.E^{-1}.(-p)_7A.1	7A	105.98	74.08	7.23	NA	-16.11	-24.49	25.57	51.66	7E-13	6.71
84	Q.Ears.p^{-1}(-p)_7A	7A	110.06	-0.64	-0.20	0.47	-0.83	-0.10	0.46	-0.12	9E-05	9.37
85	Q.FT(+p)_7A.1	7A	161.15	11.13	-30.43	-57.69	-2.20	-17.87	-24.35	-24.89	0.001	1.13
86	Q.Grains.E^{-1}.(-p)_7A.2	7A	202.68	322.33	170.50	NA	108.35	190.04	144.51	140.36	1E-07	-1.77
87	Q.FT(+p)_7A.2	7A	216.28	-68.92	-60.03	-22.36	-78.77	-44.10	-55.98	-76.73	0.0091	2.61
88	Q.TGW(-p)_7A	7A	234.92	3.40	2.09	1.65	-0.32	1.09	3.75	2.06	9E-08	1.27
89	Q.Ears.p^{-1}(+p)_7A	7A	296.59	-1.13	-0.84	-0.66	-1.52	-1.31	-1.04	-0.45	0.0015	7.57
90	Q.FT(+p)_7A.3	7A	304.72	5.48	1.36	0.04	45.57	2.32	5.53	-10.83	5E-06	3.27
91	Q.Ears.p^{-1}(+p)_7A	7A	361.69	0.77	0.65	1.26	1.17	1.09	0.87	1.15	5E-09	8.23
92	Q.Grains.E^{-1}.(+p)_7A	7A	365.23	8.39	25.63	47.54	16.11	35.62	34.45	29.52	0.0022	3.72
93	Q.P.ht(+p)_7B	7B	168.07	1.96	-3.13	1.31	0.57	-0.49	1.28	5.46	0.003	-1.33
94	Q.GY.(+p)_7B	7B	187.30	-0.64	0.93	1.34	-1.14	-2.34	0.28	0.68	3E-06	12.32

5 Chapter 5: Discussion

5.1 Thesis Overview

Wheat yield stability under drought as a complex trait is the result of various morphological and physiological responses, including water use efficiency and stomatal conductance (Kimurto *et al*., 2009), canopy temperature depression (Thapa *et al*., 2018), increased root length density (Ehdaie *et al*., 2012), senescence rate (Foulkes *et al*., 2007, Lopes and Reynolds, 2012), thousand grain weight, grains/ear and tillers/plant (Afzal *et al*., 2017), presence of long awns (Taheri *et al*., 2013) and NDVI measurements (Tattaris *et al*., 2016). The relative importance of these traits depends on timing, severity of drought, accompanying environmental factors and the extent of GxE interaction (Mir *et al*., 2012). In addition to the genetically determined morpho-physiological responses to drought, symbiotic interaction between wheat and endophytic fungus *Piriformospora indica* represents extrinsic potential factor to enhance growth and development and yield.

This PhD thesis aimed to conduct an in-depth study of the genetic architecture of wheat responses to drought, deciphering the genetic basis of both source and sink traits under field conditions, as well as investigating the ability of *P. indica* to increase yield in both well-watered and drought conditions and identifying QTL underpinning drought-resistance traits influenced by endophytic growth. To accomplish this, a representative subset of the winter wheat elite 8-founder MAGIC population was tested under contrasting water availability regimes under field conditions and adding the *P. indica* in controlled environment trials. A representative subset of the population was phenotyped throughout the growing season for crop canopy indices, yield and yield components.

5.2 The three main hypotheses examined in this study were:

1. The ability of a given wheat genotype to withstand limited periods of drought results from multiple interacting quantitative traits expressed throughout the life cycle including phenology, canopy development and architecture, and regulation of photosynthesis, evapotranspiration and canopy temperature in response to fluctuating environmental conditions.

2. There are significant heritable differences in the phenological and developmental traits between MAGIC genotypes which cause heritable differences in the final yield under contrasting water availability regimes.

3. The extent to which *P. indica* may buffer a particular wheat genotype against drought stress is conditioned by a specific set of *P. indica* response QTL, understanding of which would contribute to a better mechanistic understanding of mutualistic symbiosis.

