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Wheat Ear Sterility Project (WESP)

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This is the final report of a 42-month project (RD-2007-3438) which started in September 2009. The total cost of the work was £350,953, which was funded by Defra and BBSRC (Control of infertility in wheat by phenotype screening and genetic analysis of varieties and breeding lines, SA LINK LK09116) and including a contract for £70,832 from AHDB Cereals & Oilseeds.

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1. ABSTRACT

The overall aim of this project was to establish methods for phenotypic screening and crop data collection to support QTL mapping of variation in wheat ear sterility. This is the precursor to understanding the genetics underlying vulnerability to sterility in UK wheat, with a view to identifying DNA markers for its elimination from breeding programmes. Our approach was to undertake population phenotyping coupled with genotyping and candidate plant trait identification and validation. The key drivers to the work were threefold:

- To protect UK wheat crops against potentially devastating crop losses
- To establish the occurrence of major and subclinical effects of sterility on yield potential
- To support a strategy to improving breeding efficiency by elimination of vulnerable material in early generation selection and enable breeders to have more confidence in the deployment of diverse breeding materials

Partnering plant breeding companies supplemented an existing doubled-haploid (DH) population, Avalon x Cadenza, with four bi-parental DH populations coded as 9M, FA, LQ and TR, with approximately 100 lines in each. Populations were grown at an AHDB/BSPB RL/NL wheat site in East Lothian, Scotland. A weather station was located adjacent to the wheat plots. Previous reports of moderate to high levels of ear sterility in winter wheat variety trials at this site had been reported to to AHDB. Populations 9M, LQ and TR were grown in 2008-09, whilst all five populations were grown in 2009-10, 2010-11 and 2011-12. Over the four harvest years, a total of thirteen population x year combinations were assessed for ear sterility in the field and laboratory.

Quantitative assessments for assessing ear sterility were developed for in-field assessment of the condition, whilst lab assessments, on ears sampled prior to harvest, were based on scoring of all florets along one side of each ear (detailed assessment) or outer florets only (rapid assessment). Evidence for particular sensitivity of outer floret sterility in vulnerable varieties has been established, with differentiation between sub-clinical and yield limiting levels of sterility. For genotyping and map construction, the Avalon x Cadenza cross is a UK reference population with a map publically available. New genotyping was done initially using diversity arrays technology (DArT) on population M9. Subsequently, moderate to good segregation for sterility across populations (M9, FA, LQ and TR) allowed us to take advantage of new SNP genotyping technology using the KASP marker system.

Following genotyping and linkage mapping of all populations, QTL analysis has indicated flowering time and growth stage QTL on several chromosomes. These previously reported QTL serve as good controls for dataset integrity. More importantly, these analyses have indicated an accumulation of several weak ear sterility related QTL on specific chromosomes. None of these QTL were common across the five populations, though there was evidence for a cluster of QTL

on chromosome 7A (with three QTL). This provides evidence for a difference in the genetic control of ear sterility between varieties and populations. Environmental interactions with the level of phenotypic expression were complex. Monitoring of several weather variables indicated that reduced radiation level and air temperature increased crop susceptibility to sterility during the booting growth stage (GS41-45).

2. EXECUTIVE SUMMARY

2.1. Phenotypic screening

Phenotypic data for all populations from harvest years 2009, 2010, 2011 and 2012 was cross-checked for segregation in seed set in the two parents and across the lines, with evidence for extreme low, moderate and high levels of sterility, expressed as the percentage of florets that failed to set seed.

From analysis of percentage sterility scored in all florets within an ear (AFS), we concluded that assessment of sterility in outer florets (OFS) would be a good standard measure of the phenotype. Our results provide evidence for differential sensitivity in seed set in outer florets, with OFS and AFS being highly correlated. Sterility phenotypes ranged from low or sub-clinical levels to high (yield limiting) levels, with above 40% sterility in outer florets.

Field assessments of sterility confirmed the presence of several vulnerable (high sterility) and resistant (low sterility) lines in each population. Although there was significant season variation in OFS and AFS (e.g. variation in population mean sterility), segregation in sterility among lines, as evident from field scores and lab assessments, justified the genotyping of all five populations. The most significant findings from our phenotyping based on OFS are outlined below.

Population 9M

There was consistently higher OFS in parent 9 compared to parent M, with some lines expressing 25% sterility in 2009 and 2010, and 35% in 2011 and 2012.

Population FA

In 2011, parent F had significantly higher OFS than parent A, but parental difference was not significant in 2012. The FA population range in OFS was to 1-48% in 2011 and 3-18% in 2012.

Population Avalon x Cadenza

Cadenza had significantly higher OFS than Avalon in 2011, but not in 2012. Individual lines indicated sterility of more than 50% in both years.

Population LQ

Parent L had significantly higher OFS than parent Q 2011, but there was difference between parents in 2012. OFS ranged from 2% to 37% in 2011 and from 2% to 17% in 2012.

Population TR

Parent T was consistently weaker for sterility than parent R, with lines expressing OFS up to 15% in 2010, and above 50% in 2011 and 2012.

2.2. Genetic and quantitative trait loci (QTL) analysis

A QTL overview across a range of developmental and sterility traits indicated several growth stage or flowering time loci on several chromosomes. These previously reported QTL serve as good controls for data set integrity. Several chromosomes showed an accumulation of several weak sterility related QTL. The most significant QTL explained up to 20% variation in the flowering time and sterility traits. Several QTL were associated with different sterility traits that were scored. Apart from a cluster on 7A (three QTL), there were no common sterility QTL across populations. There was evidence for a common QTL for both a field sterility score and a lab % sterility score, within populations, though not between populations.

Overall, sterility traits were seasonally dependent with evidence for strong genotype by environment interaction. Hence, QTL effects (allele effects - not proportion of variance explained) were relatively low. A summary of genotyping and QTL is presented below.

Population 9M

The linkage map was developed using both DArT and KASP markers and comprised 590 polymorphic markers. 17 lines were removed from the analysis due to evidence of being off types or heterogeneous. The parents of this population were the most consistent in their phenotypic expression across years. Sterility QTL were discovered on chromosomes 1A, 1D, 3A, 6B and 7D. Both parents provided some protection, with the resistance allele being shared.

Population FA

A linkage map was developed using KASP markers after a parental screen had provided 185 polymorphic markers. 7 lines were removed due to being either off types or heterogeneous. There were various sterility QTL on chromosomes 3D and 7A. On 3D parent A had the protectant allele, whereas on 7A it was parent F.

Population Avalon x Cadenza

Being the UK reference population the genetic map for this population was publically available and incorporates SSR, DarT and KASP markers. Various sterility QTL were discovered on chromosomes 5A, 7A, 1B and 2D. Both parents had a protection effect. There was some consistency in QTL between 2011 and 2012. There was some consistency in QTL based on field sterility score across seasons.

Population LQ

There were 222 polymorphic markers, of which 215 were useful for mapping. 7 lines were removed due to evidence of being off types or heterogeneous. Various sterility QTL were found on 1A, 2B, 2D, 4A, 6A and 7A. Both parents had a protection effect.

Population TR

There were 206 polymorphic markers, of which 197 were useful for mapping. 7 lines were removed due to evidence of being off types or heterogeneous. Various sterility QTL were found on chromosomes 2B, 3B, 4B, and 5B. Both parents had protection effect. This population provided good field and lab QTL on 4B and 5B.

2.3. Screening tests

An objective of the study was to produce a reliable field scoring or screening method that can be used by plant breeders, and in the NL and RL variety testing system, as a practical tool. The WESP project team also considered how to improve its laboratory phenotyping procedures by assessing seed set in relation to different spikelet and floret positions.

Initially, poor correlation between field scores on a 1 to 9 scale (where 1 = no sterility and 9 = > 90% sterility) and lab assessments of OFS and AFS was attributed to difficulty in distinguishing between low and moderate levels of sterility in the field. Revising the field scoring procedure to differentiate at the lower end of the scale (for harvests 2011 and 2012) improved the correlation between field and lab scores.

3. INTRODUCTION

Flowering in wheat is the process that will ultimately deliver the grain harvested for human, animal and industrial use. Yield is a product of the number of flowers and the efficiency with which those flowers are fertilized and set seed. The UK regularly achieves high yields but it is known that some varieties have a genetic susceptibility to sterility that is manifest under certain environmental conditions. An example of this problem occurred in the winter wheat variety 'Moulin' in the mid 1980's when very low levels of seed set caused losses to growers of up to 90% in extreme cases (Law 1999).

Wheat sterility remains a serious threat and plant breeders in the UK are aware of the occurrence of high levels of sterility in some winter wheat trials. Sterility occurs when susceptible varieties are exposed to climatic stress resulting in the failure of some florets to set seed. The problem in 'Moulin' was attributed to a failure to produce viable pollen but its genetic basis, and the genetic basis of sterility in other cases, remains unknown and unpredictable.

Plant breeders in the UK are aware of the occurrence of high levels of sterility in some winter wheat trials. This condition occurs when susceptible varieties are exposed to various climatic stress resulting in the failure of some florets to set seed. Such is the threat to the development of new wheat varieties, the breeding companies Limagrain UK Ltd, RAGT Seeds Ltd and KWS UK Ltd, considered this issue a priority for collaborative research. The breeding companies and SRUC had identified breeding lines and varieties with known vulnerability to sterility. Analysis of pedigrees from previous work (Hoad et al. 1999) does not suggest that vulnerable varieties have a common origin. Therefore, it is unclear whether sterility has a single underlying genetic cause or several independent causes. Resolving this is the primary goal of our phenotypic and genetic analysis because it is crucial to devising a strategy that will address the problem.

Ear sterility has economic impact across the grain chain, as well as implications for food security. The economic impact of undetected levels of sterility is likely to be very high with consequential financial losses for growers and the UK cereal trade. A 1% loss in fertility causes an equivalent loss of grain yield (Hoad et al. 1999) and each 1% loss in wheat production equates to £18m (i.e. 15mt production at a grain price of £120 per tonne).

Climate change is likely to exacerbate this problem as a consequence of increasing variation in temperature (and temperature extremes) during critical stages of floret development. This is particularly pertinent as some high risk varieties may be arising as breeders increase genetic diversity in response to sustainable agricultural requirements, whilst testing regimes are constrained by resources to reduce the number of trials in high risk situations.

National List (NL) and Recommended List (RL) trials carried out by SRUC over the past 10 years have linked sterility to poor yields in several varieties. For example, in 1997, poor seed set resulted in a 70% yield loss in some NL varieties (Hoad et al. 1999 and in 2007 sterility was linked to low yields in several varieties in RL trials. An analysis of trials at Kelso and Aberdeen funded by AHDB (HGCA, Project Report 193) indicated that grain yield declined by 75-130 kg ha^{-1} for each % loss in ear fertility; the terminology used in earlier work (Fig. 1). Our earlier work indicated that typical levels of seed set in an unstressed crop were likely to range between 75-95%, depending on genotype and site. However, stress, including low temperatures during floret development, resulting in a fall in fertility much below 70% caused a significant loss in yield. Fig. 1 shows that in a selection on NL1 varieties yield was significantly depressed when the percentage fertility was less than 62%. The most vulnerable varieties yielded below 2.5 t ha^{-1}.

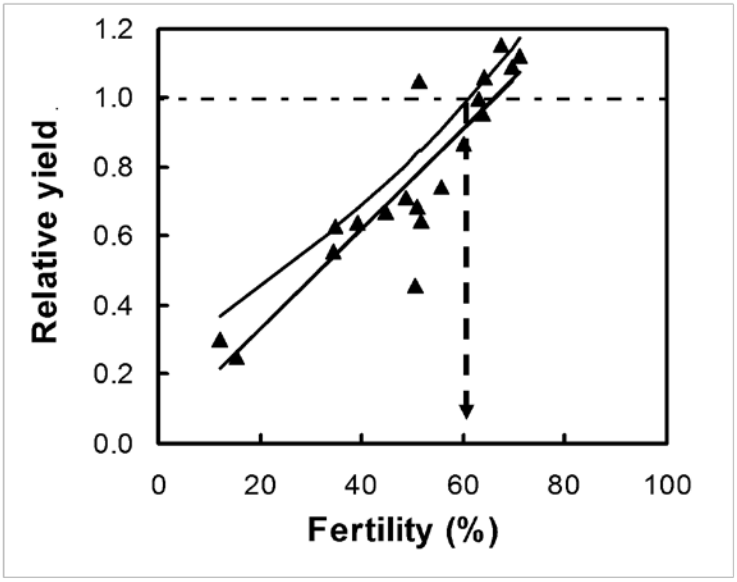


Figure 1 Relationship between ear fertility (%) and relative yield across selected varieties in NL1, 1997/98 (% yield is expressed as a proportion of % UK yield. The point at which the CI intercepts yield = 1.0 indicates the fertility below which the predicted yield for varieties falls below the expected yield, as shown by the arrow.

We also recorded high levels of sterility during field assessment of varieties in NL and RL trials in East Lothian, Scotland, 2004/5 to 2006/07 (Tables 1 and 2). An analysis from two trials in 2007 (Aberdeen and East Lothian) confirmed the link between sterility and reduced yields in RL varieties in 2006/07 (Fig. 2). Results from RL trials in 2007 gave rise to one particularly high risk variety which was just two years from commercialisation. These results illustrate two main threats to wheat yield. Firstly, a variety with a major weakness may slip through the current trialling system and give a major yield failure if adverse conditions are encountered. In any year, the UK wheat area can be dominated by a just few varieties which means there would be a serious problem should the fertility of a single variety failed. Secondly, low levels of reduced seed set may be present that are suppressing wheat yields. Eliminating varieties with inherent genetic weakness to poor seed set i.e. those with alleles linked with ear sterility will therefore, enhance yield and protect against yield failure.

The control varieties (in Tables 1 and 2; Alchemy, Claire, Einstein, Malacca and Robigus) each had relatively low sterility i.e. 1.6 or less at each site. Although sterility might not explain all variation in yield in these varieties at these sites, we speculate that moderate to high sterility i.e. scores above 2 or 3 increase the risk of a significant loss of yield.

Table 1 Sterility scores for selected varieties in NL trials at Humbie, East Lothian in 2004/05, 2005/06 and 2006/07. Sterility was recorded by visual assessment of plots prior to harvest (from mid-July to early August). Data are variety means of fungicide-treated replicate blocks. C = control variety. Scores are: 1 = none or very low sterility; 2 = low to moderate; 3 = moderate; 4 = moderate to high; 5 = very high. [1]indicates an SRUC variety code name.

	2004/05		**2005/06**		**2006/07**	
	NL1	NL2	NL1	NL2	NL1	NL2
Claire (C)	1.0	1.0	-	-	-	-
Malacca (C)	1.0	1.0	1.0	1.0	1.5	1.0
Riband (C)	1.5	1.5	-	-	-	-
Einstein (C)	-	-	1.0	1.0	1.5	1.5
Robigus (C)	-	-	1.0	1.0	1.0	1.5
Alchemy (C)	-	-	-	-	1.5	1.0
NSL WW69	-	3.0	-	-	-	-
NSL WW70	-	2.6	-	-	-	-
A53 03	-	3.0	-	-	-	-
CPBT W116	-	2.3	-	-	-	-
Socrates	-	3.0	-	-	-	-
NSL WW75	2.5	-	-	2.5	-	-
NSL WW81	3.0	-	-	4.0	-	-
CPBT W123	1.0	-	-	2.0	-	-
CPBT W124	3.3	-	-	2.0	-	-
CPBT W131	2.6	-	-	2.5	-	-
CEB 02091	2.5	-	-	2.5	-	-
LP 413/8/0	3.5	-	-	3.0	-	-
PBI 03/0092	1.0	-	-	2.0	-	-
A63 05	-	-	3.5	-	-	*n/a*
A64 05	-	-	1.7	-	-	2.5
A65 05	-	-	4.0	-	-	5.0
A66 05	-	-	2.0	-	-	2.5
NSL WW89	-	-	1.0	-	-	2.0
PBI 40537	-	-	1.5	-	-	1.5
PBI 40557	-	-	1.0	-	-	1.0
PBI 40558	-	-	1.0	-	-	2.0
PBI 40573	-	-	1.5	-	-	3.0
PBI 40593	-	-	1.5	-	-	3.0
CPBT W135	-	-	2.0	-	-	1.0
CPBT W136	-	-	1.5	-	-	2.0
CPBT W137	-	-	1.5	-	-	2.0
CPBT W140	-	-	1.5	-	-	2.0
SRUC Code A[1]	-	-	1.5	-	-	4.5
NA WW1	-	-	-	-	5.0	-
NA WW2	-	-	-	-	4.5	-
NA WW7	-	-	-	-	2.5	-
PBI 40671	-	-	-	-	2.5	-
PBI 40673	-	-	-	-	2.0	-
PBI 40675	-	-	-	-	1.0	-
CPBT W 144	-	-	-	-	2.0	-
CPBT W 146	-	-	-	-	4.0	-
CPBT W 150	-	-	-	-	1.0	-

Table 2 Sterility scores for selected varieties in RL trials at four sites in 2006/07. Sterility was recorded by visual assessment of plots prior to harvest (mid July to early August). Data are variety means of fungicide-treated replicate blocks. C = control variety. Scores are: 1 = none or very low; 2 = low to moderate; 3 = moderate; 4 = moderate to high; 5 = very high. [1]indicates an SRUC variety code name.

	RL trial location				
	East Lothian	Borders	Fife	Aberdeenshire	Average
SRUC Code B[1]	3.6	2.2	3.4	2.8	3.0
Timber	2.8	2.8	3.0	1.4	2.5
Cordiale	2.4	1.4	2.4	2.2	2.1
Marksman	2.4	1.8	1.6	2.0	2.0
Humber	1.4	2.2	1.6	1.4	1.7
Musketeer	1.2	1.8	2.0	1.2	1.6
Solstice	1.6	1.6	1.2	1.2	1.4
Oakley	1.4	1.6	1.4	1.0	1.4
Robigus (C)	1.6	1.4	1.2	1.2	1.4
Malacca (C)	1.0	1.2	1.0	1.2	1.1
Alchemy (C)	1.0	1.2	1.0	1.0	1.1
Claire (C)	1.0	1	1.0	1.0	1.0

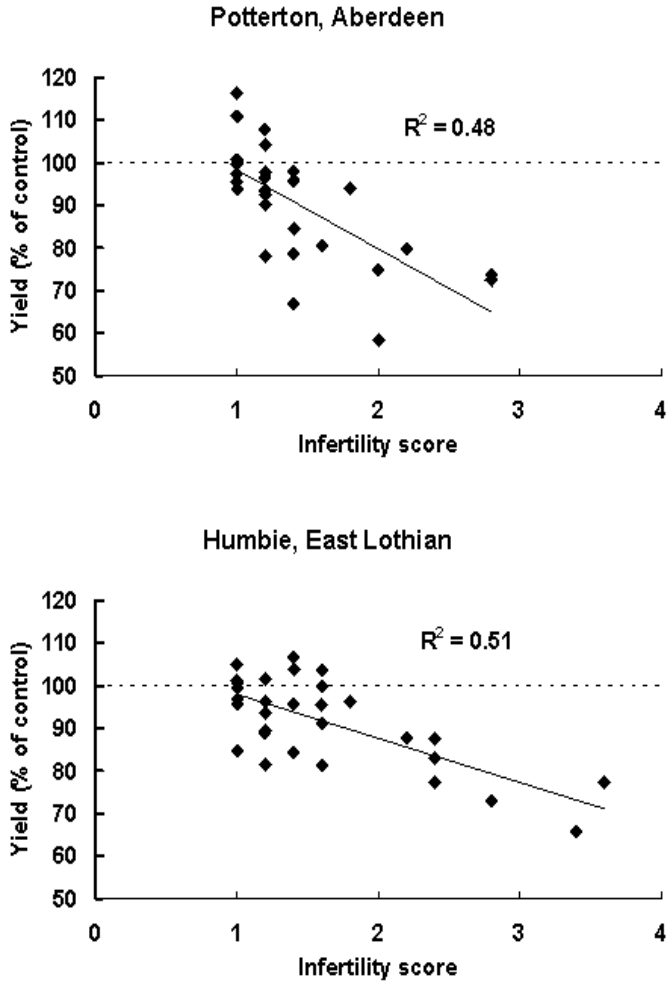


Fig. 2 Ear sterility (described as infertility in these Figures) and grain yield across varieties from two RL trials in 2007. Data are variety means of fungicide-treated replicate blocks from trials at: Potterton near Aberdeen and Humbie in East Lothian. Sterility was recorded by visual assessment of plots prior to harvest (between mid July to early August). Scores: 1 = none or very low; 2 = low to moderate; 3 = moderate; 4 = moderate to high. Note: in some plots scores of 5 (very high) were recorded.

The variety Moulin and other vulnerable genotypes had many desirable qualities. Consequently, they have been used as parents in breeding programmes. This makes it important to identify vulnerable material that exposes breeders and growers to risk. As breeders extend the range of genetic material in their programmes there is an increased risk of variety failure. This is coupled with the prediction of increasing variation in weather patterns and extremes of weather for the UK. These changes in climate are likely to exacerbate the problem of sterility as a consequence of increasing variation in temperature (and temperature extremes) during critical stages of floret development. In

addressing this problem, UK plant breeding will be adapting to, and mitigating against, climate change by optimising and securing production. In addition, fertilizers and agrochemicals are applied before sterility can be recognized and are, therefore, wasted if sterility occurs. Eliminating genetic weakness, or alleles contributing to sterility, from breeders' selection programmes will, therefore, increase the efficiency of input use.

A further significant factor is that high risk varieties for the UK may be arising as breeders use exotic germplasm to increase genetic diversity in response to sustainable agricultural requirements. Risk is increased because testing regimes are constrained by resources to reduce the number of trials in high risk situations. Results from RL trials in 2007 gave rise to one particularly high risk variety which was just two years from commercialisation. Such is the threat of sterility to the development of new wheat varieties that the breeding companies Limagrain UK Ltd, RAGT Seeds Ltd and KWS UK Ltd, who represent 95% of wheat varieties grown in the UK, consider this issue a priority for collaborative LINK research.

To address this problem, we propose to combine a reliable phenotypic screen with genetic analysis in order to identify loci that determine vulnerability to sterility. Assessments from NL and RL trials have identified varieties with wide variation in environmentally induced sterility and these are excellent parents for genetic mapping populations. Phenotyping will be led by SRUC in partnership with Limagrain, RAGT and KWS UK Ltd. JIC will coordinate the genetic mapping and undertake QTL analysis to identify loci conferring sterility. Analysis of a wider collection of breeding lines and varieties will test the association of markers with sterility to assess the reliability of marker predictions of phenotype.

Sterility is linked to climatic stress and crops are potentially vulnerable across a wide developmental phase from terminal spikelet (first node stage) to flowering. However, the most sensitive phase is likely to be between flag leaf and flowering, when stress can adversely affect floral development leading to the production of non-functional ovules or pollen. Irregularity in meiosis leading to asynchrony of development is also possible. We, therefore, also wish to test the idea that sterility is caused by differential development so that key developmental stages are more exposed to climatic stress in vulnerable genotypes. Alternatively, a specific developmental stage may be more susceptible to damage by climatic stress in vulnerable genotypes.

