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Evaluation of novel naked barley lines for β–glucan, amylose and field performance

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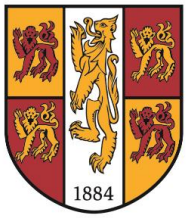

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ABSTRACT

Naked barley has recently gained worldwide interest due to health benefits claimed for its bioactive polysaccharides, β–glucan and amylose. It was side-lined by European plant breeders as less desirable for cultivation due to its poor field establishment and low yield compared to hulled barley. This project used Deiniol, a naked barley line bred at Bangor University and selected for cultivation in the UK. The aim was to improve the yield and bioactive properties of Deiniol through agronomy and breeding.

The effect of plant growth regulator (application on stem length, yield, yield components, β–glucan and amylose were studied in Deiniol and Sanette (hulled). We found that early PGR application at ZGS 32 significantly decreased the stem length from 118.2 cm to 75.5m for Deiniol and from 80.2 to 68.2 cm for Sanette (SE) ± 2.1, a 36% and 15% decrease in stem length, respectively. There was an increase in straw dry weight for both varieties; in Deiniol it increased from 180 to 595 g and in Sanette from 373 g to 406 (SE) ± 44.86g. That had consequences for the harvest index of Deiniol which was lower compared to hulled barley. Thousand grain weight increased significantly following early PGR application. The β–glucan content increased significantly in both genotypes at (p=0.01). But no effect was seen for the same treatments on amylose and amylopectin content. In contrast, we found that late PGR application on naked barley at ZGS 37 had no significant effect.

In order to identify a potential alternative naked barley line for the UK to replace long stemmed Deiniol, screening progenies (MA lines) of various crosses were carried out for stem length, β–glucan, amylose and field performance. Their β–glucan content ranged from 3.05 to 8.15 ± 0.22 g/100g. As compared to published values for current hulled barley, the β–glucan content in these lines was in the range of moderate to high

while the amylose content was in the range of normal to high. The lines associated with significantly higher grain yield and shorter straw than Deiniol could be potential alternatives to Deiniol for UK cultivation. Line MA 1 had significantly shorter stem length (61.3 ± 1.62 cm), grain yield (486.6 ± 21.8g/m^2), β–glucan content (4.08 ± 0.15 g/100g) and amylose (66.5 ± 1.27 %) to Deiniol. A new cross between Deiniol and a low-amylose starch mutant (Riso 13; hulled) was made to study the heritability of these traits. Riso 13 had high β–glucan content (8.12 ± 0.22 g/100g) whereas the F_3 line 31_2 had very high β–glucan content (14.79 ± 0.71 g/100 g) indicating that transgressive segregation, and broad sense heritability was high (approaching a value of 1). Deiniol had the highest amylose content (60.50 ± 1.87) and F_3 line 10_2 had the lowest amylose content (18.75 ± 1.87), but not quite as low as Riso 13. Another new cross between Deiniol and Propino was made with the aim of improving naked barley for UK agronomy. It was advanced to the F5 in pots, but no significant improvement was found over Deiniol for yield components.

Early PGR application did not improve the yield of Deiniol; therefore, a breeding approach is needed. MA lines with higher yield and β–glucan content than Deiniol were identified in field trials, and new crosses improved β–glucan content further. Together, these results indicate that this novel germplasm is potentially valuable for breeding naked barley for the health food sector in the UK.

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ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
B–glucan	B–glucan
bp	Base pair
cM	Centimorgan
° C	Degrees Celsius
DNA	Deoxyribonucleic acid,
EPSM	Ears per square meter
F1	First generation
F2	Second generation
F3	Third generation
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SE	Standard Error
SSD	Single seed decent
SSR	Simple sequence repeats
GI	Glycemic index
GMC	Grain moisture content
GPE	Grains per ear
GY	Grain yield
HI	Harvest index
MAS	Marker assisted breeding
N	Nitrogen
K	Potassium
P	Phosphorous

PGR	Plant growth regulator
pH	Potential of hydrogen
P	Probability
QTL	Quantitative trait loci
RIL	Recombinant inbred line
StL	Stem length
TGW	Thousand grain weight
ZGS	Zadok’s growth stages