5.3 Level of drought in the field

The research conducted in Chapter 2 showed spring 2017 to be marked by historic levels of drought: rainfall over the whole spring was less than half the historic 30-year UK average, and was especially acute during April where less than 20% of annual average and 13.6% of the onsite weather station 60-year average. As a consequence, in the rainfed block, a prolonged large SMD >100 mm lasted from 13th June to 18th July with maximum SMD peaking at 120 mm and in contrast, SMD dropped rapidly in the irrigated block compared to the rainfed by 30 mm within ten days post-irrigation and the difference between the two reached a difference of 93 mm within two months of commencement of supplementary irrigation, and soil moisture content in the irrigated blocks were shown to be twice that in the rainfed blocks in all depths. The suppressed canopy coverage reduced above ground biomass and significant loss of yield in the rainfed blocks agrees with previous field experiments under UK conditions, where SMD exceeding 75 mm was reported (Foulkes *et al*., 2001, Foulkes *et al*., 2002, Foulkes *et al*., 2007). However, these researchers reported a significant reduction in the number of grains/ear and TGW, which was not the case in this study, and an advance in flowering time by up to 9 days, while it was advanced by 2 days only under rainfed treatment in this study. The stress during late grain filling in this experiment was slightly mitigated by rainfall so it did not exceed 120 mm and during tillering stage in April, SMD ranged between 70-85 mm, significantly restricting the tillering capacity, while SMD exceeded 160 mm in (Foulkes *et al*., 2001, Foulkes *et al*., 2002), during grain filling significantly restricting grain filling and it did not exceed 50 mm during tillering, causing no significant effect on number of tillers; however, Fang *et al*. (2017) found significant reduction in ears/plant in a moderately droughted spring. These differences underline the reality that seasonal differences of timing, severity and duration of the drought can trigger markedly difference crop responses. This may point to an inherent difficulty in breeding for resilience to drought as traits that preserve the ability to tiller well during a period of early drought may require different traits and mechanisms compared to traits which preserve grain number during ear development and grain size during grain filling. On the other hand, it is possible that traits e.g. deep rooting, that might mitigate water limitation at any stage in crop development could have a generic positive impact on the resilience to all manifestations of

drought. Genetic mapping of quantitative responses offers a route to differentiating between stage-specific and all-stage drought resilience as QTL for the latter would be expected to appear consistently in a number of different drought scenarios.

Root system response measurements were not feasible in this experiment, but the average daily temperature in the topsoil layer (at 50 cm depth) was recorded throughout the growing season and showed drought stressed blocks to be warmer by 1-2^{o}C from mid-June to mid-July. Cooler topsoil under stress was reported as being associated with delayed senescence (Wraith and Hanks, 1992) and delayed cumulative soil water depletion (Wraith and Hanks, 1994) in wheat and maize, warmer soils reduced root length and were associated with 10% yield loss (Dong *et al*., 2016). By quantifying SMD and tracing its effects on various traits responses of highly replicated check variety 'Kielder', and by demonstrating that the differential treatment had been relatively evenly applied across the large spatial scale of the experiment, we provide the justification and the means to dissect the genetic basis of wheat MAGIC population responses to a moderate/severe spring drought in the unsheltered field.

5.4 Genetic responses to drought

The limited water availability had a significant negative effect on all measured traits causing an average yield loss of 3.74 t/ha in the rainfed blocks. As expected from a population whose founders were chosen to encompass wide variation in most traits (Mackay *et al*., 2014), transgressive segregation was detected as the phenotypic range of the progeny under both treatments greatly exceeded the range of the parental lines and there were significant differences among genotypes for all measured traits. Critically for the aims of this thesis, highly significant interactions between drought treatment and genotypes were found for key traits, such as GY, FT, P.ht, CTD and late season spectral indices NDVI.(1976dd) and NDVI.(2553dd). This result indicates a strong genetic basis for the detected responses, which is confirmed by moderate to high heritability of 0.45 and 0.87 for these traits, except for the NDVI measurements marked with low heritability of 0.34-0.36 which might be explained by inevitable noise in this environmental sensitive spectral index, GY had moderate heritability of 0.53, which is typical for such a polygenic trait, highly influenced by environment, especially factoring in the significant environmental variance due to drought stress treatment (Huang *et al*., 2006, Cuthbert *et al*., 2008, Tyagi *et al*., 2015, Gahlaut *et al*., 2017, Elfeki *et al*., 2018).

Previous studies investigating the association between green area peak and accumulation as well as NDVI measurements at different time points and final grain yield, reported significant positive correlations with yield ranging between 0.42 and 0.83 (Brian *et al*., 2004, Pennacchi *et al*., 2018, Guan *et al*., 2019). As treatments differed significantly in water availability, crop indices differed in their significance in the stepwise prediction model of yield, tagging mid-grain filling as the key canopy index in irrigated treatment represented by NDVI.1976, which coincides with the key stage reported by Brian *et al*. (2004), Pennacchi *et al*. (2018) and Guan *et al*. (2019) as highly correlated to grain yield. For the rainfed treatment, green canopy cover (GAI.1388) at tillering stage appeared as the key canopy index, which can be associated to a big reduction in Grains.m^{-2} and confirmed by a significant reduction in ears/unit area detected in highly replicated 'Kielder' plots. In this experiment, we believe that the observed reduction in Grains.m^{-2} was mainly a product of reduction in ears/unit area (restricted tillering), since the decrease in grains.E^{-1} was minimal.