In this project, we are choosing to use SRUC field trials at sites in eastern Scotland known to reliably induce sterility in vulnerable genotypes. There are several reasons for

preferring this approach to the use of controlled environment tests. Firstly, it enables us to analyse sufficient genotypes and populations in a cost efficient way. Secondly, as the underlying cause or causes of sterility are unknown we are not certain of being able to replicate sterility in a controlled environment. Thirdly, industry requires low cost test methods to be developed and this favours the selection of suitable field trial sites. Our approach provides the most cost-effective opportunity to associate phenotypic data with genotype variation.

Previous work on ear sterility in wheat has been linked to a wide range of climatic stress; including low temperature (Qain et al. 1985; Skinnes & Burås 1987; Demotes-Mainard et al. 1995; Subedi et al. 1998), accumulated high temperatures (Ferris et al. 1998), heat and cold stress (Langer & Olugbemi 1970), water stress (Saini & Aspinall 1981) and low radiation (Batch & Morgan 1974; Demotes-Mainard et al. 1996), as well as nutrient (boron) deficiency (Huang et al. 1995; Rawson & Noppakoonwong 1995). It is, therefore, important to establish if UK wheat is exposed to a threat from one or many of these possible sources. Once this is known, a detailed examination of variety pedigrees and the genetic make-up of both susceptible and resistant varieties and breeding lines will provide the basis for a more fundamental research on the molecular controls of floret development and fertility.

The possibility of several inter-related environmentally triggered causes of sterility in susceptible genotypes precludes the use of extensive, and expensive, glass house and controlled environment work at present. However, these approaches will be feasible once the ground work of this project is complete. Our approach is to understand phenotypic variation in relation to seasonal and genetic controls by using bi-parental mapping populations in which parents have been identified as having differential levels of sterility.

Linking climatic records to crop development across a wide range of genotypes this project will help to identify causes of sterility and differential patterns of seed in UK wheats, as a pre-requisite of future, more fundamental, controlled environment work on flowering biology. Our work linked mapping populations provided by plant breeding companies with a proven phenotype screen provided by SRUC and genetic mapping expertise from JIC. Analysing multiple populations and lines, a key component of the work, is feasible because of recently developed genotyping technology. This project brings together a number of complementary technologies. Some of these technologies have been developed within commercial breeding programmes and the opportunity has

been taken to capitalise on the willingness of the commercial parties to provide resources to the project.

The main objective is to better understand the genetics underlying vulnerability to sterility with a view to identifying DNA markers for its elimination from breeding programmes. This will be achieved by undertaking population phenotyping, genotyping and candidate QTL identification and validation.

The central hypothesis is that phenotypic expression of sterility is associated with allelic variation that is capable of being identified across breeding lines and varieties. Another premise is that sterility is associated with differential development that exposes a genotype to climatic stress and/or differential sensitivity to climatic stress between genotypes.

The partner breeding companies provide four doubled haploid (DH) populations each comprising 100 lines. This was supplemented by use of a sub-set of 100 lines from the Avalon x Cadenza mapping population that has been extensively uses by the Wheat Genetic Improvement Network (WGIN) community.

Each population derives from a cross between a genotype with proven high vulnerability to sterility and a genotype with proven low vulnerability populations. The use of these populations will provide: (1) a high level of inherent genetic variability in the condition, (2) a breadth of phenotypic expression of the condition and (3) opportunity to test for clusters of genes associated with sterility and markers linked to the condition.

4. MATERIALS AND METHODS

4.1. Plant material and sites

Five wheat populations based on doubled-haploid production from bi-parental crosses were evaluated for seed set. Population size was 96 to 98 lines, plus the two parents. These were:

(1) a sub-set of 98 lines plus parents from the Avalon x Cadenza cross sources from the John Innes Centres via the WGIN project consortium, and four populations from partner breeders:

(2) 9M, from Limagrain UK Ltd and sourced from John Innes Centre

(3) FA sourced from Limagrain UK Ltd, and

(4) LQ and (5) TR sourced from KWS UK Ltd.

Populations were autumn sown in seasons 2008-09, 2009-10, 2010-11 and 2011-12, referred to as harvest years 2009, 2010, 2011 and 2012, adjacent to AHDB Recommended List and BSPB National List wheat variety trials at SRUC's East Lothian trials centre. Site details are presented in Table 3.

In harvest year 2009, populations 9M, FA and TR were hand sown as tussocks using 50 seeds per tussock sized approximately 0.3 x 0.3 m dimension. For harvest years 2010, 2011 and 2012, all five populations were sown as mini-plots of 1.0 m length x six rows, using 200 seeds per plot to provide a target ear population 500 ears m^{-2}.

Table 3 Site details for wheat sterility trials, harvest years 2009 to 2012.

	2009	2010	2011	2012
Location	Gilchriston	Cauldshiel	Cauldshiel	Gilchriston
Elevation (m)	160	175	165	175
Soil series	Humbie	Humbie	Humbie	Humbie
Soil texture	Loam	Loam	Loam	Loam
Soil pH	6.2	6.2	6.1	6.4
Previous crops	Winter OSR, winter barley, spring barley	Grass, grass, grass	Winter OSR, winter barley, spring barley	Winter OSR, winter barley, spring barley
Sowing date	2nd Oct 2008	5th Oct 2009	5th Oct 2010	30th Sept 2011
Nitrogen fertiliser total (kg/ha)	180	160	160	160
Harvest date	5th Sept	1st Sept	1st Sept	9th Sept

4.2. Field assessment and samples

Crops were assessed for general growth and health, noting any features such as poor establishment, disease or lodging. Key crop growth stages between booting to flowering were scored during June in each year. Field assessments of sterility were made from late milk (GS77) to hard dough (GS87) during July to August.

Development of protocols for field assessment was an objective of this research and will be considered in the results. The procedure for in-field scoring was discussed with AHDB, with a view to developing a 1-9 scale for variety testing protocols.

A few days before harvest, samples of 20 ears per plot were collected for laboratory assessment. After an initial (rapid) visual score of each sample, a detailed assessment based on all florets or outer florets was made as described below.

Assessment of the outer florets would become the standard procedure for recording OFS in all populations and seasons. This was supplemented by assessment of all florets for recording AFS in population 9M in 2009 and 2010 and in population FA in 2011 and 2012.

4.3. Phenotyping ear sterility

Selection of populations to laboratory assess in each year was based on field level of sterility recorded between GS77-87 and need to cover phenotypic data across the five populations.

A sterile floret was defined as one with no grain at maturity, but which contained the remains of floral parts (e.g. carpel and anthers). To reduce the likelihood of counting late developing florets, only those florets with lemmas at least 3 mm long were recorded. The definition of sterility was adapted from Rawson and Bagga (1979) and Rawson (1995). Florets in which grain had formed but was missing were recorded as grain absent 'a' (this occurrence was rare).

A summary of the populations selected for assessment of all or outer florets only is presented in Table 4.

Table 4 Phenotypic data for all florets and outer florets in each harvest year.

	Harvest year			
Population	2009	2010	2011	2012
9M	All	All	Outer	Outer
FA		Outer – in selected lines	All	All
Avalon x Cadenza		Outer – in selected lines	Outer	Outer
LQ			Outer	Outer
TR	Outer – selected lines ony	Outer	Outer	Outer

Assessment of sterility was based on a procedure developed by Limagrain UK Ltd as described in Hoad et al. (1999). The sample of ears to be assessed was laid flat on a bench with a row of spikelets facing upwards. Starting with the spikelet at the base of the ear, the glumes of each floret where opened using a pair of forceps and the floret was recorded as either grain present or a sterile site.

The original method (Hoad et al. 1999) using a specially-designed template to number the position of each grain was numbered and record each sterile sites in red (Fig. 3). This procedure was completed for both sides of the ear. Percentage fertility was expressed as,

$$\frac{s}{s+f} x100$$

where *s* = number of sterile sites
and *f* = number of fertile sites

An example of an assessment of a single ear is shown in Fig 3.

Revised recoding templates were developed for this project; one for all scoring all florets (Fig. 4) and one for outer florets only (Fig. 5) modified for data analysis from an Excel spreadsheet.

The procedure for assessing all florets on one side of an ear (Fig. 4) uses '1' to denote a grain present, '0' is a sterile floret, and 'a' is a absent grain. A blank cell represents either no floret being present or a late/small floret with no potential to set seed.

The procedure for assessing all florets enabled seed set to be estimated for outer, inner and middle or central florets. The analysis for all and outer florets also considered florets in four spikelet positions defined as lower, middle and mid-upper i.e. three spikelets in each, and the tip portions where the ear had ten or more spikelets.

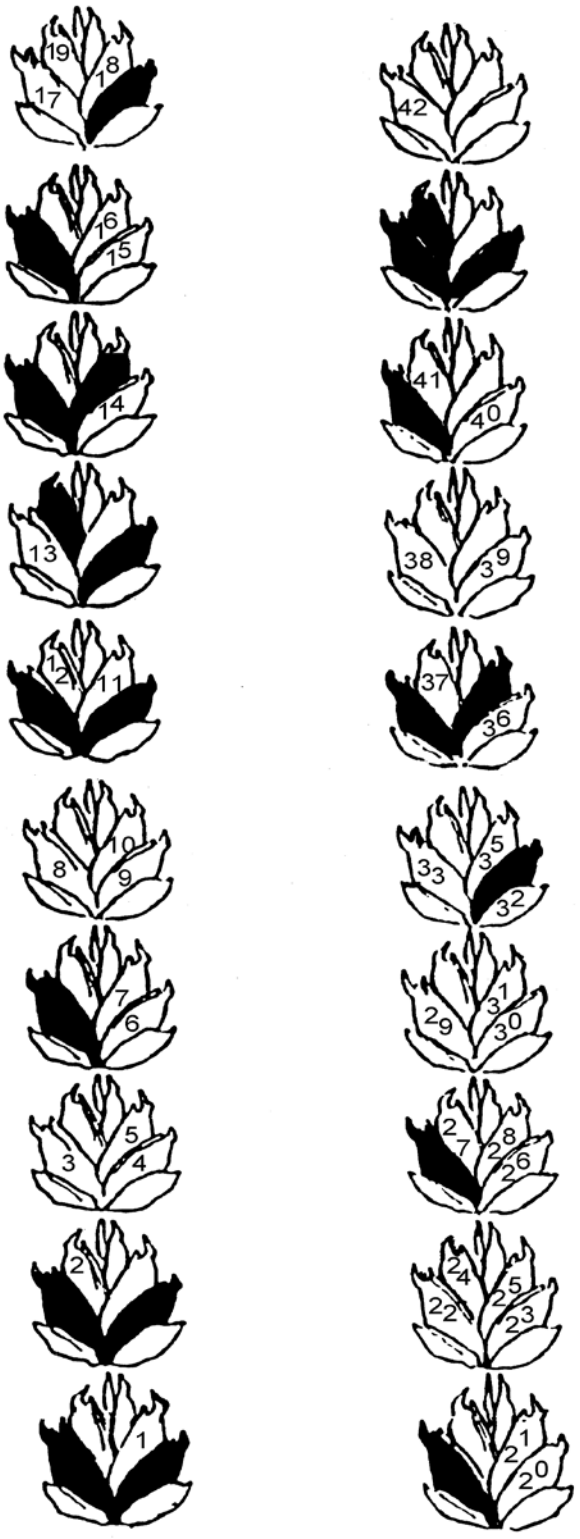


Fig. 3 Assessment of ear sterility. This example shows how each spikelet on both sides of the ear were scored for grain present (numbers) and sterile site (shaded areas). There were 22 grains and 42 sterile sites giving a % sterility of 22/(22+42) x 100 = 34.4%.

	Ear number					
Spikelet	O1	I1	M	I2	O2	Extra
12						
11	1				1	
10	1	0			1	
9	1	0			0	
8	1	1		0	1	
7	1	0		1	1	
6	1	1		1	1	
5	1	1		1	1	
4	1	1	0	1	1	
3	1	1	0	1	1	
2	1	1	0	1	1	
1	1	1		1	1	
Grain	11	7	0	7	10	0
Sterile	0	3	3	1	1	0
Missing	0	0	0	0	0	0
Length	9.8					

Fig. 4 Template for assessing all florets along one side of wheat ears, where O1,O2 are outer florets, I1 and I2 are inner florets, with M being the middle or central florets. In the this example, the ear had 11 spikelets, a total of 35 grains, eight sterile florets and no absent grain.

	Ear Number	
Spikelet	O1	O2
12		
11		
10	1	1
9	1	1
8	1	0
7	1	1
6	1	1
5	0	1
4	1	1
3	1	1
2	1	1
1	1	1
Grain	9	9
Sterile	1	1
Missing	0	0
Length (cm)	10	

Fig. 5 Template for assessing outer florets along one side of wheat ears, where O1 and O2 are the two lines of florets. In the this example, the ear had 10 spikelet, a total of 18 outer floret grains, two sterile florets and no absent grain.

The distribution of sterility within ears was determined by counting the number of sterile sites in the base, lower middle, upper middle and tip regions (quarters) of the ear. These counts were also expressed as proportions of sterile sites for the ear as a whole.

In each year, field assessment of sterility was made at soft dough stage (GS85) and / or hard dough stage (GS85). Developing protocols for field assessment was an objective of the study, and will be considered in the results. The procedure for in-field scoring was discussed with AHDB, with a view to developing a 1-9 scale for variety testing protocols.

Table 5 presents the list of crop and ear measurements from field and laboratory assessment. The various sterility traits were derived from analysis of the replicate ear assessments for each line and parent in each population.

Table 5 Crop growth and ear sterility traits used for phenotyping and QTL mapping.

Trait	Abbreviation and symbols
Crop Height	
Flowering Traits	
Ear Length	
Spikelet Number	
Field sterilty score at GS85	S_FLD_85
Field sterility score at GS87	S_FLD_87
Initial laboratory sterility score	S_LAB
Sterility % All florets	AFS
Sterility % Outer florets	OFS
Sterility % Inner florets	IFS
Sterility % excluding middle florets	exMid S
Sterility % in lower florets	Lower S
Sterility % in middle florets	Middle S
Sterility % in mid-upper florets	Mid-up S
Number of sterile florets - All florets	S All
Number of sterile florets - Outer florets	S Outer
Number of sterile florets - Inner florets	S Inner
Number of sterile florets -Lower florets	S Lower
Number of sterile florets - Middle florets	S Middle
Number of sterile florets - Mid-upper florets	S Mid-up
Number of grains - All florets	G All
Number of grains - Outer florets	G Outer
Number of grains - Inner florets	G Inner
Number of grains - Lower florets	G Lower
Number of grains - Middle florets	G Mid
Number of grains - Mid-upper florets	G Mid-up

4.4. Summary statistics

Histograms for *%S* presented (at 5% intervals) for each population in each year. Summary statistics for each population presented as mean, median, maximum, minimum, variance and CV% for ear and sterility traits. In the first instance, we were interested in: (1) the association between different crop traits or the timing of crop growth stage and sterility and (2) association between different measures of sterility.

4.5. Genotyping amd QTL analysis

Initial genotyping of the population 9M was performed using the rapid and cost-effective Diversity Arrays Technology DArT (Akbari et al. 2006) DArT technology is a chip based approach allowing simultaneous hybridisation of sample DNA to large numbers of immobilised probe sequences, resulting in fast and cost effective genome coverage with low data point costs. This is achieved by reducing sequence complexity through methylation sensitive enzyme digestion, adaptor ligation, PCR amplification with fluorescence labelling and hybridisation to a chip of pre-selected polymorphic probe sequences (chip version 2.5 contains 5,000 probes). This generates a dominant marker score for about 600 loci in a typical UK bi-parent breeding population. Although fast and cost efficient, there is significant marker clustering as well as large gaps, especially on the generally less polymorphic D genome. Thus, initial mapping using DArT will identify regions that require gap filling.

Subsequently, a more recent genotyping method that had been developed for wheat using the KASP system (from LGC Group, formerly KBioscience). This high-throughput genotyping method which is based on polymorphic SNP markers was seen as advantageous in adding additional information to mapping 9M and for genotyping populations FA, LQ and TR. Genotypic data were already available for the Avalon x Cadenza population through the WGIN consortium.

Table 6 indicates the number and type of markers used in the analysis and Table 7 presents the mapping coverage across chromosomes for each population.

Table 6 Summary of markers used in QTL analysis

Population	Mapped Markers	Unlinked Markers	Marker Type	Genetic Distance
9M	567	23	DArT (493), KASPar (98), SSR (5)	1062cM
FA	166	19	KASPar (185)	1066cM
Avalon x Cadenza	4000+	-	KASPar (852) SSR (129), DArT (42)	3576cM
LQ	204	11	KASPar (215)	1249cM
TR	169	28	KASPar (197)	1134cM

The ten parents where included in parental screens carried out by JIC using 3000 SNPs through LGC, supplemented with data from the WAGTAIL project and a further screen carried out at the University of Bristol using a 90k chip. The following sections summarise the marker coverage for each population.

Population 9M

The initial round of DArT genotyping was followed by use of KASP markers. 102 KASPar markers were selected to complement the previously mapped DArT markers, to fill gaps and anchor genetic maps with reference markers. Of the 102 KASPar markers, 98 of these were useful for the mapping. A new map using DArT and KASPar markers comprising 567 markers forming 33 linkage groups and covering 1062cM, with an additional 23 unlinked markers. There was considerable redundancy with the DArT markers, with many markers co-segreragting. Seventeen lines were removed from analysis due to no suitable phenotype of being an off-type or heterogeneous within the population (lines 3, 10, 12, 39, 40, 47, 48, 49, 50, 51, 52, 53, 54, 55, 57, 58, 59).

Population FA

In an initial round of mapping with KASPar markers there were 122 polymorphic markers, of which 98 mapped into 21 linkage groups with 24 unlinked markers. After a parental screen an additional 110 markers were selected to fill gaps. Only 63 of these were polymorphic and useable. This suggested that the parent A used in the original KASPar screen may be different to the one used in the current analysis. A new map comprised 185 polymorphic markers, 185 forming 30 linkage groups covering 1066cM, with 19 unlinked markers. Seven lines (6, 26, 27, 28, 42, 43, 81) were removed from analysis due to off-types and heterogeneity.

Population Avalon x Cadenza

This population had been genotyped previously as part the WGIN project. For the current analysis there were 852 KASPar, 129 SSR and 42 DArT markers.

Population LQ

Of 222 expected polymorphic markers selected to span across genome, 215 were identified as being useful for mapping. The map comprises 215 polymorphic markers, 204 forming 34 linkage groups covering 1249cM, with 11 unlinked markers. Five lines (6209, 6249, 6257, 6276, 6285) were removed from analysis due to off-types and heterogeneity.

Population TR

Of 206 expected polymorphic markers selected to span across genome, 197 were useful for mapping. The map comprised 197 polymorphic markers, 169 forming 35 linkage groups covering 1134cM, with 28 unlinked markers. Seven lines (3952, 4038, 4155, 4481, 4482, 4500, 4501) removed from analysis due to off-types and heterogeneity.

QTL analysis

Single marker analysis was carried out for each trait on 'QTL Cartographer' v2.5. This forms a quick method of scanning the complete genome to highlight possible QTL regions. QTL were detected using the package qtl (vs. 1.35, B ROMAN et al. 2003) for R software. QTL analyses were performed in two steps: putative QTL were identified in an initial single QTL scan and subsequently tested in a final multiple QTL model using a significant threshold calculated from the data distribution. Parents were identified as providing the protectant or resistant allele. Genome wide scans (Figs. 11 to 15) show two methods of computation, one being Haley-Knott (a popular regression method for QTL approximation) and another one built into the QTL software program. Each scan includes a LOD score (on y-axis) which is log10 of the ratio of the probability that a QTL is present rather than absent i.e a LOD score of 3 indicates that the presence of a QTL is 1000 times more probable than its absence. Each scan indicates a threshold LOD score of between 1.9 to 2.3 as an indication of significant QTL.

Table 7. Summary of mapping coverage as cM per chromosome (A, B and D genome for chromosomes 1 to 7) for each population.

Population	1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	4D	5A	5B	5D	6A	6B	6D	7A	7B	7D
9xM	54	55	62	29	112	23	43	104	35	14	56	0	45	93	17	82	56	20	68	16	84
FxA	46	59	61	111	17	8	91	65	65	90	11	0	118	24	10	22	83	0	43	88	21
AxC	109	178	125	233	141	226	183	240	116	156	111	123	183	283	212	166	136	94	219	74	150
LxQ	79	56	21	127	59	39	61	136	19	86	39	2	91	65	36	45	47	39	159	37	0
TxR	85	49	0	101	82	38	33	75	0	39	52	1	141	62	9	58	73	0	130	90	11

5. RESULTS

5.1. Phenotypic variation in grain and sterility traits

Population 9M

Outer florets, years 2008/09 to 2011/12

Across four years, parent 9 had higher level of OFS than parent M at >20% sterility in three out of four years (Fig. 6; Tables 8-11).

Across four seasons, the maximum number of sterile outer florets per ear was 8.4 (in 2008, Table 8), 6.2 (in 2010, Table 9), 10.2 (in 2011, Table 10) and 10.1 (in Table 11). The minimum number of sterile florets ranged from 0.4 (in 2009) to 2.0 (in 2012). In each year, OFS ranged from less than 4% to more than 22%.

Mean ear length was shortest at 8.8 cm in 2012 (Table 11) and longest at 9.7 cm in 2011 (Table 10). Spikelet number was least at 8.9 in 2009 (Table 10) and most at 9.7 in 2012 (Table 11). Across the four years, the mean outer floret grain number was least at 32.9 in 2009 (Table 8) and most at 36.7 in 2010 (Table 9). Among population lines, grain number in the outer florets ranged from 22.9 to 39.6 in 2009 (Table 8), 23.1 to 43.2 in 2010 (Table 9), 23.9 to 42.6 in 2011 (Table 10) and 27.3 to 38.6 in 2012 (Table 11).

Population 9M

All florets, years 2008/09 and 2009/10

The population mean for AFS was 21.3% in 2009 (Table 8) and 25.2% in 2010 (Table 9). In 2009, inner floret sterility was particularly high with a mean of 31.6% and maximum of 52.7% (Table 8). By contrast, inner floret sterility was low in 2010 (10.5% mean, 25.4% maximum; Table 9).

Outer floret grain number as a proportion of total grains was 0.57 in 2009 and 0.55 in 2010 (derived from Tables 8 and 9).

Mean grain number per whole ear was 57.8 in 2009 (Table 8) and 67.1 in 2010 (Table 9). Minimum to maximum grain number among population lines was 32.6 to 75.6 in 2009 and 42.9 to 91.2 in 2010.

The mid-ear section had on average 3 more grains than the lower ear section and 7 more grains than the upper ear section (Tables 8 and 9). The distribution of grains across the three sections, in the two seasons, were 32.2-32.7% in lower ear, 36.6-38.5% in middle ear and 25.4-26.1% in upper ear.

When assessing all florets, both parents had relatively high levels of AFS (above or close to the population mean), with parent M having relatively high sterility in the inner florets, and upper ear section, whilst parent 9 had higher OFS.