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Literature review and objective

1.1. Introduction

Barley (*Hordeum vulgare* L) is one of the earliest domesticated crop plants. It ranks as the fourth most important cereal crop in quantity produced and area cultivated (Zhu, 2017; Monteiro et al., 2018). Barley belongs to the genus Hordeum in the tribe Triticeae of the grass family. Barley harvested area worldwide was 47 million hectares with an average yield of 3.01 tonnes per hectare and 141 million tonnes total production in the 2016 agricultural season. The UK had a total harvested area of 1.12 million hectares with a yield of 5.9 tonnes per hectare and a total barley production of 6.7 million tonnes in 2016 (FAOSTAT, 2018). In the UK barley is mainly used for animal feed and malting.

Barley attains its popularity from its broad climatic adaptation and suitability to be grown in marginal soils and under saline water irrigation (Flowers and Hajibagheri, 2001). It could be used as a value-adding cash crop contributing to crop diversification and an integrated pest management approach through crop rotation (Brouwer et al., 2016).The Fertile Crescent could be the origin of domesticated barley (Lev-Yadun et al., 2000).

1.2 Barley uses

1.2.1 Barley for animal feed

Barley as one of the small grain crops surpasses other cereals in its protein content, making it ideal for ruminant feed for a wide variety of animal species (Houde et al., 2018). Indeed, it is extensively used as an animal feed (Li et al., 2001;Yu et al., 2017). Numerous processing approaches are used for barley kernels with the aim of boosting digestibility and animal performance, e.g., rolling and roasting, etc. (Vandehaar, 2005).

1.2.2 Barley for malting

Malting is a process in which grains are germinated under specific conditions with the purpose of enhancing grain modifications, followed by quick drying to stop further changes (Bera et al., 2018). Barley is the principal cereal in malt production (Gupta et al., 2010). That is due to its content of both alfa and beta amylose and less handling risks under high moisture conditions than other cereals (Guoping and Li, 2012).

1.2.3 Barley for human food

Growing of naked barley is an ancient activity dating back to soon after the early domestication of hulled barley (Zohary and Hopf, 1993), but it is now less common globally. Naked barley yields are significantly lower than hulled barley (Barabaschi et al., 2012) and naked barley crop lacks intensified breeding programmes (Atanassov et al., 2001). Naked barley with its high adaptability to harsh weather conditions of drought and poor soils meets food security targets, and it could play a crucial role in sustainable agriculture (Moza and Gujral, 2016).

Barley is used as human food in some parts of the world, e.g., Finland, Pakistan, Afghanistan, India, Ethiopia Japan, Libya, Nepal, and China (Newman and Newman, 2006; Baik and Ullrich, 2008). It is an excellent source of dietary fibre (Kumar et al., 2013). China saw the most significant increase in barley food consumption; from 2 million tonnes – 4 million tonnes between 1990-2005 (Guoping and Li, 2012). Naked barley is consumed as wholemeal flour, flakes, noodles, and unleavened tortilla bread (Baik and Ullrich, 2008).

1.3 Barley plant morphology

The barley plant life cycle starts with seed germination. Several tillers emerge from the plant's crown just underneath the soil surface. Environment and genotype influence the number of the emerging tillers. The booting stage takes place when the head emergence from within the

leaves. Heading refers to the emerging of the spike from the boot. The earliest grain filling stage is called the milk stage. Eventually the grain becomes dough-like and that is followed by kernel dry down. When the spike bases become golden brown the grains have reached maturity and no more dry matter accumulates.

Barley stem length ranges from 60 to 120 cm. Seedling roots and adventitious roots form the barley root system. The seedling roots (plate 1.1) emerge during germination and tiller formation, and adventitious roots develop after that period (Wahbi, 1995).