Grains.m^{-2} appeared as the strongest source trait correlated to yield (r=0.72) and (r=0.68) in rainfed and irrigated treatments, respectively. However, it showed a strong source-sink trade-off by having a significantly negative correlation with TGW (r=-0.44) and (r=-0.46) in rainfed and irrigated treatments, respectively. Seemingly, the increase in the number of ear-bearing tillers was accompanied by a decrease in assimilates available for individual grains. This trade-off between Grains.m^{-2} and TGW, matches the same trend detected by Jackson (2019) in two consecutive field trials using the same elite MAGIC population used in this study.

CTD significantly differed between treatments by an average of 4.9^{o}C, CT negative correlation with grain yield under drought stress was reported by Thapa *et al*. (2018) and Crain *et al*. (2016) and associated with an average increase in yield of 13% (Li *et al*., 2019). Lopes and Reynolds (2010) reported a significant correlation between cooler canopy temperature and root mass and deep-water extraction ability. CTD estimated for the MAGIC lines under both treatments, agreed with the literature, as it showed strong significant correlation (r=0.52) and (r=0.61) for rainfed and irrigation treatments, respectively.

5.5 QTL analysis

In Chapter 3, a total of 309 significant QTL were detected for the traits under both treatments, explaining individually 0.92 to 15.18% of the phenotypic variation, 53 QTL locations were associated with two or more traits and eight were major QTL detected in both environments for AUC, FT, P.ht and GY.

As GAI was measured over the growing season, QTL analysis was conducted for individual dates as well as cumulative green cover (AUC), indicating significantly associated markers with each phenotype and giving an insight on when these markers signals are peaking or decaying. Among the 110 QTL identified in both water availability regimes, five loci were detected in at least one of single GAI dates and the AUC, in addition to a novel pleiotropic locus (independent of *Rht-B1b* locus) on chromosome 4B harbouring other traits beside GAI and AUC with different size effects explained by the QTL, as follow, Q.GAI.1192_4B explained (4.35%), Q.GAI.1630(R)_4B (5.7%), Q.GAI.1630(I)_4B (4%), Q.AUC(R)_4B (4.12%), Q.AUC(I)_4B (3.83%), Q.GY.(R)_4B (1.7%), Q.NDVI.1976(R)_4B (3.6%), Q.NDVI.2553(R)_4B (1.52%) and Q.P.ht(I)_4B.2 (5%).

Major QTL for plant height tagging *Rht-D1b* and *Rht-B1b* loci on chromosomes 4D and 4B, respectively and flowering time QTL tagging known locus of photoperiod sensitivity *Ppd-D*1 on chromosome 2D were identified with big size effect of 11.23%-15.18% individually. Moreover, major loci for other traits were found to match those identified in previous research, such as the grains.E^{-1} two big size effect loci Q.Grains.E^{-1}.(R)_2A (11%) and Q.Grains.E^{-1}.(R)_4A (7.8%) fall close to the same trait loci identified in (Echeverry-Solarte, 2015) explaining 12.3% and 12.5% on chromosomes 2A and 4A, respectively.

Of 17 QTL detected for GY, only one of them was treatment-independent, explaining 3.4% and 2.14 % of the phenotypic variation in rainfed and irrigated treatments, respectively. This falls within the expectation of such a complex trait, highly influenced by environment and might propose rainfed specific QTL as candidates to describe yield underpinnings under drought stress. This was also reflected in the low GY heritability of 0.53 which matches typical value of GY heritability in the literature (Huang *et al*., 2006, Cuthbert *et al*., 2008, Elfeki *et al*., 2018) and was higher than estimated in the same population in other trials, where Jackson (2019) found GY heritability of 0.26 combined over 2 years. The low heritability might explain the limited number of GY QTL to co-locate with those found for other traits, where two loci on chromosomes 3A and 6B detected as follow, Q.GY.(I)_3A (3.7%) co-located Q.Grains.m^{-2}.(I)_3A.2 (6.4%) and Q.GY.(I)_6B (2%) coincided with both Q.CTD.(I)_6B (1.3%) and Q.GAI.1994.(I)_6B.2 (4.7%), in addition to the Q.GY.(I)_4D (3.42%) falling within the pleiotropic region tagging *Rht-D*1.