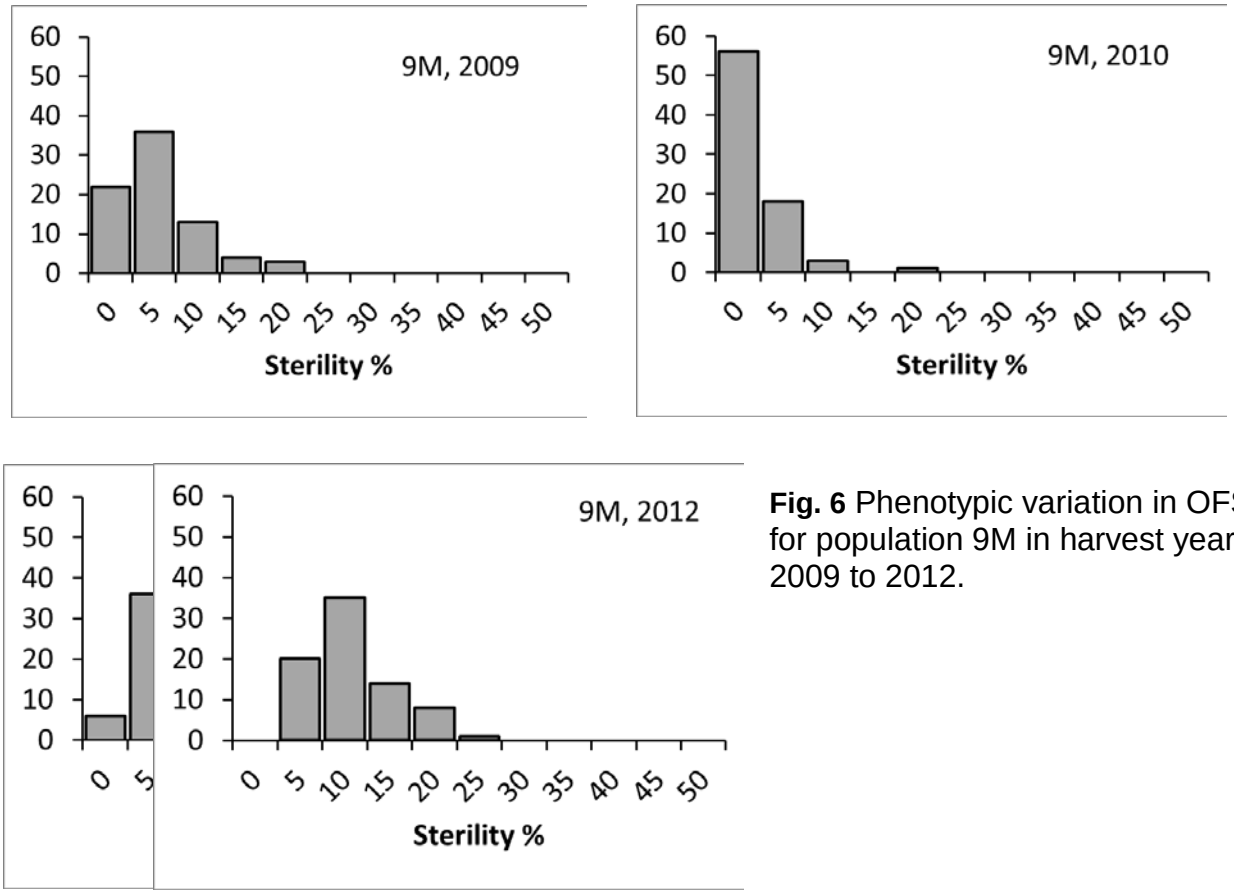


Fig. 6 Phenotypic variation in OFS for population 9M in harvest years 2009 to 2012.

Table 8 (A) Ear traits and (B) sterility traits for population 9M, harvest 2009

(A) Ear traits		Ear length (cm)	Spikelet number	Grains per ear	Grains per outer florets	Grains per inner florets	Grains per lower ear	Grains per mid ear	Grains per upper ear
Parents	9	9.09	9.31	52.13	29.63	22.50	17.25	18.00	15.75
	M	8.75	8.88	54.75	34.63	19.25	19.13	20.50	13.63
Population	Mean	8.95	8.94	57.75	32.87	23.08	18.88	22.25	14.68
	Median	9.00	9.00	58.13	33.38	24.00	19.25	22.63	15.00
	Maximum	10.19	10.44	75.63	39.63	31.63	22.88	26.50	21.13
	Minimum	6.91	6.88	32.63	22.88	9.75	13.13	14.75	4.00
	St. Dev.	0.63	0.73	8.94	3.61	4.72	2.22	2.61	3.51
	CV%	7.04	8.16	15.47	10.99	20.47	11.77	11.72	23.92

(B) Sterility traits		Sterile florets	Sterile outer florets	% sterility all florets	% sterility outer florets	% sterility inner florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	9	21.63	7.63	29.05	20.47	38.36	25.81	25.00	32.98
	M	16.88	0.88	24.16	2.46	44.60	18.18	17.17	35.12
Population	Mean	15.23	2.88	21.26	8.21	31.55	20.19	14.55	26.95
	Median	15.13	2.75	19.83	7.36	30.22	19.62	13.00	27.27
	Maximum	26.88	8.38	38.65	22.87	52.73	38.89	37.89	46.04
	Minimum	7.63	0.38	9.15	0.94	15.81	8.56	0.94	13.16
	St. Dev.	3.75	1.48	5.70	4.57	8.76	6.25	7.27	6.77
	CV%	24.60	51.51	26.81	55.72	27.76	30.95	49.92	25.12

Table 9 (A) Ear traits and (B) sterility traits for population 9M, harvest 2010

(A) Ear traits		Ear length (cm)	Spikelet number	Grains per ear	Grains per outer florets	Grains per inner florets	Grains per lower ear	Grains per mid ear	Grains per upper ear
Parents	9	8.87	9.35	70.50	36.10	30.40	22.80	25.60	20.10
	M	9.09	9.55	65.00	37.40	24.40	22.90	23.50	16.10
Population	Mean	8.89	9.63	67.13	36.66	26.11	21.64	24.54	17.49
	Median	8.85	9.60	66.70	36.33	26.20	21.80	24.60	17.40
	Maximum	10.98	11.25	91.20	43.20	36.50	28.70	29.60	25.20
	Minimum	7.12	7.31	42.92	23.08	13.00	11.30	17.69	7.54
	St. Dev.	0.76	0.78	10.67	3.15	5.48	3.34	2.94	3.51
	CV%	8.60	8.08	15.89	8.60	20.98	15.43	11.98	20.09

(B) Sterility traits		Sterile florets	Sterile outer florets	% sterility all florets	% sterility outer florets	% sterility inner florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	9	17.80	1.30	20.11	3.40	6.48	19.15	18.99	20.24
	M	25.10	0.80	28.22	2.17	15.25	21.58	25.87	35.08
Population	Mean	22.22	1.82	25.17	4.77	10.47	23.96	21.22	28.86
	Median	22.30	1.50	26.27	3.84	10.34	23.63	22.55	29.76
	Maximum	37.30	6.15	47.95	22.53	25.38	45.24	47.25	45.71
	Minimum	5.80	0.50	8.37	1.28	2.15	10.00	5.51	9.24
	St. Dev.	6.51	1.14	7.15	3.25	5.12	7.39	7.50	7.74
	CV%	29.30	63.03	28.41	68.24	48.88	30.85	35.36	26.80

Table 10 Ear and sterility traits for outer florets in population 9M, harvest 2011.

Harvest 2011		Ear length (cm)	Spikelet number	Grains per outer florets	Sterile outer florets	% sterility outer florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	9	9.54	10.25	31.70	9.20	22.05	15.83	24.17	20.00
	M	10.37	9.90	36.70	2.90	7.23	3.33	5.83	6.67
Population	Mean	9.71	9.63	34.48	3.98	10.36	8.18	8.94	10.88
	Median	9.59	9.65	34.50	3.55	9.29	7.50	8.33	10.00
	Maximum	12.10	11.30	42.60	10.20	25.01	25.56	25.83	29.17
	Minimum	6.95	7.40	23.87	1.30	3.32	1.39	1.67	0.83
	St. Dev.	0.98	0.71	3.25	1.77	4.65	4.35	4.72	6.20
	CV%	10.12	7.41	9.44	44.61	44.85	53.14	52.84	56.97

Table 11 Ear and sterility traits for outer florets in population 9M, harvest 2012.

Harvest 2012		Ear length (cm)	Spikelet number	Grains per outer florets	Sterile outer florets	% sterility outer florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	9	9.40	10.20	31.80	9.00	22.41	22.50	15.00	25.00
	M	8.27	9.15	32.50	4.10	10.94	12.50	7.50	9.17
Population	Mean	8.77	9.69	33.42	5.15	13.14	10.83	10.60	14.50
	Median	8.78	9.65	33.60	4.75	12.29	10.00	9.17	12.50
	Maximum	10.98	11.30	38.60	10.10	25.43	25.83	25.83	33.33
	Minimum	7.42	8.60	27.30	2.00	5.42	2.50	2.50	3.33
	St. Dev.	0.68	0.62	2.74	1.93	4.88	4.77	5.10	6.88
	CV%	7.78	6.37	8.20	37.39	37.15	43.99	48.13	47.46

Population FA

Outer florets, years 2010/11 and 2011/12

The mean number of outer floret grains were 34.4 and 36.1, in 2011 and 2012 respectively. Among all lines, grain number ranged from a minimum of 21.7 to a maximum of 41.3 in 2011, and a much more narrow 31.2 to 42.5 in 2012 (Tables 12 and 13).

Mean OFS was similar in each year, but the range among lines was wider in 2011 than in 2012; the minimum to maximum range in OFS was 1.2% to 48.0% in 2011 and 2.9% to 18.4% in 2012 (Fig. 7; Tables 12 and 13).

The population mean for number of sterile outer florets ranged range from a minimum of 0.4 (2011) or 1.2 (2012) to a maximum of 7.9 (2012) and 19.7 (2011).

Population FA

All florets, years 2010/11 and 2011/12

Mean ear length was 8.8 cm in 2011 and 9.2 cm in 2012, with mean spikelet number of 9.3 and 10.0 in 2011 and 2012, respectively (Tables 12 and 13). Mean total grain number per ear was 58.3 in 2011, whilst only 51.7 in 2012. Thus, there were more grains per spikelet in 2011.

Total grain number in parent F was below the population mean, whilst population A had grain number above the mean; this difference was most pronounced in 2011 (Tables 12 and 13). Among all lines in the population, the minimum and maximum total grain numbers were 29.0 and 72.6 in 2011, compared to 36.5 to 64.2 in 2012.

Outer floret grain number as a proportion of total grains was 0.59 (2011) and 0.69 (2012).

In both years, the mid-ear region had approximately 1-2 grains more than the lower ear region and 5 grains more than the upper ear. The distribution of grains across the three ear sections were 34.5-35.4% (lower section), 37.4-37.8% (middle) and 27.2-27.7% (upper).

In 2011, parent F had significantly higher AFS than parent A, with the difference in OFS being pronounced, 24.4% compared to 5.8% (Fig. 7). There was difference in AFS or OFS between the parents in 2012. Parent A was weak for sterility in the inner florets (in 2011). Parent F was weaker for % sterility in middle and upper florets (both years).

Inner floret sterility was relatively low, with seasonal maxima of 16.2% (2011) and 19.9% (2012). In both seasons, the upper ear was especially weak for sterility (>23%) i.e. above the sterility levels in the lower and mid-ear regions.

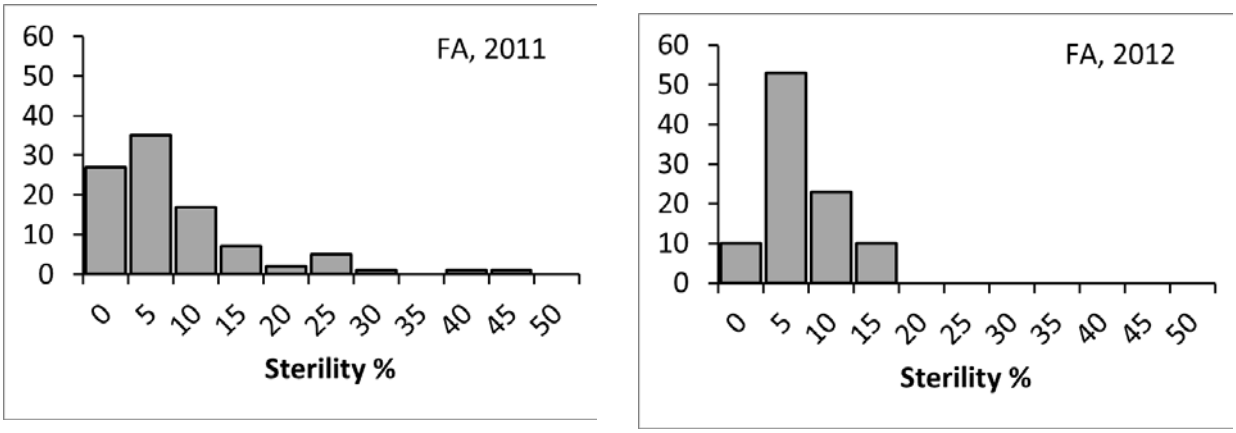


Fig. 7 Phenotypic variation in OFS for population FA in harvest years 2011 and 2012.

Table 12 (A) Ear traits and (B) sterility traits for population FA, harvest 2011.

(A) Ear traits		Ear length (cm)	Spikelet number	Grains per ear	Grains per outer florets	Grains per inner florets	Grains per lower ear	Grains per mid ear	Grains per upper ear
Parents	F	9.25	9.30	51.00	28.20	21.50	19.10	18.60	12.00
	A	9.52	10.50	60.00	39.50	20.50	17.50	20.40	16.20
Population	Mean	8.84	9.29	58.30	34.35	23.11	19.18	21.00	15.41
	Median	9.13	9.55	59.65	34.65	23.50	19.30	21.40	16.10
	Maximum	10.55	11.20	72.60	41.30	30.70	23.00	25.80	20.80
	Minimum	0.33	0.40	29.00	21.70	7.30	10.70	10.60	5.60
	St. Dev.	1.57	1.65	7.65	3.64	4.44	1.89	2.55	2.85
	CV%	17.77	17.79	13.13	10.59	19.20	9.87	12.13	18.47

(B) Sterility traits		Sterile florets	Sterile outer florets	% sterility all florets	% sterility outer florets	% sterility inner florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	F	25.00	8.80	32.69	24.37	4.69	22.69	29.75	43.23
	A	18.10	2.50	23.12	5.82	14.85	25.00	19.84	22.27
Population	Mean	17.63	3.89	23.25	10.13	9.18	21.17	20.29	26.25
	Median	16.50	2.95	22.29	8.07	9.07	21.04	19.20	23.35
	Maximum	40.40	19.70	58.74	48.04	16.15	50.91	52.65	69.35
	Minimum	7.70	0.40	10.76	1.18	1.58	10.57	7.85	8.85
	St. Dev.	6.12	3.29	8.04	8.33	3.10	5.99	7.48	11.17
	CV%	34.70	84.54	34.57	82.17	33.81	28.30	36.86	42.56

Table 13 (A) Ear traits and (B) sterility traits for population FA, harvest 2012.

(A) Ear traits		Ear length (cm)	Spikelet number	Grains per ear	Grains per outer florets	Grains per inner florets	Grains per lower ear	Grains per mid ear	Grains per upper ear
Parents	F	8.60	9.95	48.40	36.50	11.90	16.60	16.10	12.10
	A	9.23	9.50	52.40	35.40	17.00	17.20	18.80	14.60
Population	Mean	9.18	10.00	51.65	36.07	15.52	17.05	18.06	13.12
	Median	9.25	10.00	50.75	36.05	15.25	17.20	17.95	13.00
	Maximum	10.59	11.40	64.20	42.50	24.80	19.70	22.30	17.20
	Minimum	7.58	8.68	36.50	31.20	3.30	13.20	12.70	8.90
	St. Dev.	0.74	0.61	4.93	2.26	3.56	1.22	1.63	1.65
	CV%	8.05	6.08	9.55	6.25	22.96	7.16	9.01	12.54

(B) Sterility traits		Sterile florets	Sterile outer florets	% sterility all florets	% sterility outer florets	% sterility inner florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	F	17.60	3.00	26.52	7.62	17.20	25.11	26.34	28.57
	A	17.60	2.60	25.09	6.72	17.71	26.81	20.68	25.89
Population	Mean	14.90	3.74	22.26	9.38	11.96	20.78	19.55	23.66
	Median	14.25	3.26	21.07	8.43	12.20	20.52	18.39	23.29
	Maximum	24.20	7.90	35.09	18.35	19.91	32.43	32.27	39.86
	Minimum	6.10	1.20	9.56	2.90	4.09	6.25	6.73	8.62
	St. Dev.	4.28	1.67	5.74	3.97	3.81	5.59	5.65	6.91
	CV%	28.69	44.81	25.77	42.32	31.83	26.92	28.90	29.19

Population Avalon x Cadenza

Outer florets, years 2010/11 and 2011/12

Mean for ear length was 8.0 cm in 2011 and 8.7 cm in 2012, with mean spikelet number of 9.7 and 10.3 in 2011 and 2012, respectively (Table 14).

Mean number of grain in outer florets was similar in both years with 32.7 in 2011 and 33.0 in 2012 (Table 14). The population minimum and maximum grain numbers were also similar between seasons; from 8.30 to 40.5 in 2011, compared with 8.7 to 42.8 in 2012.

Parent A had similar moderate OFS in both years, whereas parent C had relatively high OFS 2011 (27.8%) and low OFS in 2012 (6.1%) (Fig. 8). Parent A had low levels of sterility in middle florets, in both years. Both parents had high upper floret sterility in 2011, but low upper floret sterility in 2012 (Table 14).

The population mean for sterile outer florets was 6.1 florets in 2011 and 8.2 florets in 2012, with mean OFS among lines was of 15.8% in 2011, compared to 20.6% in 2012. However, the range of sterile sites among lines was the widest of any population. The population minimum for sterile outer florets was 0.9 in 2011 and 2.1 in 2012, with a maximum of 21.2 in 2011 and 28.4 in 2012. This resulted in OFS from 2.4% to 72.0% in 2011 and from 5.0% to 78.7% in 2012 (Fig. 8).

On average, % sterility was highest in upper florets, but with severe weakness evident in some lines at lower and middle ear regions.

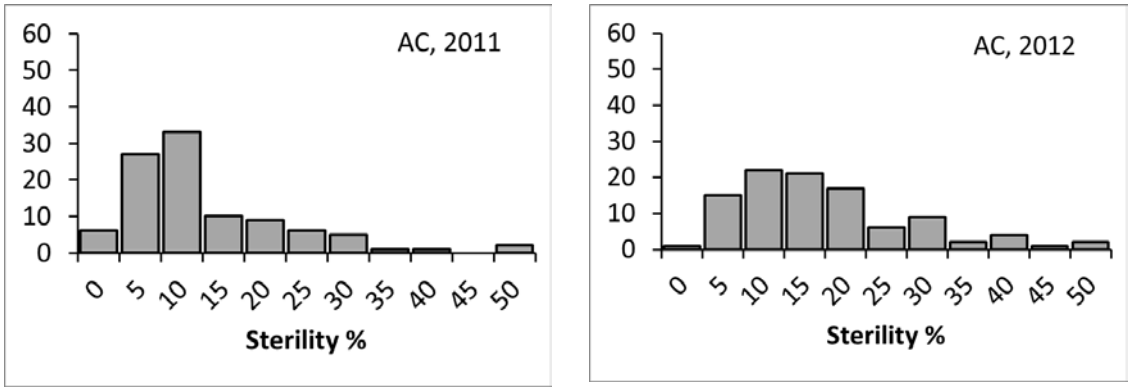


Fig. 8 Phenotypic variation in OFS for population Avalon x Cadenza in harvest years 2011 and 2012.

Table 14 Ear and sterility traits for outer florets in population Avalon x Cadenza, (A) harvest 2011 and (B) 2012.

(A) Harvest 2011		Ear length (cm)	Spikelet number	Grains per outer florets	Sterile outer florets	% sterility outer florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	A	9.16	10.20	35.00	5.80	14.37	10.83	4.17	15.83
	C	8.57	10.20	29.40	11.40	27.82	15.83	15.83	37.50
Population	Mean	7.96	9.69	32.65	6.06	15.79	12.31	11.06	19.19
	Median	7.86	9.70	33.80	5.05	12.75	10.00	8.75	15.00
	Maximum	9.86	10.95	40.50	21.20	72.02	73.33	71.67	61.67
	Minimum	6.11	7.40	8.30	0.90	2.36	1.67	0.83	0.83
	St. Dev.	0.87	0.52	4.58	3.88	10.85	9.25	9.64	14.21
	CV%	10.87	5.37	14.02	63.97	68.74	75.13	87.14	74.05

(B) Harvest 2012		Ear length (cm)	Spikelet number	Grains per outer florets	Sterile outer florets	% sterility outer florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	A	9.30	10.43	37.00	4.70	11.05	11.67	6.67	3.33
	C	9.14	10.73	40.30	2.60	6.12	6.67	4.17	4.17
Population	Mean	8.67	10.33	32.97	8.24	20.60	15.19	14.04	22.85
	Median	8.51	10.38	34.15	7.20	18.37	13.33	10.83	20.00
	Maximum	13.84	11.43	42.80	28.40	78.73	79.17	75.00	63.33
	Minimum	5.71	8.63	8.70	2.10	5.00	2.50	0.83	2.50
	St. Dev.	1.14	0.55	5.37	4.54	11.85	10.39	11.55	13.58
	CV%	13.15	5.35	16.29	55.09	57.54	68.44	82.25	59.40

Population LQ

Outer florets, years in 2010/11 and 2011/12

Population mean ear length was 8.2 cm in 2011 and 9.3 cm in 2012, with mean spikelet number of 8.4 and 9.3 in 2011 and 2012, respectively (Table 15).

Mean outer florets grain number was lower in 2011 (29.8 grains) compared to 2012 (34.7 grains). The population minimum and maximum grain numbers were from 21.3 to 34.4 in 2011 and from 29.3 to 39.4 in 2012.

The mean number of sterile outer florets across the population was low compared to other populations, being 3.6 in 2011 and 2.5 in 2012. Consequently, mean OFS was 10.8% in 2011, but only 6.8% in 2012 (Fig. 9).

In 2011, % sterility was highest in the lower florets (11.6%) and least in the upper florets (7.6%), whilst in 2012 levels in the lower, middle and upper florets were similar to the overall mean of 6.8%

In 2011, parent L had (a significantly) higher number of sterile florets and % sterility than parent Q; the level of sterility in L increased from the lower florets to the tip. In 2012, parent L had only slightly higher sterility than parent Q, but was significantly weaker in lower and upper florets, whilst mid ear florets has relatively low sterility.

The number of sterile outer florets for the population ranged range from a minimum of 0.5 (2011) or 0.7 (2012) to a maximum of 12.1 (2011) and 6.6 (2011). This resulted in a minimum and maximum OFS of 1.5 to 36.7% in 2011 and 1.7 to 17.1% in 2012.