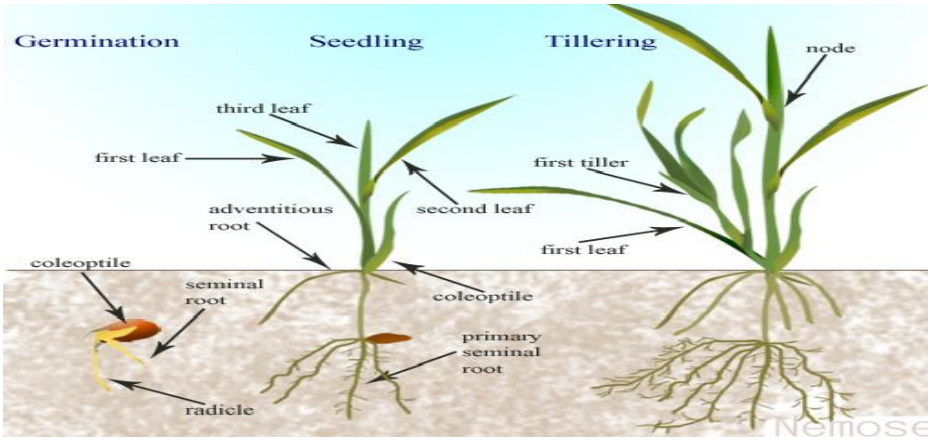

Plate 1.1 Barley vegetative stages
Source: **http://www.geochembio.com/biology/organisms/**

The barley hollow stem of 5 to 7 internodes represents a vital source of carbohydrates at the grain filling stage (Bidinger *et al*., 1977). It is reported that the stem's capacity to support grain is subject of genotype and drought stresses (Ehdaie *et al*., 2008; Abeledo et al. 2004). Barley spikes (plate 1.2) confer to the plant its distinctive characteristics. Plants can be categorized as six- or two-row based on the number of grains in the spike (Frégeau-Reid et al., 2001). Each spike comprises several spikelets which are arranged alternately along a central axis called the rachis (plate 1.2). At each rachis node there are three florets (flowers); one on the centre and two on the laterals. In two-row barley, the lateral florets are sterile or not fully developed and do not produce grain; so, the grains are arranged in two rows on each side of the

rachis. In six-row barley all three florets are fertile, and they produce three grains on each side of the rachis,

Each floret is protected by two bracts: the lemma and the palea. These bracts enclose the carpel. The carpel comprises the ovary with its feather-like stigmas, three stamens which support the anthers (pollen grains), and the ovule. After fertilisation, the ovule produces the kernel.

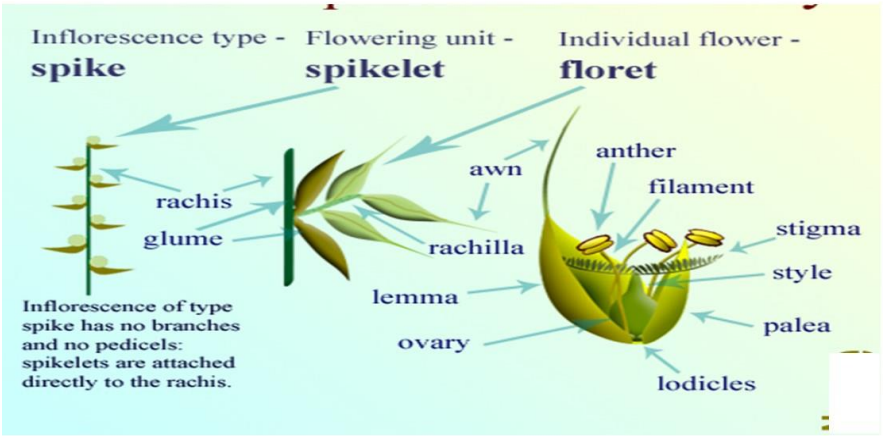

Plate 1.2 Reproductive organs
Source: http://www.geochembio.com/biology/organisms/barley/

The awns appear at the tip of each fertile floret. They are mostly stiff and break off during grain threshing.

1.4 Barley cultivation and crop growth stages

Barley production starts with the selection of a suitable variety. At this stage a number of factors should be taken into consideration including desired end use, plant growth habit, seed availability, resistance to diseases and growing season (spring or winter). Winter barley must be exposed to cold conditions in order to enhance flowering (vernalisation-sensitive).