In GY as a complex trait, epistatic SNP pairs are expected to explain significant variation beside the main effect QTL as found by Assanga *et al*. (2017), Jiang *et al*. (2017) and Sehgal

et al. (2017). In this study, after stringently selecting the most significant interactions, we identified six two-way interactions, two rainfed-specific and four irrigated-specific, highlighting either positive or negative effect allele combinations causing average decreases/increases of 1.5-2 t/ha. As might be expected, we found cases of co-location between one or both of the interacting SNPs pair with QTL previously identified for other traits, mainly those of crop canopy indices. Moreover, one of the two interacting SNPs for rainfed yield (wsnp_CAP12_c1503_764765) on 2D is tagging the *Ppd-D*1 photoperiod sensitivity locus that is the same case found by Sehgal *et al*. (2017). More investigation is needed for the epistatic SNPs associated with phenotypes measured in this experiment, which could provide a better in depth understanding of shared genetic basis of grain yield and its source and sink traits components.

5.6 MAGIC responses to *Piriformospora indica*

Improving wheat performance and increasing yield is always slowed down by the polygenic nature of yield and significance of environmental parameters, giving rise to the possibility of utilizing the promising potential of endophyte inoculation to improve plant performance and breed for better response to inoculation (Fester and Sawers, 2011, Galván *et al*., 2011).

Piriformospora indica is one of the most promising potential symbiotic fungi. It belongs to the order Sebacinales, an order widely involved in mutualistic symbiosis, increases host plant resistance to biotic and abiotic stresses, with availability in soils of almost all habitats (Weiß *et al*., 2011). The interaction of *P.indica* with other microorganisms of endo/epiphytic populations, regarding diversity and density reveal paradoxical reports according to plant part, where significant increase found in fungal and bacterial diversity in the soil and root microflora of *P.indica* inoculated wheat (Rabie, 2015) or no effect in aerial parts microorganisms of barley, probably as it does not colonise plant shoots (Gravouil, 2012).

The wide host range of *P.indica* and its beneficial impact on nutrients mediation, inducing biotic and abiotic stress resistance and yield increase is detailed in Chapter 4. However, *P.indica* is native to hot dry environment dominating its native habitat of Thar desert in India, Rabiey *et al*. (2017) reported the ability of *P.indica* to successfully colonize spring and winter wheat and survive the UK winter and summer conditions and Varma *et al*. (2014) found it to promote seed germination in temperatures as low as 4^{o}C, indicating the climatic resilience of such species.

In the experiment conducted in Chapter 4, drought negatively affected yield and growth and development of the MAGIC lines, with significantly varying degrees among genotypes, confirming the wide range of responses in MAGIC that was found in the field trial (Chapter 3). Drought reducing ears.p^{-1}, TGW and grains.E^{-1} by 31.5%, 13.9% and 57.3%, respectively, leading to a loss of 60.5% of yield. Drought imposed throughout flowering and grain filling is reported to significantly reduce numbers of grains/ear as consequence of reduced fertility (Onyemaobi *et al*. 2016). However, others monitoring drought in pot experiments found this reduction in grains number associated with increased TGW such as Lehnert *et al*. (2018); we found drought to decrease them both.

The main factor of yield increase as a response to *P.indica* inoculation was response of grains.E^{-1} as it showed strong significant correlation (r=0.94) with GY response, confirming the significance of this trait under drought, followed by response of ears.p-1 (r=0.76), while no significant correlation was detected between yield response and other traits response to *P.indica*, suggesting the endophyte's potential to mitigate negative effects of drought relies mainly on sustaining tillering and potential fertility, but to a much lesser extent on assimilate translocation to the fertilized grains. *P.indica* generally increased GY, however, the effect size was bigger under well-watered conditions, in contrast to findings of stronger effect under stresses reported for mycorrhiza (Lehnert *et al*. 2018) and *P.indica* (Hubbard *et al*., 2014, Vahabi *et al*., 2016).

The significant interaction between genotypes and *P.indica* either as a main effect or in the three way interaction with drought treatment, indicates heritable genetic variation controlling individual genotype responses to *P.indica* inoculation. Since successful colonization was confirmed via microscopic inspection of sampled roots, the varying degrees in response might be due to differences in colonization rate, which was also observed in barley where cultivars varied in their colonization rate of *P.indica* (Gravouil, 2012); more future experiments are needed to evaluate colonization rates in MAGIC RILs.