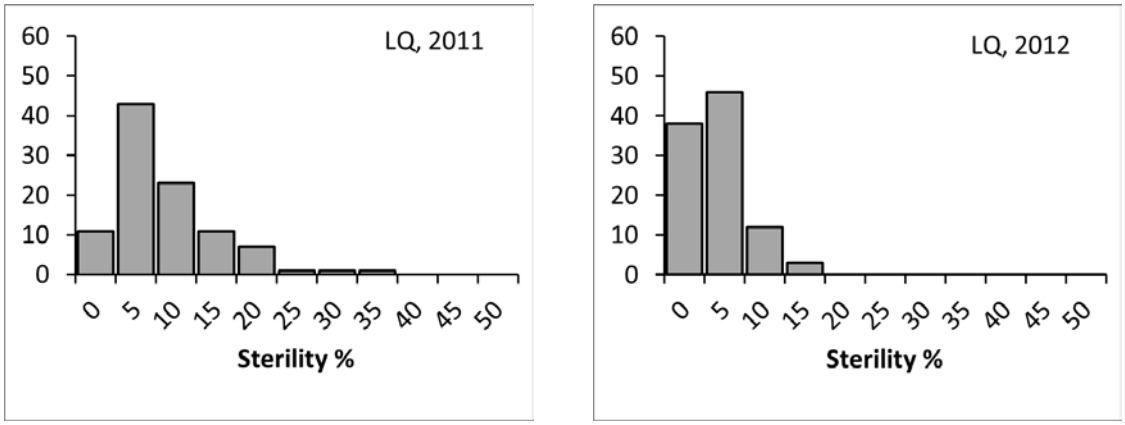


Fig. 9 Phenotypic variation in OFS for population LQ in harvest years 2011 and 2012.

Table 15 Ear and sterility traits for outer florets in population LQ, (A) harvest 2011 and (B) 2012.

(A) Harvest 2011		Ear length (cm)	Spikelet number	Grains per outer florets	Sterile outer florets	% sterility outer florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	L	8.05	8.00	24.20	7.80	24.19	14.17	23.33	27.50
	Q	8.55	8.25	31.20	1.60	4.81	5.00	6.67	1.67
Population	Mean	8.17	8.36	29.79	3.57	10.82	11.55	10.44	7.58
	Median	8.19	8.35	30.40	3.05	8.94	10.00	8.33	6.67
	Maximum	9.28	9.05	34.40	12.10	36.65	42.50	34.17	32.50
	Minimum	7.05	7.30	21.30	0.50	1.49	0.00	0.83	0.00
	St. Dev.	0.45	0.32	2.78	2.11	6.58	7.35	6.92	5.11
	CV%	5.53	3.78	9.34	59.17	60.80	63.67	66.27	67.36

(B) Harvest 2012		Ear length (cm)	Spikelet number	Grains per outer florets	Sterile outer florets	% sterility outer florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	L	8.53	8.50	31.10	2.80	8.26	7.50	3.33	12.50
	Q	8.37	8.80	32.90	2.30	6.49	4.17	6.67	7.50
Population	Mean	9.27	9.32	34.67	2.51	6.77	6.69	6.54	6.40
	Median	9.23	9.35	34.60	2.40	6.38	5.83	5.83	5.83
	Maximum	10.43	10.10	39.40	6.60	17.13	25.00	21.67	15.00
	Minimum	7.34	8.20	29.30	0.70	1.73	0.83	0.00	0.83
	St. Dev.	0.58	0.39	2.18	1.22	3.39	4.57	4.27	3.49
	CV%	6.30	4.15	6.28	48.71	50.10	68.37	65.37	54.44

Population TR

Outer florets, years in 2010/11 and 2011/12

Population mean ear length was 8.4 cm in 2010, 9.0 cm in 2011 and 10.2 in 2012, with mean spikelet number of 9.4, 10.3 and 10.3 in 2010, 2011 and 2012, respectively (Table 16).

Mean number of outer floret grains was 36.3 (2010), 37.6 (2011) and 37.7 (2012). The population minimum and maximum grain numbers were 28.0 and 41.3 in 2010, 23.0 and 43.4 in 2011 and 15.4 and 43.3 in 2012.

The mean number of sterile outer florets across the population was very low at 1.0 in 2010 and low at 3.6 in both 2011 and 2012.

In 2010, mean OFS was 2.8%, whilst in 2011 and 2012 the levels were 8.7 and 9.1%, respectively (Table 16). In 2010 and 2012, the pattern of % sterility was consistent across different parts of the ear, whilst in 2011 sterility was highest in upper florets 9.5%.

In all three years, parent T had higher sterility than parent R, with parent T being above the population mean. OFS for parent T was 4.3% in 2010, 13.4% in 2011 and 17.2% in 2012.

Parent T had above average sterility in each part of the ear, whilst parent R was relatively weak in the upper ear only, with relatively low sterility in the lower and mid ear.

The number of sterile outer florets for the population ranged range from a minimum of 0.1 on (2010) or 0.5 (2011 and 2012) to a maximum of 4.2 (2010), 20.3 (2011) and 27.5 (2012). This resulted in a minimum to maximum range in OFS of 0.2% to 10.6% in 2010, 1.2% to 46.6% in 2011 and 1.1% to 65.9% in 2012 (Table 16).

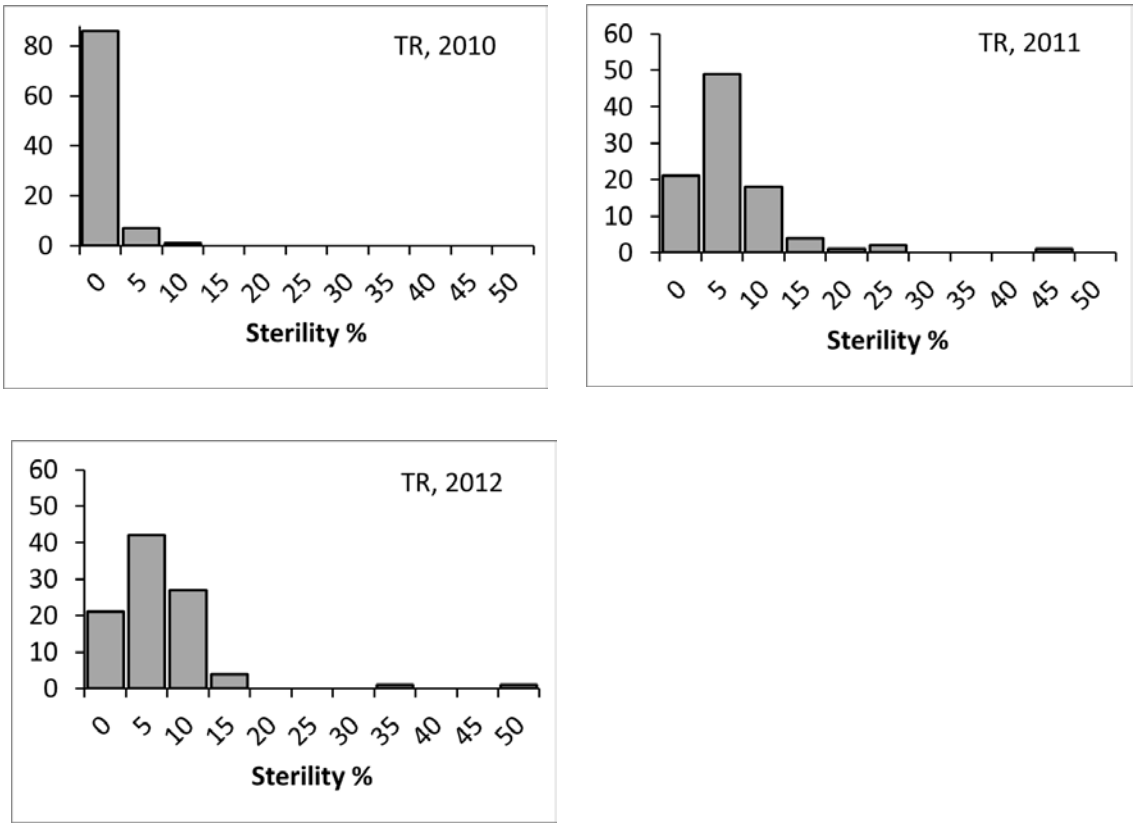


Fig. 10 Phenotypic variation in OFS for population TR in harvest years 2011 and 2012.

Table 16 Ear and sterility traits for outer florets in population TR, (A) harvest 2010, (B) 2011 and (C) 2012.

(A) Harvest 2010		Ear length (cm)	Spikelet number	Grains per outer florets	Sterile outer florets	% sterility outer florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	T	9.13	10.45	40.00	1.80	4.28	4.17	3.33	3.33
	R	8.75	9.55	37.40	0.80	2.06	1.67	0.00	2.50
Population	Mean	8.40	9.39	36.26	1.04	2.75	2.27	2.09	2.46
	Median	8.40	9.43	36.55	0.90	2.36	1.67	1.67	1.67
	Maximum	9.70	10.60	41.30	4.20	10.59	10.00	10.00	10.00
	Minimum	6.55	7.25	28.00	0.10	0.23	0.00	0.00	0.00
	St. Dev.	0.57	0.64	2.40	0.71	1.82	1.82	1.73	2.14
	CV%	6.77	6.77	6.61	68.63	66.23	80.34	83.18	87.17

(B) Harvest 2011		Ear length (cm)	Spikelet number	Grains per outer florets	Sterile outer florets	% sterility outer florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	T	9.76	10.70	37.10	5.70	13.35	14.17	9.17	16.67
	R	8.43	9.50	34.80	3.20	8.62	1.67	5.00	17.50
Population	Mean	9.04	10.31	37.57	3.62	8.73	5.43	6.29	9.47
	Median	9.04	10.30	37.85	3.05	7.13	5.00	5.83	6.67
	Maximum	11.61	11.40	43.40	20.30	46.56	39.17	38.33	50.83
	Minimum	7.05	9.00	23.00	0.50	1.17	0.00	0.00	1.67
	St. Dev.	0.71	0.63	3.22	2.75	6.36	5.06	5.03	8.10
	CV%	7.86	6.14	8.57	75.85	72.93	93.06	80.09	85.59

(C) Harvest 2012		Ear length (cm)	Spikelet number	Grains per outer florets	Sterile outer florets	% sterility outer florets	% sterility lower florets	% sterility middle florets	% sterility upper florets
Parents	T	10.78	11.35	37.60	7.80	17.19	20.00	15.00	10.83
	R	9.75	10.00	38.60	1.40	3.70	1.67	2.50	5.83
Population	Mean	10.24	10.34	37.69	3.57	9.05	6.52	5.24	7.50
	Median	10.27	10.45	37.60	3.05	7.95	5.00	4.17	6.67
	Maximum	12.13	11.90	43.30	27.50	65.90	50.00	50.00	61.67
	Minimum	7.90	8.05	15.40	0.50	1.10	0.83	0.00	0.83
	St. Dev.	0.72	0.78	3.72	2.92	7.48	6.01	5.35	6.83
	CV%	7.08	7.52	9.86	81.56	82.64	92.26	102.06	91.04

5.2. Association between ear sterility and other traits

Population 9M

All floret sterility

There was a significant correlation between percent sterility and plant height in 2010 (Table 18).

Percent sterility had a significant negative association with ear length (2009 and 2010) and with spikelet number (2010).

In 2009, AFS was negatively and significantly associated with growth stage (except at late June); thus slower (backwards) crop development was correlated with increased sterility. By contrast, in 2010, sterility was significantly and positively correlated with growth stage; hence, more rapid (forward) crop development correlated with sterility.

AFS was significantly correlated grain number and sterility in different portions of the ear e.g. outer florets or mid-ear florets.

Lab and field sterility scores were strongly correlated in 2010, but only weakly so in 2009.

Table 18 Association between AFS and other plant and sterility traits in population 9M, harvests 2009 and 2010.

	2009		2010	
	Correlation coefficient	P	Correlation coefficient	P
Plant height cm			0.368	0.001
Ear length	-0.435	<0.001	-0.298	0.009
Spikelet number	-0.394	<0.001	-0.114	0.330
GS on 3rd June 2009	-0.362	0.001		
GS on 14th June 2009	-0.248	0.032		
GS on 19 June 2009	-0.285	0.013		
GS on 22nd June 2009	-0.135	0.249		
GS on 11th June 2010			0.248	0.032
GS on 14th June 2010			0.248	0.032
GS on 19th June 2010			0.301	0.009
GS on 22nd June 2010			0.144	0.219
Grain number all florets	-0.721	<0.001	-0.601	<0.001
Grain number outer	-0.546	<0.001	-0.263	0.023
Grain number lower	-0.649	<0.001	-0.509	<0.001
Grain number middle	-0.792	<0.001	-0.703	<0.001
Grain number mid-upper	-0.628	<0.001	-0.626	<0.001
Sterile florets All	0.866	<0.001	0.893	<0.001
Sterile florets outer	0.525	<0.001	0.425	<0.001
Sterile lower florets	0.886	<0.001	0.925	<0.001
Sterile middle florets	0.908	<0.001	0.920	<0.001
Sterile mid-upper florets	0.397	<0.001	0.665	<0.001
Sterility % all florets	--	--	--	--
Sterility % outer	0.571	<0.001	0.477	<0.001
Sterility % inner	0.845	<0.001	0.794	<0.001
Sterility % lower	0.906	<0.001	0.927	<0.001
Sterility % middle	0.922	<0.001	0.939	<0.001
Sterility % mid-upper	0.810	<0.001	0.936	<0.001
Field sterility GS87	0.226	0.052	0.333	0.004
Initial lab score	-0.145	0.213	0.610	<0.001

Population 9M

Outer floret sterility

For harvest years 2009 and 2010, OFS is compared with other grain and sterility traits for the whole ear (Table 19), whilst in years 2011 and 2012 it is compared with outer florets only (Table 20).

There was no association between OFS and plant height.

OFS was negatively and significantly associated with ear length in two years only (2009, 2010), but with spikelet number only in 2009.

OFS was not significantly associated with crop growth stage.

With the exception of mid-upper ear region (2009 and 2010), OFS was significantly correlated with grain number and other ear sterility traits.

OFS was strongly correlated with field and initial lab scores in 2010, 2011 and 2012, but not in 2009.

Table 19 Association between OFS and other plant and sterility traits in population 9M, harvests 2009 and 2010.

	2009		2010	
	Correlation coefficient	P	Correlation coefficient	P
Plant height cm			0.0358	0.760
Ear length	-0.474	<0.001	-0.3173	0.006
Spikelet number	-0.390	0.001	-0.0642	0.584
GS 3rd June 2009	-0.223	0.054		
GS 14th June 2009	-0.122	0.298		
GS 19 June 2009	-0.109	0.353		
GS 22nd June 2009	-0.023	0.845		
GS 11th June 2010			0.103	0.379
GS 14th June 2010			0.149	0.202
GS 19th June 2010			0.100	0.392
GS 22nd June 2010			0.062	0.599
Grain number all	-0.540	<0.001	-0.407	<0.001
Grain number outer	-0.740	<0.001	-0.414	<0.001
Grain number lower	-0.516	<0.001	-0.501	<0.001
Grain number middle	-0.563	<0.001	-0.453	<0.001
Grain number mid-upper	-0.515	<0.001	-0.359	0.002
Sterile florets All	0.388	0.001	0.310	0.007
Sterile florets outer	0.981	<0.001	0.964	<0.001
Sterile lower florets	0.519	<0.001	0.431	<0.001
Sterile middle florets	0.601	<0.001	0.366	0.001
Sterile mid-upper florets	-0.065	0.582	0.045	0.700
Sterility % all florets	0.571	<0.001	0.477	<0.001
Sterility % outer				
Sterility % inner	0.242	0.037	0.133	0.254
Sterility % lower	0.588	<0.001	0.549	<0.001
Sterility % middle	0.633	<0.001	0.403	<0.001
Sterility % mid-upper	0.354	0.002	0.296	0.010
Field sterility GS87	0.108	0.355	0.276	0.017
Initial lab score	0.091	0.437	0.376	0.001

Table 20 Association between OFS and other plant and sterility traits in population 9M, harvests 2011 and 2012.

	2011		2012	
	Correlation coefficient	P	Correlation coefficient	P
Plant height cm	-0.189	0.105	0.065	0.579
Ear length	-0.149	0.202	0.037	0.751
Spikelet number	-0.073	0.535	0.073	0.535
GS 8th June 2011	0.118	0.312		
GS 13th June 2011	0.088	0.452		
GS 20th June 2011	-0.012	0.919		
GS 22nd June 2011	0.173	0.137		
GS 10th June 2012			-0.029	0.804
GS 19th June 2012			-0.079	0.501
GS 25th June 2012			0.026	0.822
Grain number outer	-0.628	<0.001	-0.627	<0.001
Potential grain number	-0.073	0.535	0.073	0.535
Sterile florets (outer)	0.983	<0.001	0.983	<0.001
Sterile lower florets	0.765	<0.001	0.693	<0.001
Sterile middle florets	0.871	<0.001	0.768	<0.001
Sterile mid-upper florets	0.862	<0.001	0.898	<0.001
Sterile tip	0.448	<0.001	0.491	<0.001
Sterility % lower	0.765	<0.001	0.693	<0.001
Sterility % middle	0.871	<0.001	0.768	<0.001
Sterility % mid-upper	0.862	<0.001	0.898	<0.001
Field sterility GS87	0.237	0.041	0.449	<0.001
Initial lab score	0.435	<0.001	0.671	<0.001

Population FA

All floret sterility

There was no correlation between sterility and plant height (Table 21).

AFS was positively and significantly correlated with ear length and spikelet number in 2011, but not in 2012.

AFS had a positive and significant association with crop growth stage in 2011, but only a weak negative association with growth stage in 2012. The former highlights an association between advanced crop growth and increased sterility.

AFS was highly correlated with all grain and other sterility measures in both 2011 and 2012.

There were highly significant correlations between AFS and field scores at grain soft dough and grain hard dough, as well as with the initial lab score in both years.

Table 21 Association between AFS and other plant and sterility traits in population FA, harvests 2011 and 2012.

	2011		2012	
	Correlation coefficient	P	Correlation coefficient	P
Plant height cm	-0.036	0.725	-0.043	0.677
Ear length	0.274	0.007	-0.099	0.339
Spikelet number	0.202	0.048	0.161	0.117
GS 5th June 2011	0.201	0.050		
GS 13th June 2011	0.234	0.022		
GS 20th June 2011	0.211	0.039		
GS 10th June 2012			-0.183	0.074
GS 19th June 2012			-0.141	0.171
GS 25th June 2012			-0.175	0.088
Grain number all	-0.771	<0.001	-0.483	<0.001
Grain number outer	-0.690	<0.001	-0.322	0.001
Grain number inner	-0.705	<0.001	-0.467	<0.001
Grain number lower	-0.666	<0.001	-0.559	<0.001
Grain number middle	-0.803	<0.001	-0.541	<0.001
Grain number mid-upper	-0.800	<0.001	-0.497	<0.001
Sterile florets All	0.965	<0.001	0.955	<0.001
Sterile florets outer	0.925	<0.001	0.674	<0.001
Sterile inner florets	0.887	<0.001	0.890	<0.001
Sterile lower florets	0.870	<0.001	0.862	<0.001
Sterile middle florets	0.946	<0.001	0.901	<0.001
Sterile mid-upper florets	0.912	<0.001	0.879	<0.001
Sterility % all florets	--	--	--	--
Sterility % outer	0.922	<0.001	0.702	<0.001
Sterility % inner	0.214	<0.001	0.708	<0.001
Sterility % lower	0.885	<0.001	0.894	<0.001
Sterility % middle	0.969	<0.001	0.910	<0.001
Sterility % mid-upper	0.946	<0.001	0.880	<0.001
Field sterility GS85	0.775	<0.001	0.412	<0.001
Field sterility GS87	0.746	<0.001	0.635	<0.001
Initial lab score	0.780	<0.001	0.643	<0.001

Population FA

Outer floret sterility

OFS was positively and significantly correlated with ear length in 2011 and with spikelet number in 2012.

OFS had a positive and significant correlation with crop growth stage in 2011, but not in 2012.

With the exception of % sterility in inner florets (2011 and 2012), OFS was significantly correlated with grain number and other ear sterility traits.

There were highly significant correlations between OFS and field scores at grain soft dough and grain hard dough, as well as with the initial lab score in both years.

Table 22 Association between OFS and other plant and sterility traits in population FA, harvests 2011 and 2012.

	2011		2012	
	Correlation coefficient	P	Correlation coefficient	P
Plant height cm	-0.068	0.510	0.076	0.464
Ear length	0.345	0.001	0.082	0.425
Spikelet number	0.153	0.137	0.354	<0.001
GS 5th June 2011	0.256	0.012		
GS 13th June 2011	0.287	0.005		
GS 20th June 2011	0.201	0.050		
GS 10th June 2012			-0.096	0.351
GS 19th June 2012			-0.103	0.317
GS 25th June 2012			0.056	0.590
Grain number all	-0.765	<0.001	-0.416	<0.001
Grain number outer	-0.787	<0.001	-0.366	<0.001
Grain number inner	-0.645	<0.001	-0.351	<0.001
Grain number lower	-0.601	<0.001	-0.520	<0.001
Grain number middle	-0.775	<0.001	-0.443	<0.001
Grain number mid-upper	-0.808	<0.001	-0.470	<0.001
Sterile florets All	0.869	<0.001	0.650	<0.001
Sterile florets outer	0.996	<0.001	0.990	<0.001
Sterile inner florets	0.733	<0.001	0.368	<0.001
Sterile lower florets	0.702	<0.001	0.417	<0.001
Sterile middle florets	0.832	<0.001	0.490	<0.001
Sterile mid-upper florets	0.873	<0.001	0.619	<0.001
Sterility % all florets	0.922	<0.001	0.702	<0.001
Sterility % outer	--	--	--	--
Sterility % inner	-0.085	0.410	0.026	0.798
Sterility % lower	0.732	<0.001	0.485	<0.001
Sterility % middle	0.870	<0.001	0.528	<0.001
Sterility % mid-upper	0.918	<0.001	0.650	<0.001
Field sterility GS85	0.843	<0.001	0.436	<0.001
Field sterility GS87	0.796	<0.001	0.753	<0.001
Initial lab score	0.860	<0.001	0.769	<0.001

Population Avalon x Cadenza

Outer floret sterility

In both years there was a significant negative correlation between OFS and plant height. OFS was significantly and negatively correlated with spikelet number in both years and with ear length in 2012 (Table 23).

OFS was negatively correlated with early-mid June growth stage in 2012, and with early June growth in 2011. In both years, there was a significant positive correlation between days to ear emergence (from WGIN data) and OFS. Hence, slower crop development was correlated with increased sterility.

There was very strong positive correlation between OFS and other ear and sterility traits.

The correlation between field sterility scores at soft dough and hard dough, and the initial lab assessment, was significant.

Table 23 Association between OFS and other plant and sterility traits in population Avalon x Cadenza, harvests 2011 and 2012.