Ado's (ZGS) decimal code provides good descriptions of the early growth stages of cereals including barley. The code can be applied to a range of climatic conditions and regions (Tottman et al., 1979). Barley growth stages are as follows: germination (ZGS00–ZGS09), seedling growth (ZGS10–ZGS19), tillering (ZGS20–ZGS29), stem elongation (ZGS30–ZGS39),

booting (ZGS40–ZGS49), ear emergence (ZGS50–ZGS59), flowering (ZGS60–ZGS69), milk development (ZGS70–ZGS79), dough development (ZGS80–ZGS89) and ripening (ZGS90–ZGS99). The stem elongation stage consists of ZGS30 (ear at 1 cm erect), ZGS31 (first node detectable), ZGS33, (third node detectable), ZGS37 (flag leaf just visible) and ZGS39 (flag leaf blade all visible).

Crop rotation is thought to benefit barley cultivation, though barley should not be in direct rotation with other small grained crops due to overlapping of pests and diseases. Soil pH levels ranging from 5.5 to 6.5 are optimal for barley cultivation in well-drained soils. It has been reported that soil structure, texture, temperature and water holding capacity are crucial for seedlings emergence and field uniformity (Czyz, 2004; Małecka et al., 2012). Planting dates and the rate of seeding depend on the growing environment. Certified seeds should be used for planting to guarantee purity and improve germination. Planting density, the number of productive ears per plant, the number of grains per ear and average grain weight determine cereal grain yield.

1.4.1 Soil fertility management

Soil nutrient tests should take place before planting in order to determine optimum nitrogen, phosphorous, and potassium fertilizer levels needed. The fertilizer quantity required depends on the existing soil nutrient content and the previous crop grown on that ground. Nitrogen fertilizer can be applied before planting to avoid excessive leaching from the soil during heavy rainfall. Crop rotation can help to improve soil quality and reduce the amount of fertilizer needed.

1.4.2 Lodging

The tendency of a crop to lean or fall down is known as lodging and is caused by multiple factors including strong winds, rain, certain types of soil and long stem length. Lodging

leads to difficulties in combine harvesting and reduces grain yield and quality. The selection of a shorter stemmed variety for planting, avoidance of excessive nitrogen fertilization and/or the application of plant growth regulators can help minimizing the risk of lodging. Stem breakage mainly occurs in the lower internodes of barley, but it may take place in the middle internodes (bracking) or below the spikes (necking) (Perrott, 2017). Extreme lodging results in destruction of the canopy, reduced photosynthesis, reduced dry matter accumulation, lower grain filling, lower yield and lower grain quality (Kashiwagi et al., 2005).

1.4.3 Plant growth regulators

Plant growth regulators (PGRs) are naturally - or synthetically - produced compounds that can alter plant development and growth in a desirable way. The use of PGRs began in the 1930s and current worldwide sales are worth approximately $1.2 billion (Rademacher, 2015). Auxin, gibberellins, cytokinins, ethylene and abscisic acid are the most widely known plant hormones. These plant hormones are affected by the application of PGR resulting in, for example, stem shortening and yield reduction or increase (Herelius, 2017). The ability to modify plant growth depends on plant growth stage, crop variety and growing conditions, particularly day length. and cultural practices (Rajala, 2003; Supronien et al., 2006; Wiersma et al., 2011; Braumann et al., 2018a).

Plant growth regulators can be utilized to induce stem shortening (Ma and Smith, 1991; Berry et al., 2004), thus increasing lodging resistance and facilitating mechanical harvesting (Naylor and Munro, 1989) and producing plants capable of supporting heavier spikes with higher carbohydrate content (Ma et al., 1994: Rajala, 2004).

Yield increases have been reported following application of Trinexapac ethyl on wheat (Zagonel et al., 2002) and application of an Ethephon, and Trinexapac ethyl mix on barley cultivar Kymppi (Rajala and Peltonen-Sainio., 2002). However, application of the later PGR blend on the cultivar Saana was found to reduce the yield. Similar results were obtained by

Rajala and Peltonen-Sainio (2001) who concluded that PGRs had a modest potential for manipulating yield and yield components in barley.