In previous research, *P.indica* was found to increase root mass (Ghabooli and mondai, 2016), increase leaf water potential (Hussin *et al*., 2017), enhance the electron transfer chain and photosystem activity (Ghaffari *et al*., 2019) and increase Fv/Fm (Hubbard *et al*., 2014). In this thesis, *P.indica* successfully colonised winter wheat MAGIC lines and increased yield and growth parameters under both water availability regimes, but the detailed physiological and biochemical basis of these responses need to be addressed in future experiments.

The QTL analysis conducted to dissect candidate QTL associated with wheat response to *P.indica* colonisation under drought, revealed significant impact on major known QTL, such as totally masking the effect of reduced height locus *Rht-B*1 on chromosome 4B and dropping the effect size of *Rht-D*1 on 4D from 11.43% to 4% upon colonisation. This alteration in effect sizes of genes associated with DELLA protein and gibberellic acid pathway coincides with previous reports of reduction in expression of some members of the DELLA gene family, such as RGA1 in Arabidopsis colonised plants (Pan *et al*., 2017) and upregulation of GA in maize and tomato (Liu *et al*., 2019).

An interesting comparison of QTL specific to presence/absence in *P.indica* with results of QTL analysis in field trials of contrasting soil water availability showed cases where the same loci were found in dry field conditions and fungus-free drought pots, while others detected in well irrigated pots and above average rainfall fields. For example Q.TGW(+p)_2D was found co-locating with a grain weight QTL in 2016 rainy year and missed in 2015 dry year and Q.FT(+p)_5A coincided with the locus associated with FT in 2016 rainy year (Jackson, 2019). Also Q.GY.(-p)_3B which is missed in the inoculated plants was co-locating with a previously detected QTL for number of grains/m^2 in drought stressed field in 2017 (chapter 3). These findings indicate that the mitigating effect of *P.indica* might be equivalent to increased water availability, which could be validated by more experiments using the same MAGIC population either in the field or in controlled environments.

5.7 Limitations of the study

Limitations varied according to the nature of each experiment. In 2017 field trial it was necessary to subset from the >1000 RILs of the MAGIC population, even after selecting representative 384 RILs, the population founders and a check variety under two water availability regimes in two replicate; this resulted in 1600 field plots which were challenging to phenotype regarding man-power and the time needed, especially the proximal sensing ones.

Piriformospora indica is non-native to the UK, implying the experiment to be confined in glass houses and polytunnels, missing the natural field conditions and the ability to use the 'phenocart' platform and drone-based measurements to phenotype around 1000 pots, this might be tackled by looking for *P.indica* closely related species from the widely spread Sebanicales and investigate it for the potential of improving wheat performance, in addition to confounding heat effect in glass house during above average hot days. Another difficulty in interpreting the genetic analysis of wheat X *P.indica* interaction, is the lack of studies

looking at QTL underpinning wheat responses to *P.indica*, as most published literature reported only the impact of *P.indica* on wheat yield and morpho-physiological responses in few number of cultivars in each study.

A time limitation of the PhD span was a main reason of having experiments not repeated over years. However, the year-replication bottleneck was compensated by the available literature reporting genetic study on the same MAGIC population (using 800 RILs) over two consecutive years of the 2015 and 2016 growing seasons (Jackson, 2019).

5.8 Future work

This PhD project found the MAGIC population to encompass transgressive segregations in the tested RILs with potential yield sustainability under drought. These lines could be used as a breeding material for drought resistant cultivars under mild drought episodes expected in the UK. The high correlation between yield and UAV measurements, indicates these traits potential to be applied in yield predictive models; however, the variation among key indices under different water availability regimes implies the need to carry out more field trials with different scenarios of drought timing and severity. There is an opportunity that these indices, especially canopy temperature depression under drought are associated with root system traits and architecture, stomatal density and stomatal conductance which need to be investigated in future trials.

The genotype X *P.indica* responses and the QTL identified in association with these responses, give an insight into the need of validating these QTL by replicating them over years and include canopy indices, root traits, physiological parameters and biochemical responses and test their association to underpinning QTL and their response to *P.indica* inoculation under drought.

5.9 Concluding remarks

This study adds evidence for the potential of sporadic drought episodes in the UK to significantly reduce wheat grain yield, highlights the availability of MAGIC genotypes with the ability to sustain yield under drought, identifies key traits for predicting yield under given water availability regimes, shows the potential of *Piriformospora indica* in enhancing yield in both well-watered and drought conditions and identifies QTL underpinning wheat responses to drought and those associated with wheat responses to *P.indica* colonisation. The results presented in this study have the potential to be used in wheat breeding programs for drought tolerance and wheat symbiosis with endophyte fungi.

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