	2011		2012	
	Correlation coefficient	P	Correlation coefficient	P
Plant height (cm)	-0.211	0.035	-0.255	0.011
Ear length (cm)	-0.088	0.384	-0.303	0.002
Spikelet number	-0.202	0.044	-0.299	0.002
GS on 03/06/2011	-0.203	0.043		
GS on 08/06/2011	-0.078	0.439		
GS on 13/06/2011	-0.038	0.706		
GS on 15/06/2012			-0.326	0.001
GS on 19/06/2012			-0.348	<0.001
GS on 25/06/2012			-0.144	0.153
WGIN days to ear emergence	0.204	0.042	0.342	<0.001
Grain number	-0.934	< 0.001	-0.951	<0.001
Potential grain number	-0.202	0.044	-0.299	0.002
Sterile florets (outer)	0.986	<0.001	0.990	<0.001
Sterile lower florets	0.825	<0.001	0.870	<0.001
Sterile middle florets	0.906	<0.001	0.908	<0.001
Sterile mid-upper florets	0.833	<0.001	0.938	<0.001
Sterile tip	0.511	<0.001	0.476	<0.001
Sterility % lower	0.825	<0.001	0.871	<0.001
Sterility % middle	0.906	<0.001	0.898	<0.001
Sterility % mid-upper	0.833	<0.001	0.923	<0.001
Field sterility GS85	0.494	<0.001	0.742	<0.001
Field sterility GS87	0.524	<0.001	0.635	<0.001
Initial lab score	0.589	<0.001	0.899	<0.001

Population LQ

Outer floret sterility

OFS was significantly correlated with plant height in 2011, but not in 2012 (Table 24).

OFS was significantly and negatively correlated with spikelet number in both years and with ear length in 2012.

OFS was significantly positively correlated with early June growth stage in 2012, but otherwise there was no association between crop growth stage and OFS. Thus, more advanced crop development was correlated with increased sterility.

With the exception of the ear tip, there was very strong positive correlation between % sterility and different ear sterility traits, and a significant negative correlation with potential grain number.

The correlation between field sterility scores at soft dough and hard dough, and the initial lab assessment, was significant.

Table 24 Association between OFS and other plant and sterility traits in population LQ, harvests 2011 and 2012.

	2011		2012	
	Correlation coefficient	P	Correlation coefficient	P
Plant height (cm)	0.199	0.050	0.136	0.185
Ear length (cm)	0.029	0.779	-0.202	0.048
Spikelet number	-0.362	<0.001	-0.321	0.001
GS on 05/06/2011	0.016	0.873		
GS on 09/06/2011	-0.011	0.918		
GS on 13/06/2011	-0.062	0.545		
GS on 12/06/2012			0.396	<0.001
GS on 19/06/2012			0.018	0.859
GS on 25/06/2012			-0.110	0.284
Grain number	-0.931	<0.001	-0.761	<0.001
Potential grain number	-0.362	<0.001	-0.321	0.001
Sterile florets (outer)	0.997	<0.001	0.995	<0.001
Sterile lower florets	0.915	<0.001	0.825	<0.001
Sterile middle florets	0.949	<0.001	0.872	<0.001
Sterile mid-upper florets	0.819	<0.001	0.721	<0.001
Sterile tip	-0.009	0.927	0.048	0.644
Sterility % lower	0.915	<0.001	0.825	<0.001
Sterility % middle	0.949	<0.001	0.872	<0.001
Sterility % mid-upper	0.819	<0.001	0.721	<0.001
Field sterility GS85	0.635	<0.001	0.704	<0.001
Field sterility GS87	0.535	<0.001	0.520	<0.001
Initial lab score	0.643	<0.001	0.433	<0.001

Population TR

Outer floret sterility

There was a significant negative correlation between OFS and plant height in 2012. OFS and spikelet number were positively and significantly correlated in 2010 only.

OFS was negatively and significantly with mid-June crop growth stage in 2010 and with all June growth stages in 2012 i.e. advances development was correlated with sterility.

There were very strong positive correlations between OFS and different ear sterility traits, and a significant negative correlation with grain number.

The correlation between field sterility scores at soft dough and hard dough, and the initial lab assessment, was significant.

Table 25 Association between OFS and other plant and sterility traits in population TR, harvests 2010, 2011 and 2012.

	2010		2011		2012	
	Correlation coefficient	P	Correlation coefficient	P	Correlation coefficient	P
Plant height cm	-0.008	0.937	-0.012	0.810	-0.216	0.036
Ear length	-0.057	0.586	0.211	0.101	0.117	0.263
Spikelet number	0.210	0.042	0.177	0.088	0.043	0.684
GS on 05/06/2010	-0.164	0.115				
GS on 09/06/2010	-0.146	0.161				
GS on 13/06/2010	-0.207	0.045				
GS on 12/06/2011			0.047	0.625		
GS on 19/06/2011			0.067	0.441		
GS on 25/06/2011			-0.024	0.907		
GS on 12/06/2012					-0.305	0.003
GS on 19/06/2012					-0.341	0.001
GS on 25/06/2012					-0.263	0.010
Grain number	-0.056	0.590	-0.668	<0.001	-0.699	<0.001
Potential grain number	0.210	0.042	0.248	0.088	0.043	0.684
Sterile florets (outer)	0.711	<0.001	0.781	<0.001	0.798	<0.001
Sterile lower florets	0.650	<0.001	0.740	<0.001	0.812	<0.001
Sterile middle florets	0.813	<0.001	0.917	<0.001	0.847	<0.001
Sterile mid-upper florets	0.993	<0.001	0.996	<0.001	0.908	<0.001
Sterile tip	0.621	<0.001	0.769	<0.001	0.745	<0.001
Sterility % lower	0.711	<0.001	0.767	<0.001	0.798	<0.001
Sterility % middle	0.650	<0.001	0.725	<0.001	0.812	<0.001
Sterility % mid-upper	0.813	<0.001	0.902	<0.001	0.847	<0.001
Field sterility GS85	0.580	<0.001	0.847	<0.001	0.568	<0.001
Field sterility GS87	0.230	0.026	0.700	<0.001	0.438	<0.001
Initial lab score	0.623	<0.001	0.871	<0.001	0.491	<0.001

5.3. QTL Analysis

QTL meta-analysis to assess robustness of QTLs indicated that the sterility traits, AFS, OFS, number of sterile florets and number of grains were the most prominent, or significant, with QTL peaks for each population, and across seasons. The analysis below highlights (i) parental and population OFS values for each year, (ii) a genome QTL scan using across years data and (iii) a QTL summary. The genome QTL scan used two methods of computation (Haley-Knott and another one built into the QTL software program) providing virtually identical scores. The scans include a $\log_{10}$ likelihood-ratio or LOD score (y axis), with score above 2 to 2.2. being indicative of a significant QTL.

Population 9M

Table 26 summaries OFS for parents 9 and M, and the population, with parent 9 scoring higher than parent M for this trait. The genome scan indicates several weak QTL (Fig. 11). A QTL summary is given in Table 27.

Various sterility QTL (2009 and 2010 data) were discovered on chromosome 1A, with the OFS QTL peak (in 2010) accounting for 13% of the phenotypic variation. Parent 9 was identified as providing the protection allele. There was also a reverse effect for sterility in the middle ear region in 2011.

On 1D, a QTL for OFS accounted for 15% of the phenotypic variation. Data for individual years were not significant, but mean values for years 2010 to 2011 and years 2010 to 2012 were significant. There was evidence for the QTL peak to be off the end of this linkage group of markers. There were no other sterility QTL in this region. Parent 9 provided the protectant allele.

On 3A, a QTL for ear tip sterility was evident in two years, 2011 and 2012. This accounted for 6-12% of the phenotypic variation. This QTL was co-located with plant height. Parent M was the protectant.

On 6B, an OFS QTL co-located with various sterility traits in 2012 and with grain number in 2010. This QTL peak accounted for 12% of the variation. Parent 9 was the protectant.

On 7D, a weak OFS QTL was evident (in several years), but was significant for years 2009 and 2012 combined, when it accounted for 11% of the phenotypic variation. There was evidence for co-location of OFS with middle-ear sterility in 2012. Parent M provided the protectant allele.

Table 26 Summary of OFS for population 9M including parents in four harvest years.

	2009	2010	2011	2012
Parent 9	20.5%	3.4%	22.1%	22.4%
Parent M	2.5%	2.2%	7.2%	10.9%
Population	8.1%	4.8%	10.4%	13.4%

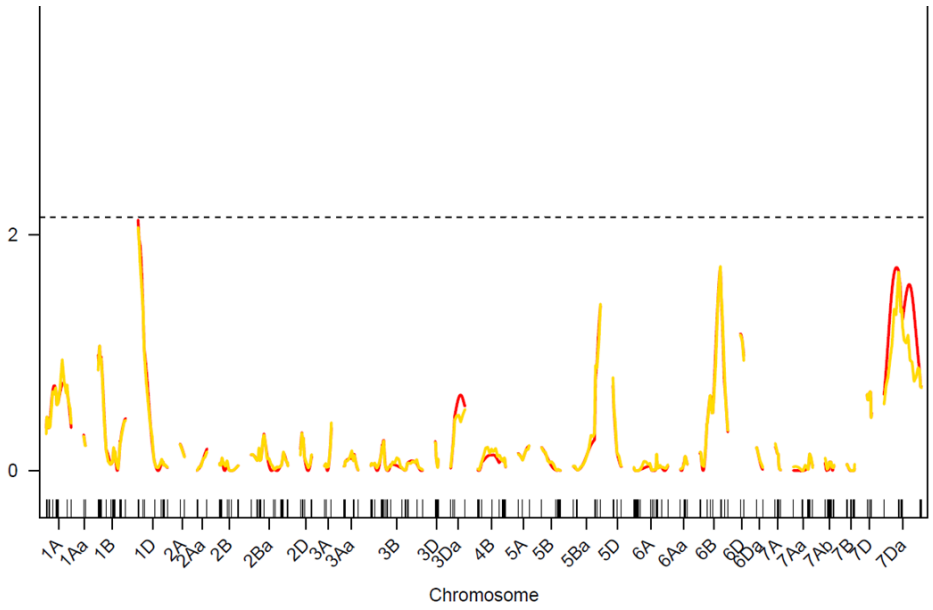


Fig. 11 Genome wide scan for OFS (mean of all years) in population 9M. Two methods of computation (Haley-Knott and in-built QTL software program) provide near indentical approximations. A threshold LOD score (on y-axis) is 2.2.

Table 27 Summary of QTL and co-location of traits in population 9M.

QTL location	QTL	Co-location	Years	% variation	Additive Effect	Protective Parent	Closest Marker	Comments
1A	AFS	Various sterility traits	09 10	13	2.6 (AFS in 2010)	9	wPt6654	Sterility effects in mid ear regions were reversed in 2011
1D	OFS	None	Means	15	3.7	9	wPt7953	Only significant on year means data
3A	Ear tip	None	11 12	6-12	0.1	M	BS00021981	Co-located with height QTL
6B	OFS	Various sterility traits and grain number	10 12	12	1.0	9	wPt4542	Other traits 15cM away
7D	OFS	Various sterility traits	11 09 12?	11	1.3	M	wPt743310	

Population FA

The main chromosomes of interest were: 1A, 3D, 5A and 7A. The main sterility related QTL were on 3D, accounting for 10-15% of the phenotypic variation in both 2011 and 2012.

Depending on the trait, there were strong additive effects from both parents.

Phenotyping of F x A was based on the lab scoring of all florets. To be consistent with other populations which scored outer florets only, it was agreed that the main method of phenotyping would be outer florets.

In 2011, parent F had significantly higher % sterility than parent A, but not in 2012 (Table 28). The genome wide scan indicated two QTLs, with several other weaker peaks (Fig. 12).

On 3D, a QTL for the number of sterile florets for the whole ear was evident in 2011 and 2012, accounting for 10% of phenotypic variation. Parent A was the protectant (Table 29) There was evidence for other sterility QTL explaining 10-15% of phenotypic variation: these included % sterility scores, as well as sterility in different parts of the ear.

On 7A, a QTL for % sterility in all florets was evident in 2011 and 2012, this accounted for 11% of variation, with parent F being the protectant. This QTL co-located with QTL for spikelet number and with field sterility scores in 2012.

Additional QTL were noted for spike architecture on chromosome 5A, grain number (harvest 2012) on 3A and crop height on 6A (data not shown).

Table 28 Summary of OFS for population FA including parents in 2011 and 2012.

	2011	2012
Parent F	24.4%	7.6%
Parent A	5.8%	6.1%
Population	10.1%	9.4%

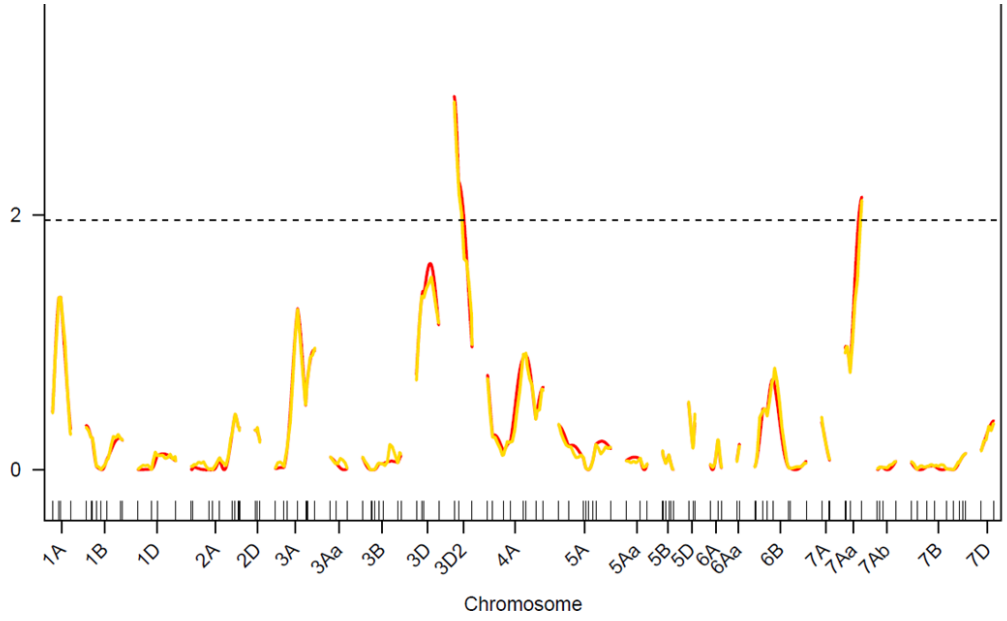


Fig. 12 Genome wide scan for OFS (mean of all years) in population FA. Two methods of computation (Haley-Knott and in-built QTL software program) provide near indentical approximations. A threshold LOD score (on y-axis) is 2.0.

Table 29 Summary of QTL and co-location with other traits in population FA.

QTL location	QTL	Co-location	Years	% variation	Additive Effect	Protection Parent	Closest Marker	Comments
3D	Number of sterile florets (whole ear)	Various sterility	11 12	10	2.1	A	BS00023079	Robust over both years
7A	AFS	Various sterility	11 12	11	1.6 (significant is 2012)	F	BS00030391	Co-locates with QTL for spikelet number. QTL also discovered from field score in 2012

Population Avalon x Cadenza

The QTL overview indicated some weak developmental traits on chromosomes 1D, 3A, 4A and 7B, with some LOD values at 3 or above, and % variation at 15% or above. As expected there were several strong height QTL's, though loci associated with Rht height reduction on 4D was surprisingly weak. There were several weak or moderate QTL's for different ear sterility traits, especially on chromosomes 5A and 7A. Some notable QTL were:

- Field scores of sterility were most evident on 2D, 5A and 6B
- Lab % sterility scores were associated with 1B, 5A and 7A
- Lab sterile floret (absolute value) associated with 1B, 5A and 7A, with poor seed set in the ear tip linked to 5D
- Floret number was associated with 5B and 7B
- Grain number was associated with 1B, 5A and 7A
- Spikelet number associated with 5B and 7B
- Ear length (cm) strongly associated with 2D

There was evidence for some sterility QTL's to be present across markers on 5A and 7A.

Cadenza had significantly higher OFS in than Avalon in 2011, but not in 2012 (Table 30). A genome wide scan indicated significant QTLs at 5A and 7A, with several other weaker QTLs (Fig. 13).

On 5A, a QTL for OFS was evident in both 2011 and 2012. The QTL accounted for 11-14% of phenotypic variation and Cadenza provided the protectant allele. This region was also noted for QTL based on field scores of sterility at GS85.

On 7A, an OFS was present in 2011. This accounted for 11% of the phenotypic variation, with Cadenza being the protectant.

On 1B, QTL for % sterility in middle part of the ear (in 2012) and middle to upper ear (in 2011) accounted for approximately 11% of the variation, with Avalon providing the protectant allele.

On 2D, a field score QTL (in 2011) accounted for 11% of the variation, with Avalon being the protectant.

Table 30 Summary of OFS for population Avalon x Cadenza including parents in 2011 and 2012.

% Sterility OF	2011	2012
Avalon Parent	14.6 %	11.1 %
Cadenza Parent	27.8 %	6.1 %
Population	15.5 %	20.4 %

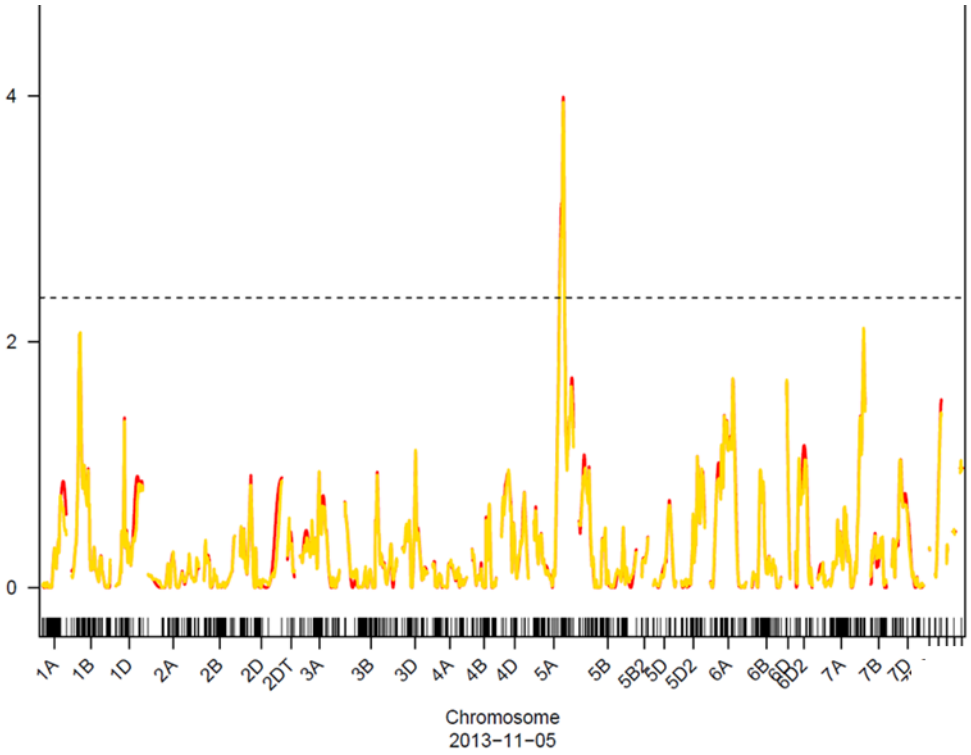


Fig. 13 Genome wide scan for OFS (mean of all years) in population Avalon x Cadenza. Two methods of computation (Haley-Knott and in-built QTL software program) provide near indentical approximations. A threshold LOD score (on y-axis) is 2.3.

Table 31 Summary of QTL and co-location with other traits in population Avalon x Cadenza.

QTL location	QTL	Co-located traits	Years	% variation	Additive Effect	Protection Parent	Closest Marker	Comments
5A	OFS	Various sterility traits	2011 2012	11-14	4.3	Cadenza	gwm126	A good target for further study. Co-located with a field score at GS85
7A	OFS	Various sterility	2011 2012	10	4.1	Cadenza	BS00000663	Environmentally sensitive, 2011 only
1B	Mid ear and upper ear % sterility	None	2011 2012	11	3.7 (for mid ear sterility)	Avalon	BS00022135	Seasonal dependant
2D	Field score at GS85	Field/Lab sterility	2011	11	2.9	Avalon	BS00009575	Close to Rht8.

Population LQ

In 2011, parent L had significantly higher OFS than parent Q, but not in 2012 (Table 32). The genome wide scan indicated several significant QTL, with several other weaker QTL (Fig. 14).

On 1A, a weak OFS (in 2011) QTL was present. This co-located with several other sterility traits. The QTL peak appeared to be just beyond the distal marker and accounted for 9% of phenotypic variation. Parent Q provided the resistant allele.

On 1B, another OFS (in 2011) QTL was evident. This also co-locate with other traits. Its peak was adjacent to the distal marker and accounted for 12% of the variation. By contrast to 1A, parent L provided the resistant allele.

On 2D, a QTL for % sterility in the mid to upper ear was present in 2012 only. This co-located with field scores for sterility. The QTL peak appeared to be just beyond the distal marker; it accounted for 11% of phenotypic variation. Parent L was the protectant.

A third OFS QTL was present on 4A. This was evident in both 2011 and 2012, accounting for 8% of variation in 2012. The protectant parent was Q.

Two stronger OFS QTL were present on 6A and 7A. Both were evident in 2011 and 2012, with Q being the protecting parent. The QTL on 6A accounted for 13-17% of phenotypic variation and was co-located with a field score and with crop height (in 2011 only). The QTL on 7A accounted for 8% of phenotypic variation.

Table 32 Summary of OFS for population LQ including parents in 2011 and 2012.

% Sterility OF	2011	2012
Parent L	24.2%	8.3%
Parent Q	4.8%	6.5%
Population	10.7%	6.8%

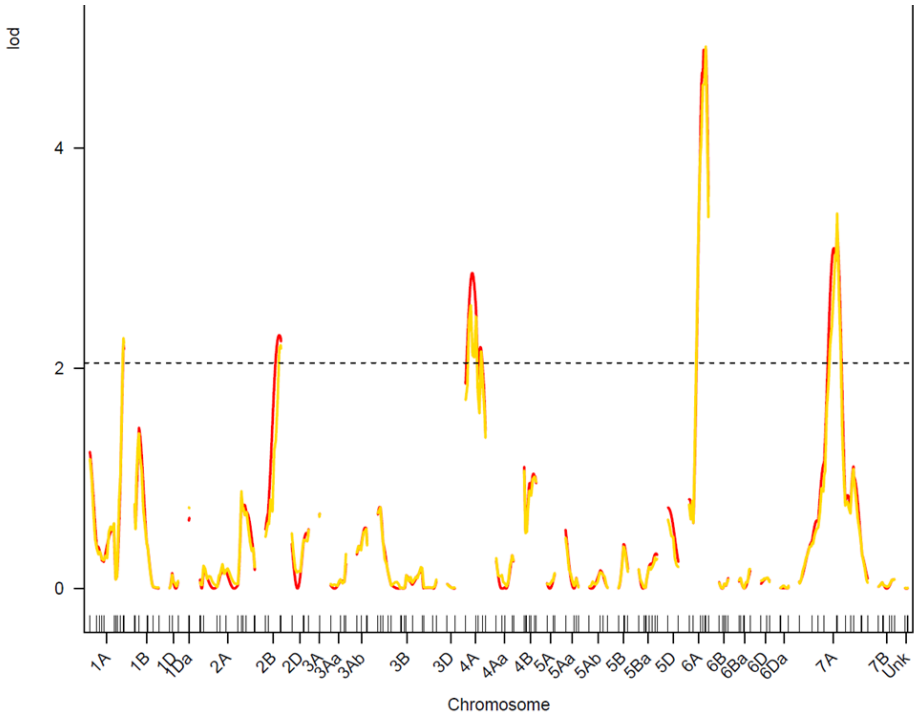


Fig. 14 Genome wide scan for OFS (mean of all years) in population LQ. Two methods of computation (Haley-Knott and in-built QTL software program) provide near indentical approximations. A threshold LOD score (on y-axis) is 2.1.