The PGR trinexapac-ethyl (Moddus) controls lodging by shortening the stem length and also strengthens the stem and root structure (Wiersma et al., 2011; Matysiak, 2006). Zagonel et al. (2002) found that trinexapac-ethyl application resulted in less dry matter and increased stem diameter. However, it has also been reported that PGR-treated seeds produced more tillers (Woodward and Marshall, 1987:Naylor and Munro, 1989) with similar results reported for foliar PGR application (Zagonel et al., 2002).

There is increasing public concern over the use of agrochemicals such as PGRs in food production. As a result, their application has become restricted and consequently they have a relatively small market, they are more complex to work with and substantial investments are needed for research and market development (Rademacher, 2018).

1.4.4 Weed control

Under suitable growing conditions, barley can outcompete weeds. A carefully designed rotation is one of the most important aspects of weeds management. Herbicides can be used for weed control with a number of pre- and post-emergence applications available for suppressing weed seed germination.

Glyphosate (herbicide) products could be used before harvesting to control weeds, facilitate harvesting and safeguard grain quality and food safety. Its application should be carefully controlled in order to reduce chemical residues in harvested grains. Spraying the crop with glyphosate especially during wet seasons can decrease greeny canopy, non-mature tillers, and aid grain harvesting and storage. This can be particularly valuable in wet seasons. The optimum moisture level for glyphosate application is 30% at ZGS 87 (the hard dough stage of barley growth, which takes place one to three weeks before harvesting (AHDB, 2018).

1.4.5 Disease management

The use of genetic disease resistant varieties is considered a highly cost-effective way of controlling disease in crops. If the genetic approach fails to eradicate disease, fungicides can also be used. It is important to protect crops from foliar and stem base diseases in early spring, especially during growth periods which are crucial in determining grain yield. Specifically, disease can affect the number of ears per m^2 (the number of survived fertile shoots), grains per ear (during the production of spikelet) and thousand grain weight (during grain development and filling). During early spring (from emergence up to ZGS 22) it is vital that mildew is controlled. The timing of fungicide application is influenced by the date of sowing and disease the specific disease risk relating to the geographical location and the variety grown.

There are three principal spraying times for spring barley. Most spring barley is well protected by one or two fungicide applications at ZGS39–59. 60% of yield response is coming during this period of fungicide application and/or at (ZGS25–31) which is the best time to spray against rhynchosporium, brown rust, net blotch and mildew. This contributes to 40% grain yield increase.

1.5 Breeding Barley

Plant breeding is a method of manipulating the plants genetic composition with the purpose of increasing their value and improving human welfare. Plant breeding uses crossing between varieties and selects desirable plant types that are better suited for cultivation, produce higher yield and are disease resistant. Crop yield stability and sustainability are of a major importance to plant breeders. These traits include resistance to pests and diseases, tolerance to abiotic stresses, nutrient- and water-use efficiencies. In addition to the development of marginal land adapted crops with a greater emphasis on minor crops.

Plant breeders continuously confront endless tasks while developing new crop varieties such as the identification of parents, rounds of crossing and back crossing and evaluation and

selection in field trials. Changes in agricultural practices can arise following the development of new genotypes with specific agronomic characteristics (such as semi-dwarf varieties allowing increased use of nitrogen). Breeders have to pre-empt, the constant change in the agricultural environments and their living organisms such as new races of pathogens. New agricultural lands used for crop production can expose crops to changing growth conditions. Finally, consumer preferences and their needs and wants change so breeders must respond.

Despite the continuation of yield improvement from conventional plant breeding there is a growing need to use advanced biotechnology such as DNA markers to speed up selection and maximize success probabilities. DNA markers are used for detecting the allelic variation present in - or linked to - the genes controlling desired traits. DNA markers application in plant breeding is called marker-assisted selection (MAS) and it is a component of the discipline of molecular breeding.

1.5.1 Marker assisted selection

The discovery of molecular markers was an important development for plant breeders. A genetic marker is a sequence of DNA that can be tested in an assay and used for genotyping (Collard, et al., 2005). It acts as a flag or marker for genes of interest that can be used to select the desired genotype during MAS (Kebriyaee et al.,2012).