Table 33 Summary of QTL and co-location with other traits in population LQ.

QTL location	QTL	Co-located traits	Years	% variation	Additive effect	Protection Parent	Closest Marker	Comments
1A	OFS	With other sterility effects	2011	9	2.2	Q	BS00021759	QTL peak is beyond distal marker
1B	OFS	With other sterility effects	2011	12	2.4	L	BS00110209	QTL peak is adjacent to the distal marker
2D	Sterility % in mid-upper ear	With a field score QTL in 2012	2012	11	1.1	L	BS00011109	QTL peak is beyond distal marker. Co-locates with a field score.
4A	OFS	---	2011 2012	8	2.1 (OFS in 2011)	Q	BS00003914	Small but stable effect. A 10cM shift in 2012
6A	OFS	Various sterility	2011 2012	13-17	2.9	Q	BS00023119	Field Score QTL also discovered (2011 and 2012). Co-locates with QTL for crop height 2011 (parent L increasing)
7A	OFS	Various sterility	2011 2012	8	2.2	Q	BS00022895	Peak was highest in 2012

Population TR

Parent T had higher levels of % sterility than parent R in each year (Table 34). The genome wide scan indicated two main QTL based on the all-years mean for % sterility (Fig. 15).

On 2B, a OFS QTL was present in 2012 only; it co-located with other sterility traits and accounted for 9% of phenotypic variation. T was the resistant parent.

On 3B, a second OFS QTL was also present in 2012 only. By contrast, parent R provided the resistant allele, with 5% of the phenotypic variation explained.

Two more significant OFS QTL were located on 4B and 5B. Both were present in 2011 only. On 4B, the QTL was co-located with several other sterility traits, including a field score. It accounted for 13% of phenotypic variation, with parent R being the protectant. The OFS QTL on 5B also co-located with other sterility traits, including a field score of sterility. This QTL accounted for 15% of phenotypic variation with parent T providing the resistant allele.

Table 34 Summary of *OFS* for population TR including parents in 2011 and 2012.

% Sterility OF	2010	2011	2012
Parent T	4.3%	13.7%	17.2%
Parent R	2.1%	8.6%	3.7%
Population	2.8%	8.7%	9.1%

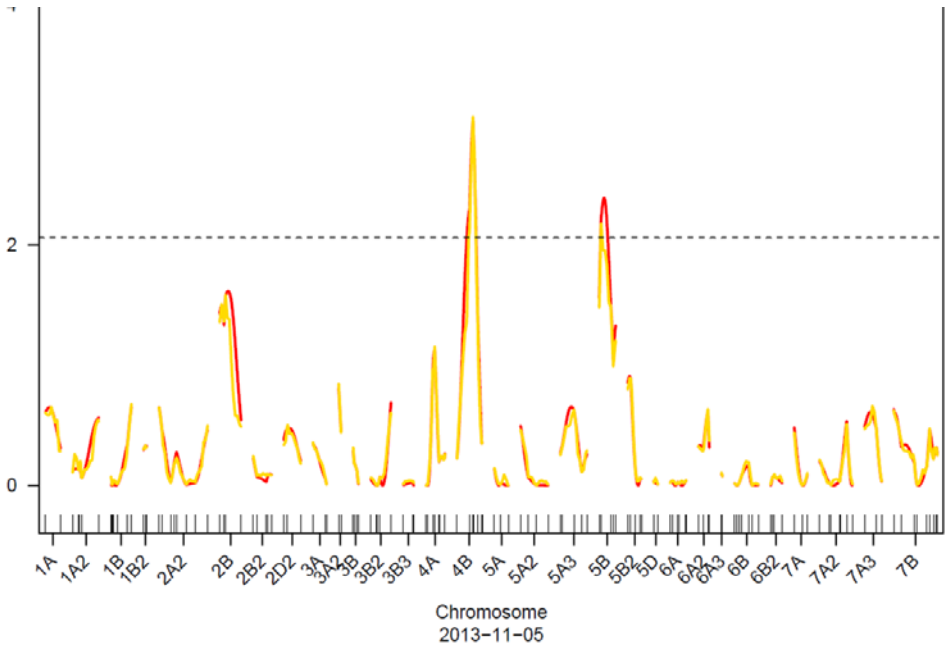


Fig. 15 Genome wide scan for OFS (mean of all years) in population LQ. Two methods of computation (Haley-Knott and in-built QTL software program) provide near indentical approximations. A threshold LOD score (on y-axis) is 2.1.

Table 35 Summary of QTL for OFS and co-location with other traits in population TR.

QTL location	QTL	Traits	Years	% variation	Additive.Effect	Protection Parent	Closest Marker	Comments
2B	OFS	Various sterility	2012	9	1.3	T	BS00072058	Not significant in 2010 or 2011
3B	OFS	---	2012	5	1.2	R	BS00059416	Not significant in 2010 or 2011
4B	OFS	Various sterility	2011	13	2.2	R	BS00067428	Co-locates with other sterility effects, including a field score QTL in 2011
5B	OFS	Various Sterility	2011	15	2.1	T	BS00106043	Co-locates with other sterility effects, including a field score QTL in 2011

A cross-population genome schematic for the most significant *OFS* QTL is presented in Fig. 16. This meta-analysis of the OFS trait was provides a consensus check to validate QTL effects across environments and genetic backgrounds. The analysis highlights an accumulation of several weak to moderately-strong ear sterility related QTL on specific chromosomes. None of these QTL were common across the five populations, although there was evidence for a cluster of QTL on 7A (with three QTL). This provides evidence for a difference in the genetic controls for sterility between varieties.

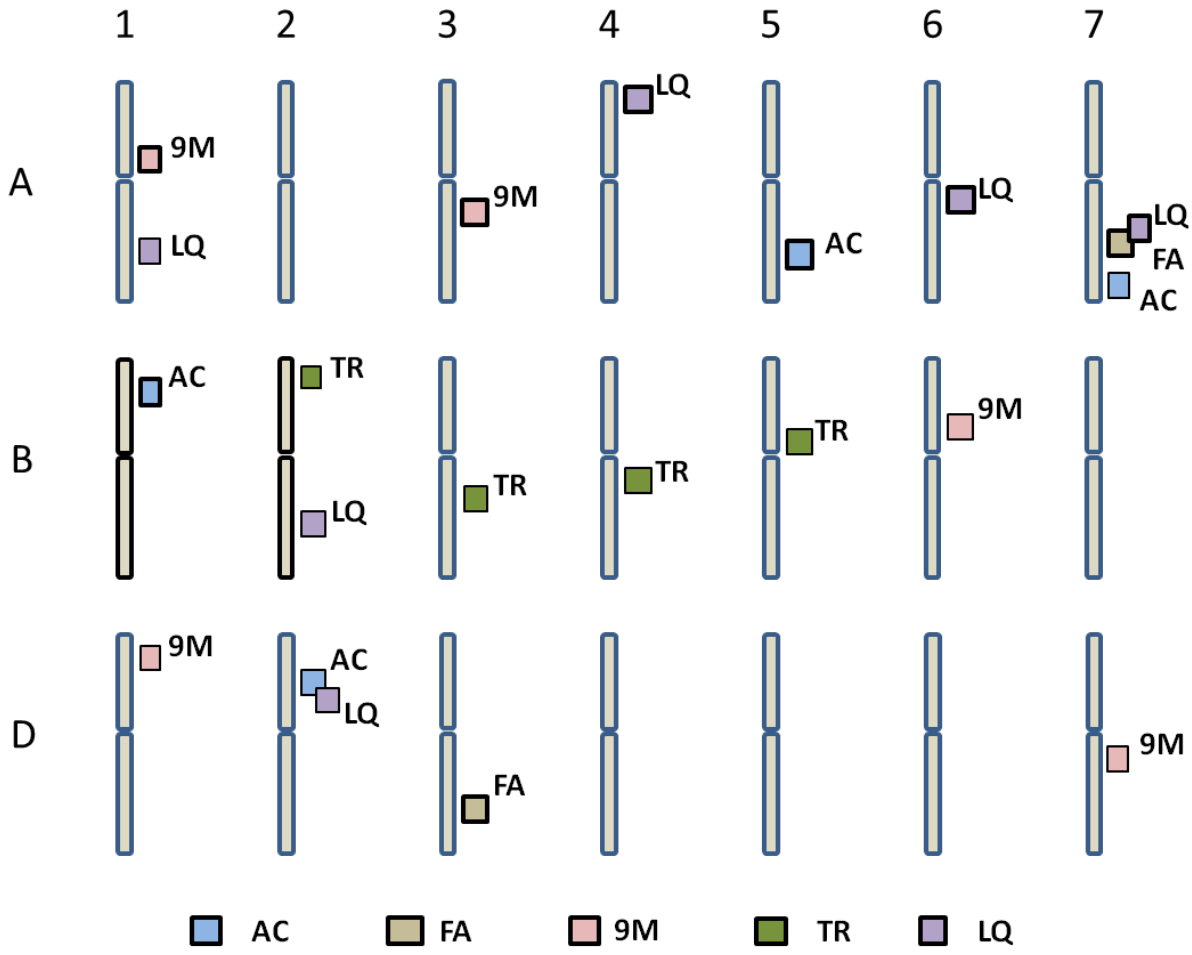


Fig. 16 A cross-population genome schematic for the most significant OFS QTL.

5.4. Phenotyping expression of sterility and weather conditions

5.4.1. Crop development and weather

The date of five key growth stages for each population, in each season, are presented in Table 36. A summary of weather conditions from 1st April to 30th June is shown in Tables 37 to 42.

These data provide a guide to the potential effects of weather conditions on seed set i.e. the timing of each growth stage or growth phase.

As a reference, growth stages for population 9M in years 2009 and 2010 would be typical of crop development in commercially-grown wheat and wheat variety trials in south-east Scotland, with:

- Start of booting GS41 in late May
- Mid booting GS45 early June
- First ear spikelet visible GS51 towards mid June
- Ear fully emerged GS59 just after mid June
- Flowering between GS65-69 20th to 22nd June

Across the populations, crop spring growth i.e. stem extension was relatively early in 2011, but late in 2012. These extremes extended throughout the remainder of crop development. Consequently, crops in 2011 were at growth stages - from mid booting to ear emergence were 4-10 days earlier than average, with flowering approximately 4 days later than typical. By contrast, crops in 2012 were 7-9 days later than average - from mid booting to ear emergence, with flowering approximately 7 days late.

Although there was wide variation within populations, FA and TR tended to be later in developmental phases from GS41 to GS65-69. Populations Avalon x Cadenza and LQ were earliest on average, with population 9M intermediate.

The overall seasonal differences on crop development were consistent with relatively warm April and early May temperatures in 2011, and with relatively cold April to early May in 2012.

Mean daily temperature during late spring and early summer were relatively high in 2009 and 2010, and moderate in 2011 and 2012. Rainfall was high in 2012.

Spring daily minimum temperatures were lowest in 2012, low-moderate in 2010 and relatively high in 2009 and 2011.

Throughout April to June, solar radiation was relatively high in 2009 and 2011, but low in 2010 and 2012. Wind speeds were relatively high in 2009 and 2012 (Figs. 17 and 18).

5.4.2. Association between sterility and weather

Correlations between date of growth stage and sterility among lines presented in Tables 18 to 25 could be negative or positive. Furthermore, some population by season combinations indicated no association between crop development and sterility. Thus, there was lack of agreement or consistency in the direction of correlation (positive or negative) between growth stage and sterility between different populations within a season, or for the same population between seasons.

For example, in 2009, sterility was negatively and significantly associated with growth stage i.e. advanced crop growth stages correlated with low sterility. By contrast, sterility was significantly and positively correlated with growth stage in 2010 i.e. with backwards crop development.

A more precise assessment of weather effects on ear sterility can be provided by more detailed analysis of weather patterns in relation to development of each population. Tables 41 to 44 summarise the most significant effects of temperature and radiation on sterility in population FA in 2011. This was chosen as an example of wide phenotypic expression within a population, with differentiation between the parents, and data for both outer florets and all florets.

The association of several weather conditions on OFS for population FA in 2011 indicates several weak but significant trends as possible explanatory environmental causes of sterility.

A reduction in minimum daily temperature (i.e. night temperature) by 1^{o}C at crop booting stage (GS45) and preceding days increased sterility by 1% to 10.5%, with the effect becoming more pronounced following several days of low temperature between 5 to 10 days before GS45 (Table 41).

The difference between daily maximum to minimum temperature had both positive and negative effects on OFS. Most pronounced was an increase in OFS by 2.6% to 4.0% for each 1^{o}C increase in the min-max difference when the crop was between 5 to 10 days before GS45 (Table 42).

The effect of radiation on sterility was not consistent, but the most significant effect was an increase in OFS of 4.6% to 5.8% for a decrease in radiation by 100 W m^{-2} during the period 1 to 7 days before GS45 (Table 43).

A composite of low temperature and radiation also indicates a particular sensitivity in the growth phase between 1-7 or 5-7 days before GS45, when low radiation and minimum temperature increased OFS by 5.3% and 2.5%, respectively (Table 44).

Table 36 Date of five key growth stages (booting, ear emergence and flowering) columns for each population in each season. Dates are the mean of all lines in the population. The number in **parenthesis** indicates the number of days +/- mid-booting (GS45). The sterility data are for the population mean and parents.

			2009	2010		2011					2012				
			9M	9M	TR	9M	FA	AC*	LQ	TR	9M	FA	AC*	LQ	TR
Booting	Start of booting, flag leaf sheath extending	GS41	26 May [**-4**]	27 May [**-7**]	01 June [**-6**]	24 May [**-6**]	29 May [**-7**]	23 May [**-6**]	23 May [**-5**]	24 May [**-5**]	1 June [**-5**]	2 June [**-6**]	2 June [**-6**]	31 May [**-7**]	1 June [**-7**]
	Mid booting	GS45	31 May [**0**]	3 June [**0**]	7 June [**0**]	30 May [**0**]	5 June [**0**]	29 May [**0**]	28 May [**0**]	29 May [**0**]	6 June [**0**]	8 June [**0**]	8 June [**0**]	7 June [**0**]	8 June [**0**]
Ear emergence	First spikelet visible	GS51	9 June [**9**]	10 June [**7**]	13 June [**6**]	7 June [**8**]	12 June [**7**]	3 June [**5**]	3 June [**5**]	5 June [**7**]	14 June [**8**]	18 June [**10**]	15 June [**7**]	15 June [**8**]	15 June [**8**]
	Ear fully emerged	GS59	18 June [**18**]	19 June [**16**]	22 June [**15**]	15 June [**16**]	20 June [**15**]	12 June [**14**]	12 June [**15**]	15 June [**17**]	25 June [**19**]	26 June [**18**]	23 June [**15**]	25 June [**18**]	25 June [**17**]
Flowering	Anthesis half-way to completed	GS65-69	20 June [**20**]	22 June [**19**]	25 June [**18**]	20 June [**21**]	24 June [**19**]	16 June [**18**]	17 June [**20**]	21 June [**23**]	27 June [**22**]	28 June [**22**]	27 June [**19**]	27 June [**20**]	28 June [**20**]
Sterility in outer florets	Population mean	*OFS*	8.21	4.77	2.75	10.36	10.13	15.79	10.82	8.73	13.14	9.38	20.6	6.77	9.05
	Parent 1		20.47	3.4	4.28	22.05	24.37	14.37	24.19	13.35	22.41	7.62	11.05	8.26	17.19
	Parent 2		2.46	2.17	2.06	7.23	5.82	27.82	4.81	8.62	10.94	6.72	6.12	6.46	3.70

***** Avalon x Cadenza

Table 37 Summary of daily weather at East trials site for calendar periods from 1st April to 30th June and expected crop growth phases for South-east Scotland for 2009. Growth phases are summarised from SRUC Crop Protection Report.

Date	Growth phase	Decimal growth stage	Temperature mean (°C)	Temperature mimumum (°C)	Temperature maximum (°C)	Solar radiation (kW m^{-2})	Rainfall (mm)	Wind speed (m s^{-1})
April 1 to 15	Tillering to psuedostem erect (GS30)	GS23-30	9.3	5.8	13.3	0.27	0.55	3.5
April 16 to 30	End of tillering to start of stem extension	GS25-31	9.5	6.6	12.8	0.35	0.61	3.3
May 1 to 15	Stem extension to second node	GS31-32	9.3	5.5	13.4	0.47	1.17	4.8
May 16 to 31	Stem extension to flag leaf sheath emergence	GS37-41	12.4	8.6	16.8	0.49	1.35	3.0
June 1 to 15	Booting and ear emergence	GS45-57	11.1	7.2	15.2	0.50	1.79	2.3
June 16 to 30	Ears fully emerged and flowering	GS59-69	14.3	11.8	17.8	0.43	0.19	2.9

Table 38 Summary of daily weather at East trials site for calendar periods from 1st April to 30th June and expected crop growth phases for south-east Scotland for 2010. Growth phases are summarised from SRUC Crop Protection Report.

Date	Growth phase	Decimal growth stage	Temperature mean (°C)	Temperature mimumum (°C)	Temperature maximum (°C)	Solar radiation (kW m^{-2})	Rainfall (mm)	Wind speed (m s^{-1})
April 1 to 15	Tillering to psuedostem erect (GS30)	GS23-30	6.79	3.90	11.00	0.34	1.95	2.63
April 16 to 30	End of tillering to start of stem extension	GS25-31	8.35	5.11	13.02	0.32	0.63	3.30
May 1 to 15	Stem extension to second node	GS31-32	6.66	3.26	11.10	0.35	1.44	2.32
May 16 to 31	Stem extension to flag leaf sheath emergence	GS37-41	11.50	7.45	16.69	0.38	0.78	1.81
June 1 to 15	Booting and ear emergence	GS45-59	12.16	8.74	16.62	0.32	1.99	1.97
June 16 to 30	Ears emerged and flowering	GS59-69	15.15	10.56	20.69	0.36	0.20	1.67

Table 39 Summary of daily weather at East trials site for calendar periods from 1st April to 30th June and expected crop growth phases for south-east Scotland for 2011. Growth phases are summarised from SRUC Crop Protection Report.

Date	Growth phase	Decimal growth stage	Temperature mean (°C)	Temperature mimumum (°C)	Temperature maximum (°C)	Solar radiation (kW m^{-2})	Rainfall (mm)	Wind speed (m s^{-1})
April 1 to 15	Tillering to psuedostem erect (GS30)	GS23-30	10.83	7.45	14.91	0.40	0.04	2.45
April 16 to 30	End of tillering to start of stem extension	GS25-31	10.05	4.65	16.04	0.49	0.23	1.75
May 1 to 15	Stem extension to second node	GS31-32	11.00	6.54	16.47	0.48	0.41	2.07
May 16 to 31	Stem extension to flag leaf sheath emergence	GS37-41	10.22	7.07	13.82	0.40	1.46	2.18
June 1 to 15	Booting and ear emergence	GS45-59	11.43	7.37	16.14	0.43	1.68	1.69
June 16 to 30	Ears emerged and flowering	GS59-69	12.69	9.34	16.86	0.35	4.33	1.06

Table 40 Summary of daily weather at East trials site for calendar periods from 1st April to 30th June and expected crop growth phases for south-east Scotland for 2012. Growth phases are summarised from SRUC Crop Protection Report.

Date	Growth phase	Decimal growth stage	Temperature mean (°C)	Temperature mimumum (°C)	Temperature maximum (°C)	Solar radiation (kW m^{-2})	Rainfall (mm)	Wind speed (m s^{-1})
April 1 to 15	Tillering to psuedostem erect (GS30)	GS23-30	5.42	2.34	8.92	0.25	5.43	3.37
April 16 to 30	End of tillering to start of stem extension	GS25-31	6.04	3.86	8.87	0.25	4.20	3.29
May 1 to 15	Stem extension to second node	GS31-32	6.76	3.96	10.51	0.32	4.16	3.12
May 16 to 31	Stem extension to flag leaf sheath emergence	GS37-41	10.97	6.74	15.80	0.38	2.15	1.80
June 1 to 15	Booting and ear emergence	GS45-59	10.24	7.46	13.54	0.33	3.40	2.06
June 16 to 30	Ears emerged and flowering	GS59-69	12.19	9.28	15.96	0.29	6.66	1.92

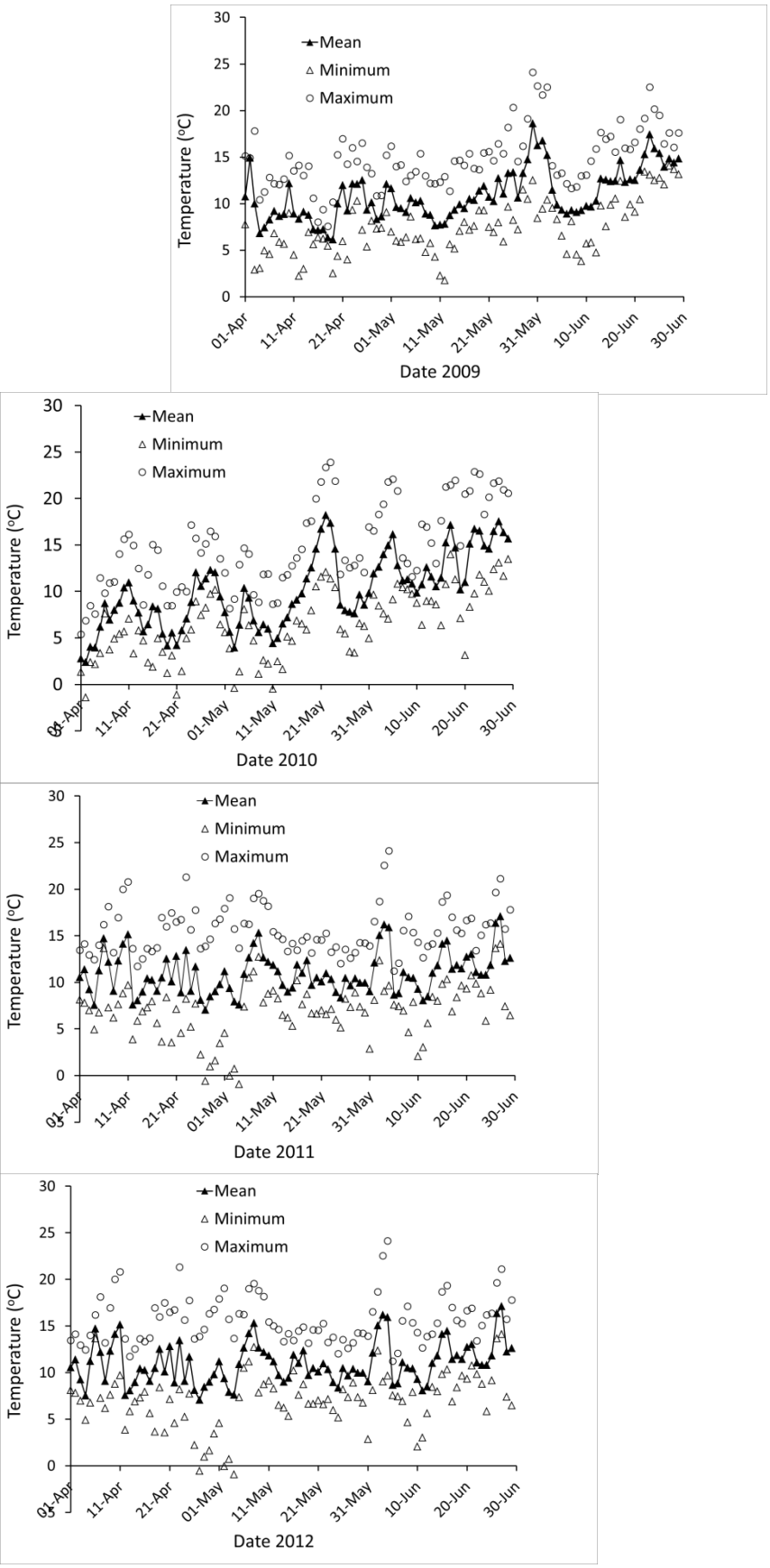
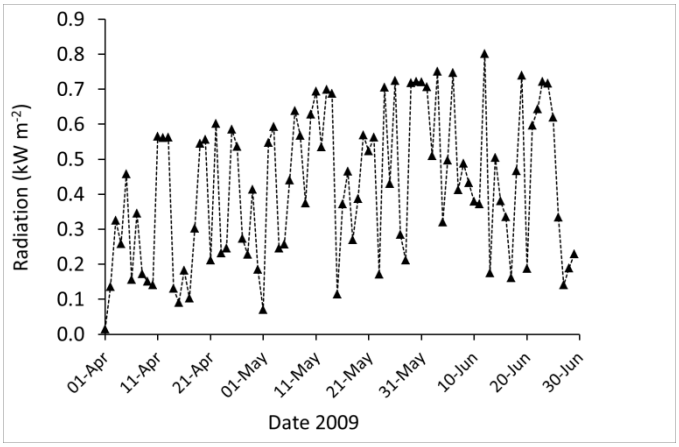
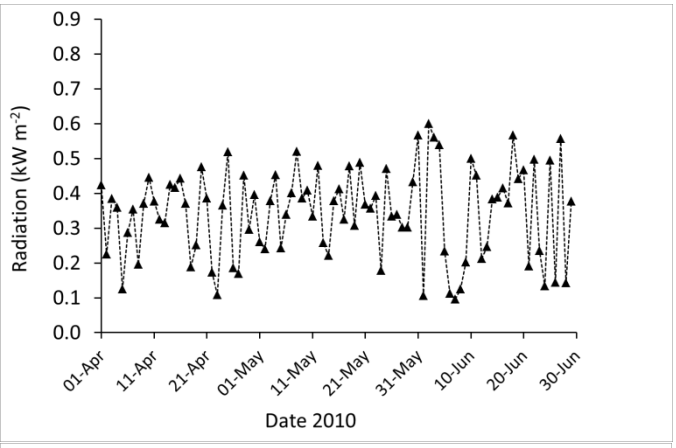
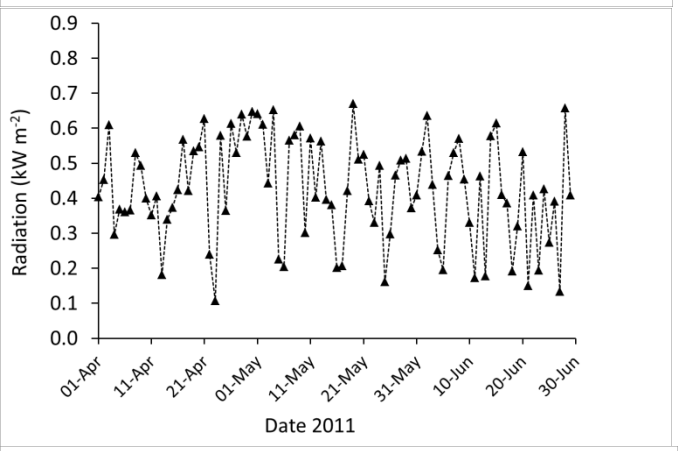


Fig. 17 Daily mean minimum and maximum temperature from 1st April to 30 June in years 2009 to 2012.







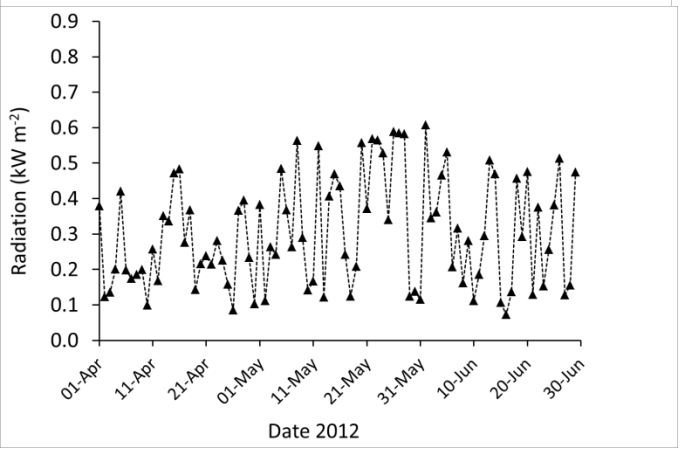


Fig. 18 Solar radiation as daily mean from 1st April to 30 June in years 2009 to 2012.

Table 41 Influence of minimum daily temperature on OFS in population FA during season 2010/11.

Minimum temperature on day or days preceding GS45	Correlation coefficient (r)	Slope of line - A negative value indicates the % increase in sterility per 1 ^{0}C reduction in minimum temperature	Significance level 0.05 = * 0.01 = ** Non sig = ns
Day of GS45 (day 0)	0.255	-0.961	*
6 days before (-6)	0.252	-1.786	*
Mean of day 0 and previous day (-1)	0.228	-1.039	*
Days -5 to -7	0.203	-2.255	*
Days -5 to -9	0.232	-5.195	*
Days -6 to -10	0.289	-10.558	*

Table 42 Influence of maximum to minimum temperature difference on OFS in population FA during season 2010/11.

Difference in temperature on day or days preceding GS45	Correlation coefficient (r)	Slope of line - A positive value indicates the % increase in sterility per 1 ^{0}C difference in the min to max temperature	Significance level 0.05 = * 0.01 = ** Non sig = ns
Day of GS45 minus 3 (-3)	0.288	-1.247	**
Day -6	0.172	+1.119	Ns
Day -7	0.244	+1.917	*
Day -9	0.246	+2.549	*
Days -1 to -3	0.238	-1.195	*
Days -1 to -4	0.258	-1.564	**
Days -1 to -5	0.279	-2.223	**
Days -1 to -7	0.247	-3.633	*
Days -5 to -9	0.226	+2.691	*
Days -6 to -10	0.266	+3.479	**
Days -7 to -9	0.293	+3.482	**
Days -7 to -10	0.276	+4.039	**

Table 43 Influence of daily mean solar radiation on OFS in population FA during season 2010/11.

Difference in temperature on day or days preceding GS45	Correlation coefficient (r)	Slope of line - A negative value indicates the % increase in sterility per 100 W m^{-2} reduction in daily radiation	Significance level 0.05 = * 0.01 = ** Non sig = ns
Day of GS45 minus 10 (-10)	0.195	+1.592	Ns
Days -1 to -4	0.167	-3.951	Ns
Days -1 to -5	0.222	-4.655	*
Days -1 to -7	0.248	-5.811	*
Days -5 to -7	0.195	-2.485	Ns
Days -7 to -9	0.194	+2.879	Ns
Days -7 to -10	0.214	+2.909	*

Table 44 Influence of daily minimum temperature and radiation multiple on OFS in population FA during season 2010/11.

Difference in temperature on day or days preceding GS45	Correlation coefficient (r)	Slope of line - A negative value indicates that lower radiation x minimum temperature increased % sterility	Significance level 0.05 = * 0.01 = ** Non sig = ns
Day of GS45 minus 6	0.207	-1.926	*
Day -10	0.237	+2.895	*
Mean of day 0 and previous day (-1)	0.196	-1.292	Ns
Days -1 to -5	0.192	-5.247	Ns
Days -1 to -7	0.236	-5.297	*
Days -5 to -7	0.215	-2.534	*

5.4.3. Relationship between sterility and yield

Bulking seed of selected lines for population 9M from harvest 2010 gave an opportunity to test the relationship between sterility and grain yield. For harvest 2011, both parents and sixteen lines, expressing a range of OFS and AFS in 2009 and 2010 were grown in yielded plots at SRUC's East Lothian site. Fig. 19 shows that across these lines the relationship between OFS and yield to be a reduction of 67 kg grain ha-1 per 1% increase in sterility.

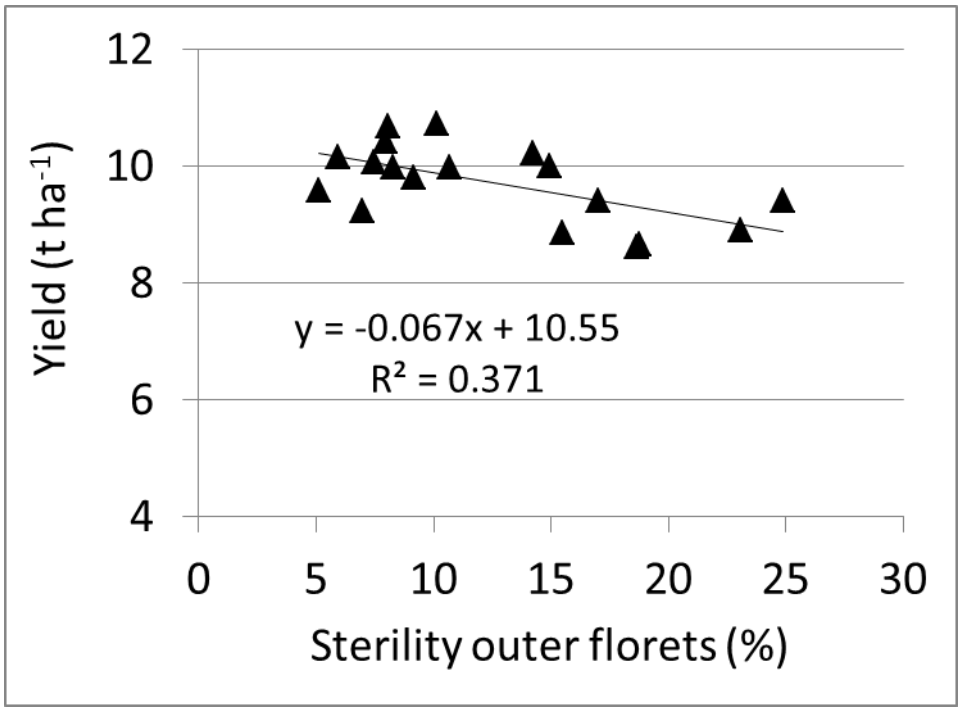


Fig. 19 Relationship between sterility in outer florets and grain yield in lines from population 9M grown in East Lothian 2011.

In 2012, the same lines from 9M were grown in yielded plots in Cambridgeshire and East Lothian. Here, the objective was to examine the relationship between sterility and yield at locations that might experince low and moderate-high levels of ear sterility at Cambridge and East Lothian, respectively. In the same lines, OFS ranged from 3.8% to 8.9% in Cambridge (Fig. 20) and from 5.1% to 20.6% in East Lothian (Fig. 21). Interestingly, there was higher level of yield loss per % increase in sterility at Cambridge compared to East Lothian, with a yield reduction of 135 kg ha^{-1} per 1% change in OFS at Cambridge, compared to
49 kg ha^{-1} in East Lothian.

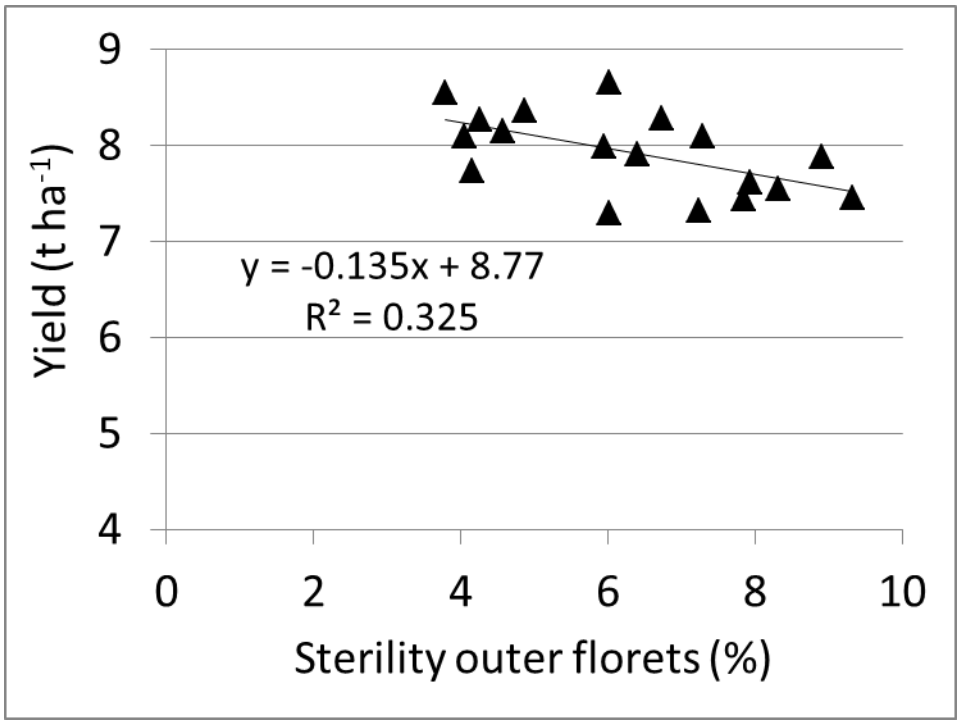


Fig. 20 Relationship between sterility in outer florets and grain yield in lines from population 9M grown in Cambridgeshire 2012.

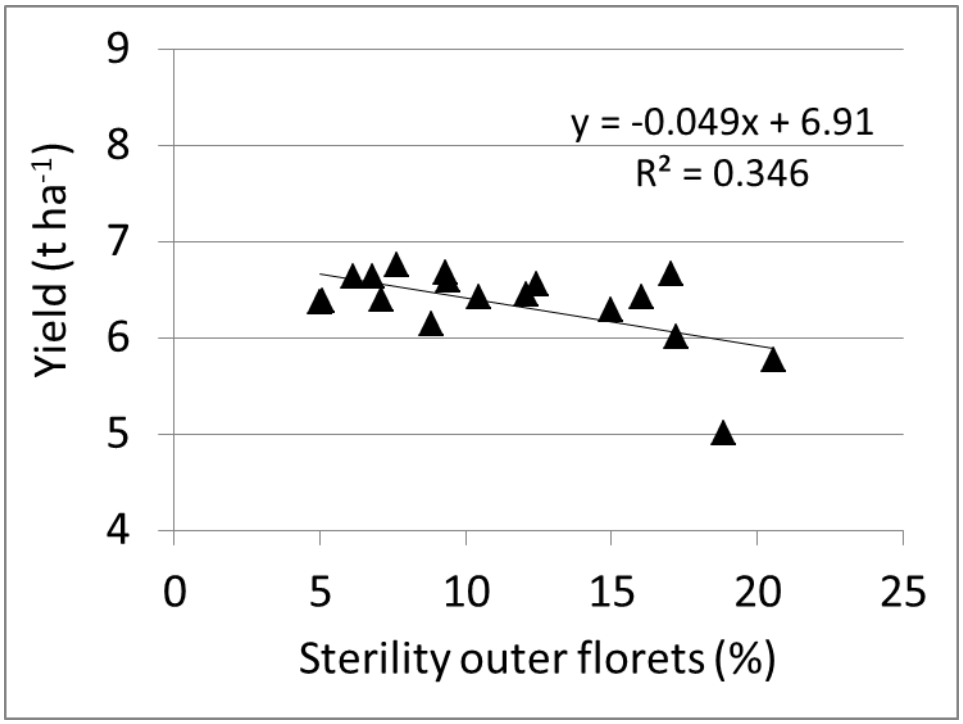


Fig. 21 Relationship between sterility in outer florets and grain yield in lines from population 9M grown in East Lothian 2012.

The relationship between OFS among lines grown at Cambridge and Edinburgh in 2012 was poor (Fig. 22).

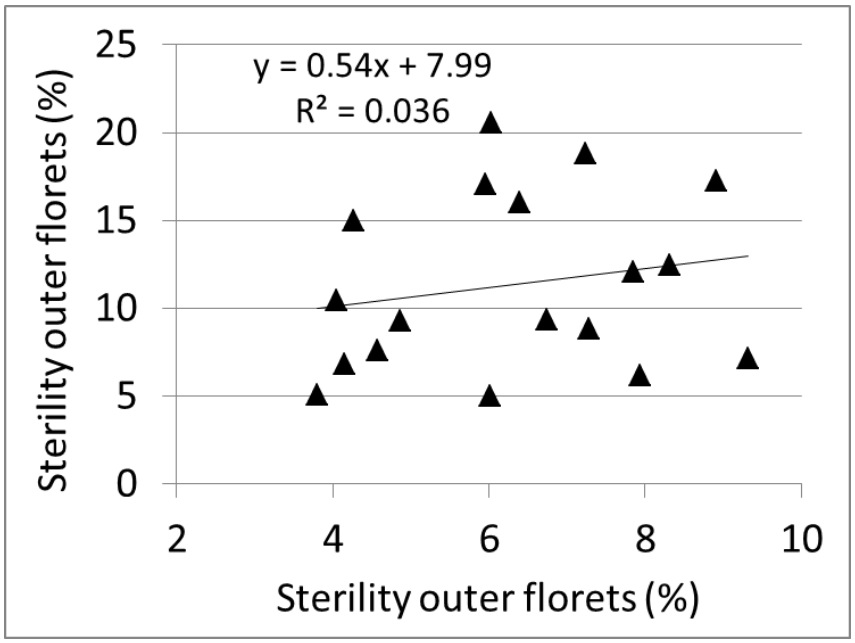


Fig. 22 Relationship between sterility in outer florets in lines from population 9M grown in Cambridgeshire (x-axis) and East Lothian (y-axis) in 2011.

5.5. Development of phenotypic screening protocols for sterility

5.5.1. Assessment of sterility in the field

Two early examples of scales used by SRUC to score sterility in field plots are presented in Table 40. Both scales were used by SRUC between 2004 and 2008, prior to this current project and are comparable with the scales (methods) used for data presented in Tables 1 and 2 and Fig. 2. Sterility was assessed by looking, close up, across a plot to derive a representative score. An experienced assessor may be able to estimate sterility in a 'group of ears' across a unit area e.g. 0.5 m^2 of a plot. More precision and less bias may added by scoring several hand-held ears within each plot

Table 40 Assessment of sterility in the field. (a) A five-point scale used to assess the level of sterility. This scale was used to assess wheat plots in RL and NL trials across several sites in 2004/05, 2005/06 and 2006/07. (b) A seven-point scale used to assess the level of sterility, including an estimate of potential yield loss. This scale is a modified version of (a) and will form the basis of field assessments in the Work Plan, described above.

(a)

Score	Level of sterility
1	none or very low
2	low-moderate
3	moderate
4	moderate to high
5	very high

(b)

Score	Level of sterility	Expected yield loss
1	none or very low	None
2	low	Undetected
3	low-moderate	Low or some yield loss
4	moderate	Yield loss expected
5	moderate-poor	Yield loss expected
6	poor	High yield loss
7	very poor	Very high yield loss

Protocols for field scoring of sterility were modified during the project. These 9-point scales range for no sterility (1) to extremely high (9). Fig 24 indicates the scale used in 2009 and 2010. While this scale and description was adequate when a wide range of sterility was present e.g. from 2 to 7, it was less to differentiate between lower scores e.g. from 2 to 5. A revised scale, with new descriptions, was introduced. This improved the correlation between the lab assessments and field score. Tables 20 to 25 indicate relatively high and significant correlation coefficients.

Score	Description
1	Very good ear – no sterility
2	No sterility
3	Suspicion of low level (<5%)
4	Evidence of low level (~ 0%)
5	Moderate levels (15-20%)
6	Moderate to high (20-40%)
7	High (>40%)
8	Very high (>60%)
9	Extreme (>80%)

Fig. 24 SRUC / WESP field sterility score, version for harvest 2009 and 2010.

Score	Description
1	Very good ear – no sterility
2	Very good ear – trace levels
3	Weakness across many ears, or occasional weak ears
4	Low to moderate across most ears, or moderate in a few
5	Moderate with 6-8 florets across most ears
6	Moderate to high, with weakness in outer florets, or very weak tips
7	High with thinning across most ears, often with very poor tips
8	Most ears are thin, with a few grains only
9	All ears are thin, with a few or no grains

Fig. 25 SRUC / WESP field sterility score, version for harvest 2011.

For some season x population combinations, use of a curvilinear equation e.g. quadratic improved the fit between the field score and laboratory assessment. Fig. 26 shows the relationship between a field sterility score at GS87 and the lab assessment for population LQ in 2011. Fig. 27 shows a strong relationship between the field and laboratory scores when using the lab scores for all florets. Encouragingly, the field score was also strongly related to the lab assessment of outer florets (Fig. 28).

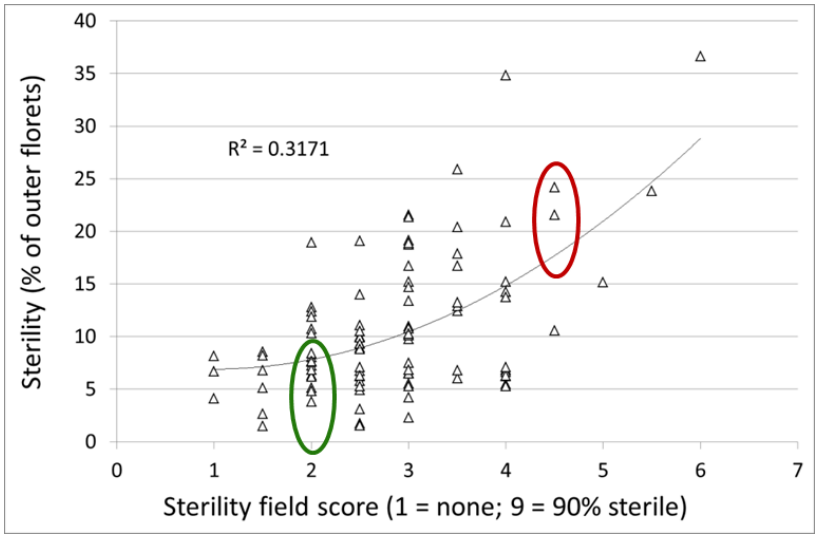


Fig. 26 Relationship between field sterility score on 1 to 9 scale (x-axis) and laboratory assessment of sterility in all florets (y-axis) for population LQ in 2011. The green (lower) and red (upper) circles indicate clusters within which the resistant (Q) and susceptible (L) parents are located.

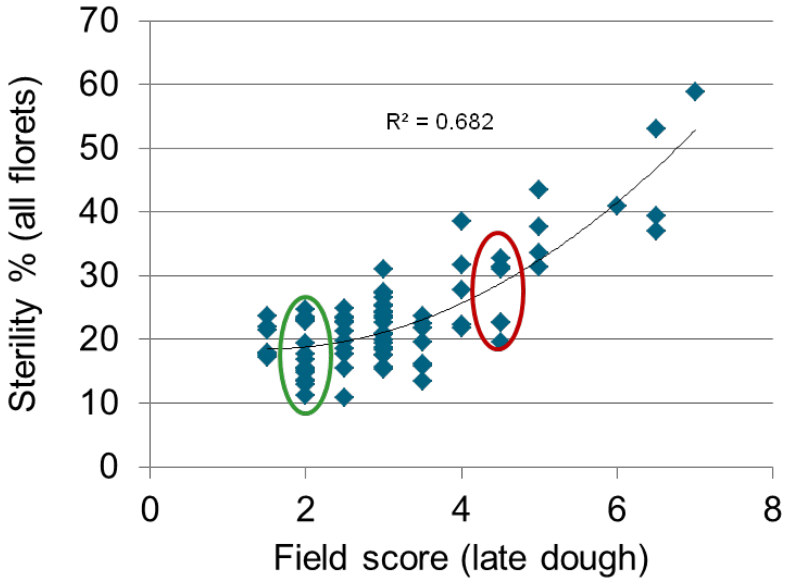


Fig. 27 Relationship between field sterility score at late dough growth stage on 1 to 9 scale and laboratory assessment of sterility % in all florets for population FA in 2011. The green (lower) and red (upper) circles indicate clusters within which the resistant (A) and susceptible (F) parents are located.

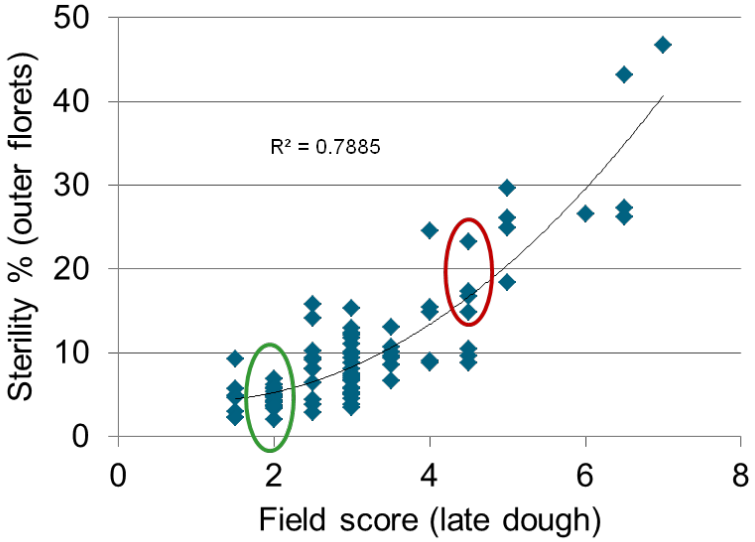


Fig. 28 Relationship between field sterility score at late dough growth stage on 1 to 9 scale and laboratory assessment of sterility % in outer florets for population FA in 2011. The green (lower) and red (upper) circles indicate clusters within which the resistant (A) and susceptible (F) parents are located.

6. DISCUSSION

6.1. Phenotyping and expression of sterility

In each season, there was a wide range of phenotypic expression – from very few sterile florets (< 5%) to moderate-high levels (>30%).

The field screen during the project had not experienced a “Moulin year” in which there was devastating sterility (i.e. across many lines).

In each population, one parent tended to be weaker than the other. However, there was also seasonal variation when neither parent was weak for sterility. In no season were both parents weak for sterility.

The year with the most differentiation between a strong and weak parent was 2011.

We suggest that the best metric for a seasonal measure of sterility is the population mean, rather than the parent values. Both parents appeared to have some protective function, as evident in the QTL analysis.

Consideration of varietal pedigree needs to be followed up – with consideration of genetic links through grandparents. For example, pedigree through to Moulin, Rendezvous, Cordiale and Cadenza, from which weaknesses in seed set are implicated.

Phenotype data provided insight into the value of measuring seed set in either all florets or outer florets only. This relates to how best to assess different ear types (genotypes) and how to account for inherent variation in seed set (across genotypes) that may be independent of climate-induced sterility.

As all genotypes should have the potential to set seed in all outer florets, then the ‘outer-floret’ method remained the most useful comparison for all genotypes or ear types. Nevertheless, assessment of all florets would help identify patterns of seed set and yield loss across ear types.

Assessment of all florets would over-estimate sterility in genotypes that only set seed in outer florets, or in a limited number of inner florets, regardless of environmental conditions.

Protocols for assessing sterility need to take into account the fact that assessment of outer florets should have high accuracy and low bias, whereas assessment of all florets could be more subjective when assessing inner florets, especially 5th or 6th florets in a spikelet.

Environmental effects on ear sterility were evident in the correlations between crop growth stage and ear sterility. These associations represent a main effect of advanced (forward) and delayed (backwards) crop growth has had on the expression sterility in a season. The highly variable weather between and within seasons means that unravelling precise weather triggers requires a more detailed assessment of crop growth stage.

The lack of consistency in correlations between crop growth stage and sterility within populations and/or seasons would support multiple trigger points for inducing sterility.

Phenotypic data included ear sterility and plant development (as growth stages). In the QTL analysis, several developmental traits and sterility scores had statistical significance with lod values above 2, and with % of phenotypic variation at 14-20%.

6.2. QTL analysis

Overall, there were a large number of weakly significant QTL in all populations. Apart from a cluster of QTL on 7A (with three QTL), there were no strong common regions for QTL across the population genome.

The QTL summary implicated multiple QTL from different parents, or possible epistatic effects. There was some evidence of markers revealing linkage groups.

Chromosomes 1A, 5A and 7A had accumulated several weak sterility related QTL's. Plant development and sterility QTL's were often correlated, by seasonally dependant.

Some encouragement was provided when two or different sterility related traits were co-located. However, these association were not necessarily common across populations.

Key questions to address include; are common regions not yet covered in our analysis and what proportion of the genome might be missing?

QTL analysis indicated that population LQ was of particular value, though some QTL need more marker work to validate regions.

The project considers a case for backcrossing several lines from population LQ to clean up QTL on 4A, 6A and 7A and from Avalon x Cadenza for a QTL on 5A.

Other key observations included:

Chromosomes 2B and 3B had flowering time or growth stage QTL's consistent with previous reports elsewhere.

Chromosome 5A carries a gene for ear morphology (Q gene) and a VRN locus for ear free threshing, as reported elsewhere.

9M had QTL on 1A short and 7A. The latter appears to be co-located with a QTL on Avalon x Cadenza.

In 7A, there was evidence for a sterility QTL in FA, with other sterility QTLs (with better coverage) in Avalon x Cadenza.

In Avalon x Cadenza there was a sterility effect evident in 5A, though this was not in the same location as ear morphology in FA.

6.3. Improving field assessment of sterility

Early in the project there was discussion about the poor correlation between laboratory assessments and field scoring of sterility. Improving this relationship required re-evaluation of the field scoring system. For example, differentiating between low or moderate levels of sterility that might be present in a large proportion of ears, or high levels of sterility in a few ears.

Another issue was that the correlation between lab and field was improved when all florets were included in the lab assessment.

Revision of the field scoring protocol included a more detailed descriptive guide to improve the association between outer floret sterility and field scores.

Revision of the field scoring protocol improved the correlation between the lab and field scores. In some seasons, or season x population combinations, the relationship between lab and field sterility was curvilinear e.g. a quadratic equation gave an improved fit.

6.4. Ongoing and future work

SRUC and JIC will continue field monitoring in one or more populations. We will focus on traits that are most robust, and consistent across seasons. Unstable QTL, or evidence for genotype by environment interaction is scientifically interesting, but hard to work with.

In terms of future plant resources, the WESP consortium will maintain populations for scoring in future field trials. Varietal pedigree through to Moulin, Rendezvous, Cordiale and Cadenza, from which weaknesses in seed set are implicated, will also be considered.

JIC will continue the project interests through back crossing for development of near isogenic lines (NILs) towards identifying more promising QTL stacks.

NILs will be developed to validate regions for the following robust QTL:
FxA on chromosome 3D
Avalon x Cadenza on chromosomes 5A and 7A
LxQ on chromsomes 1A, 4A, 6A and 7A

Lines homozygous across the regions of four QTL (1A,4A, 6A, 7A) for the LxQ population were analysed to establish the benefits of stacking protective QTL for OFS (Fig. A) Here, the resistance to sterility (reduced % sterility) is increased with an increase in the number of QTL.

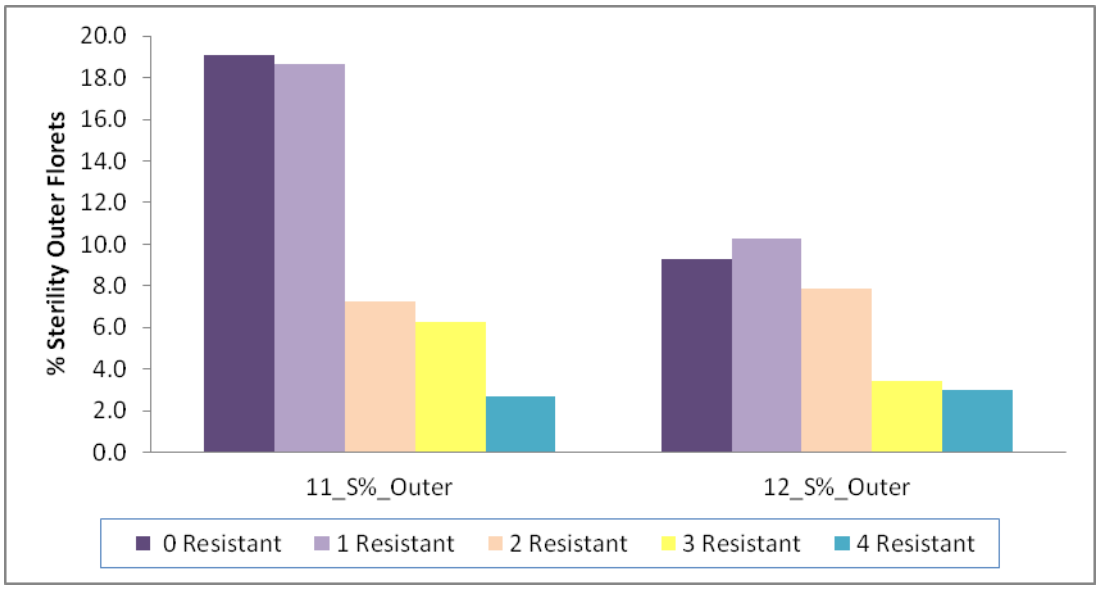


Fig. A. Influence of stacking resistant QTL from the LxQ population based on OFS scored in 2011 and 2012. The bars show lines with either 0, 1, 2, 3 or 4 protective QTL for OFS, recorded as % sterility in the outer florets in 2011 (11_S%_Outer) and 2012 (12_S%_Outer).

The same QTL stacking analysis was performed to analyse the two most significant OFS QTL in the Avalon x Cadenza population (5A/7A) (Fig. B).

Fig. B Influence of stacking resistant QTL from the Avalon x Cadenza population based on OFS scored in 2011 and 2012. The bars show lines with either 0, 1 or 2 protective QTL for OFS, recorded as % sterility in the outer florets in 2011 (11_S%_Outer) and 2012 (12_S%_Outer).

Pyramiding these effects with NILs will enable the validation of the effects and provide suitable germplasm for additional fine mapping. Once developed the NILs will provide a powerful resource to establish mechanisms behind QTL with controlled environments experiments looking at critical growth periods. JIC have established lines in population LxQ with 0, 1, 2 and 3 QTL. JIC and SRUC are currently assessing sterility in a collection of > 30 LXQ lines grown under field conditions.

SRUC and JIC would also explore the possibility of undertaking some further work on creating conditions to express the sterility phenotype in the field or glasshouse using parents and extremes of lines. Preliminary observations from on plant shading and low temperature are presented below.

Figure C indicates how shade (for 7 days in glasshouse grown plants) affects seed set in the spring wheat variety Paragon. Booting (GS 41-45) and ear emergence (GS 46-60) were particularly sensitive growth phases.

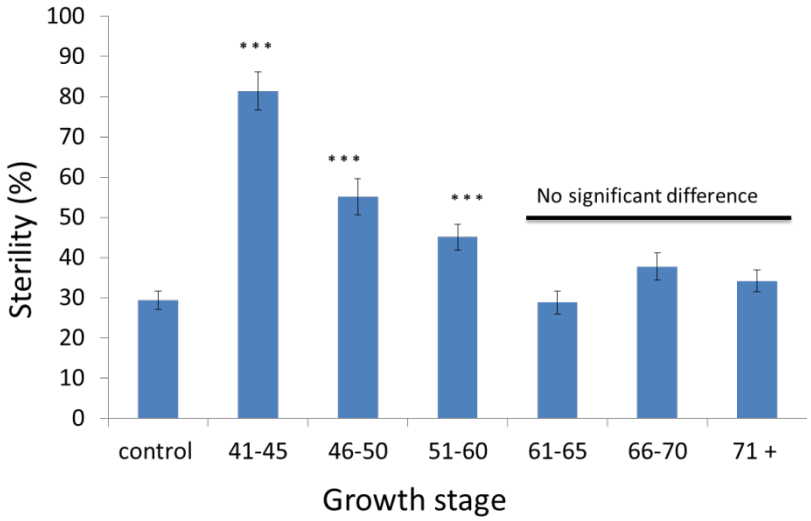


Fig. C The effect of shade (7 days) at different crop growth stages from booting (GS41 to GS71) on percent sterility in the whole ear of spring wheat variety Paragon. Data supplied by Ross Alexander (formerly at SRUC) and Steven Miller, SRUC.

When the variety Paragon was exposed to periods of cold temperature between early stem extension to flowering, the most sensitive growth phases for seed loss were at stem extension, GS30-34 and GS 35-40, as shown if Fig. D.

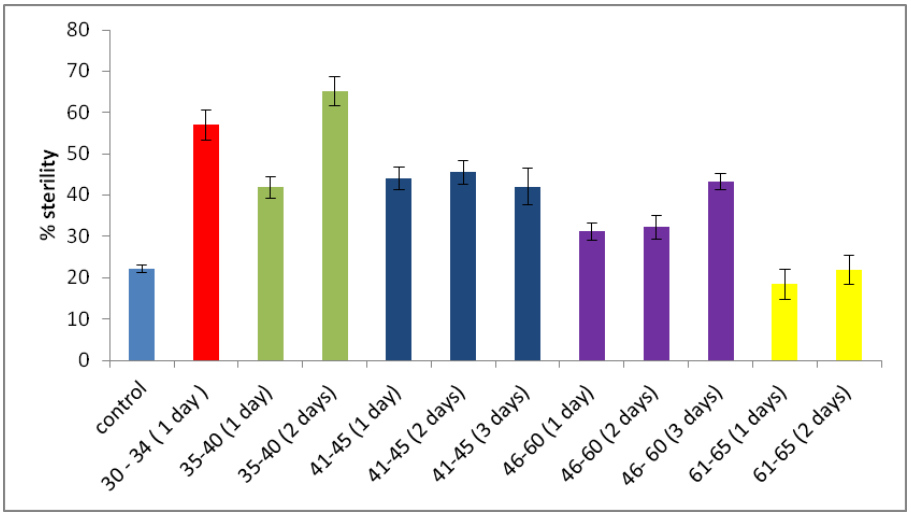


Fig. D The effect of shade (7 days) at different crop growth stages from booting (GS41 to GS71) on percent sterility in the whole ear of spring wheat variety Paragon. Data supplied by Ross Alexander (formerlt at SRUC) and Steven Miller, SRUC.

Work is ongoing to identify precise developmental growth stages or physiological tipping points that make plants more prone to poor seed set and losses in yield. Of particular interest are the processes involved in the transition from vegetative to reproductive growth.

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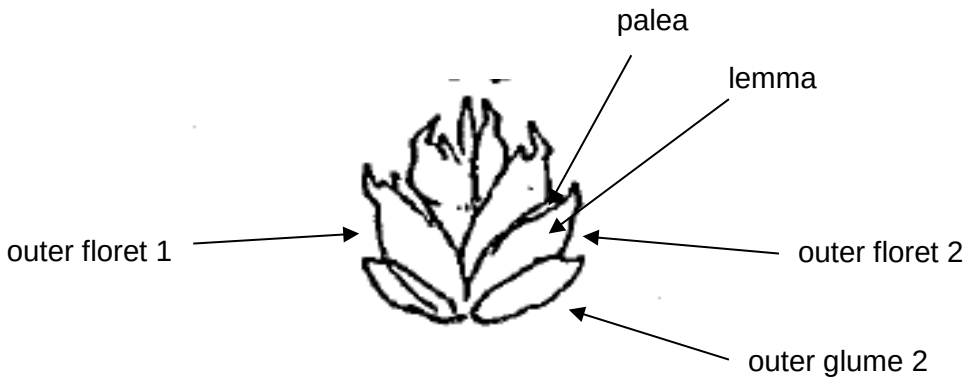
8. APPENDIX

8.1 Assessing ear sterility

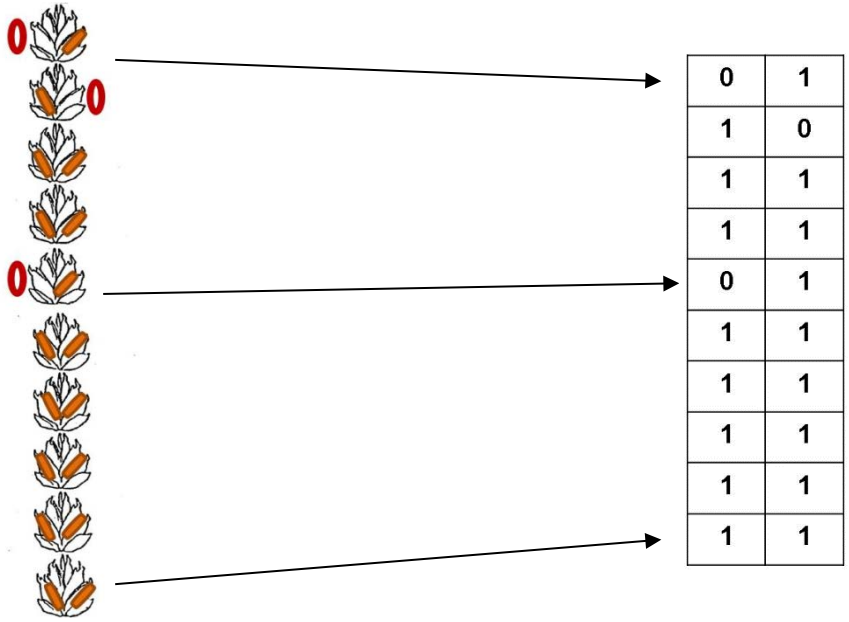
Lab assessment of outer florets

Sterility is assessed in the two outer florets of all spikelets along one side of an ear, in twenty ears per variety or line. This is best done by holding the ear in both hands. Starting at the base of the ear, use tips of index fingers to locate the outer glumes, then open the outer florets by gently pulling down on the lemma: this should separate it from the palea – revealing the inside of the floret. The structure of a single spikelet is shown below.



Each floret is scored as either ‘grain present’ or ‘sterile site’. A sterile floret is defined as one with no grain at maturity, but which contains the remains of floral parts (e.g. carpel/stigma/lodicules). Florets in which grain had formed but is missing is recorded as ‘grain absent’. Although not common, missing grain is identified by an empty floret without remains of floral parts. Very small florets (< 3mm) are not scored for sterility, as their potential to set seed is low. This occurrence is rare in the outer florets. Note: if the lowest spikelet has just one floret, then start assessments on the other side of the ear.

The image below demonstratex how sterility in outer florets should be recorded using an Excel spreadsheet. Each grain present (orange colour) is recorded as '1'. A sterile site (denoted by '**0**') is reorded as 0. A missing grain is recorded as 'a'. A very small floret (< 3mm) is left as a 'blank' cell. In the ear below, there are ten spikelets; with 17 grains and 3 sterile sites.



0	1
1	0
1	1
1	1
0	1
1	1
1	1
1	1
1	1
1	1

Percentage sterility is expressed as:

$$\frac{s}{s+g} x100$$

where *s* = number of sterile sites and *g* = number of grains. In the example above, sterility is 15%. All calculations should be made automatically in the Excel spreadsheet.

Ear length should also be recorded in cm from base of the lowest spikelet to the tip of the uppermost floret, but excluding the awn.

Note: Although this assessment should be non-destructive, prising apart glumes or the palea and lemma can cause damage to a spikelet or loss of grain. All ears should be retained for further assessment or a quality check, as required. Unless there is a specific QC requirement, there is no need to label individual ears.

An example of an Excel spreadsheet in which five ears have been assessed for sterility is shoen below. Here, ear 2 has relatively poor seed set and one absent grain, whilst the other ears have lower levels of sterility.

Ear Sterility Recording Sheet **Outer florets only** **Harvest 2010**

Key	**1**	grain present
	0	sterile floret
	blank	floret absent or with no potential to produce grain e.g. late developing
	a	grain absent

Population	**9M**	**Assessor**	S Hoad
Line	**1**	**Date of assessment**	29/09/10

	Ear 1		**Ear 2**		**Ear 3**		**Ear 4**		**Ear 5**	
Spikelet	O1	O2	O1	O2	O1	O2	O1	O2	O1	O2
12										
11										
10										
9					1	1	1	1	1	1
8	1	1	0	1	1	1	1	1	0	1
7	0	1	1	0	1	1	1	1	1	1
6	1	0	a	0	1	1	1	1	1	1
5	1	1	0	0	0	1	1	1	1	1
4	1	1	1	1	1	1	0	1	1	1
3	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1
Grain	7	7	5	5	8	9	8	9	8	9
Sterile	1	1	2	3	1	0	1	0	1	0
Missing	0	0	1	0	0	0	0	0	0	0
Length	9.3		9.0		10.1		9.5		9.3	