The first step in marker assisted selection is to link markers to traits which involves phenotyping of many individuals from a mapping population and linkage mapping in to identify markers segregating in that population that are associated with the traits. Another approach uses genome wide association analysis which avoids the need for mapping populations but requires large numbers of genotypes for phenotyping and good genome coverage with markers. Once markers are known to be associated with traits, they can be used in segregating populations for the selection of the traits to which they are associated.

1-5-2 Marker assisted selection applications in plant breeding

Collard and Mackill (2008) listed five major areas for the application of MAS in plant breeding: Marker-assisted evaluation of breeding material; marker-assisted backcrossing; pyramiding, early generation selection; and combined MAS. It is likely that whole genome-based methods such as Genomic Selection methods could be developed in the future.

1.5.2.1 Marker-assisted evaluation of breeding material

التعليق [KS1]: I have removed sub headings between 1.5.2.1 and 1.5.2.6 because they are not needed. (they all refer to your first major area so should come under the same sub-heading.

This uses molecular markers to screen lines for crossing and new breeding lines during development. Markers can reveal basic important information such as parent or cultivar identity and purity, levels of genetic diversity between cultivars, , and hybrid confirmation. DNA Markers are utilized to affirm the true identity of individual plants. High genetic purity levels are fundamental for cereal crossings targeting hybrid-vigour (heterosis) exploitation.

Plant breeding relies much on high levels of genetic diversity for progressive selection achievements. The broadening and characterization of core genetic material is essential for the determination of diverse traits for the purpose of hybridization with superior cultivars.

Hybrid crop production involves the determination of heterotic groups using DNA markers. The heterotic groups are needed for developing inbred lines which could be used for producing elite hybrids.

Understanding how allele frequencies shift within populations is a critical information for breeders as it assesses and monitors specific alleles or haplotypes and it can be used in designing appropriate breeding strategies.

1.5.2.2 Marker-assisted backcrossing

Backcrossing (BC) is a plant breeding technique most frequently used for the incorporation of one or a few genes into an elite variety. Generally, the parent used for backcrossing has a large number of desirable attributes but lacks for a few traits and features.

There are three strategies of marker-assisted backcrossing: Firstly, combining both phenotyping and mapping as screening for the target gene or QTL. The marker may then be used to replace time consuming phenotypic screening. Secondly recombinant selection, this technique can be used for selection for traits during the seedling's pre-reproductive-stage. It could be used for recessive allele selection, which is difficult to achieve using conventional ways. Genetic markers are either intergraded with conventional schemes or alternatively used to substitute them in line development.

1.5.2.3 Marker-assisted pyramiding

Pyramiding is the process of integrating a number of genes or QTLs together into a single genotype. It is difficult to determine plant having more than one gene using conventional breeding. In addition, individual plants in conventional breeding must be assessed for all traits investigated. And also, it could be very difficult to evaluate plants from specific population types (e.g. F_2) or for traits with destructive bioassays with conventional breeding. On the other hand, pyramiding could be done more accurately using DNA markers than conventional breeding. DNA markers are non-destructive and a single DNA sample can be used without phenotyping.

The most commonly known pyramiding application was the combining of several disease resistance genes into a single genotype. The reason behind that was to develop durability or stability in disease resistance. Pathogens become more resistant to single-gene host over time because of the emergence of new plant pathogen strains.

1.5.2.4 Early generation marker-assisted selection

Marker assisted selection is advantageous in early generations because plants with undesirable gene combinations can be taken out of the programme. This facilitates focusing on a small group of high-priority lines in subsequent generations. Furthermore, MAS is mostly efficient in early generations as the probability of recombination between the marker and QTL is

increasing. MAS at early generation is disadvantaged by the high cost of genotyping large plants numbers. A single large-scale MAS (SLS–MAS) was suggested at early generation and could be performed on F_2 or F_3 populations derived from elite parents. This method is based on flanking markers (less than 5 cM, on both sides of a target locus) for up to three QTLs in a single MAS step. These QTLs contribute for the largest proportion of phenotypic variance and they are stable in various environments. Fixing homozygous alleles in self-pollinated crops as early as possible is an important goal screening can be done at the F_5 or F_6 generations when most loci are homozygous.

1.5.2.5 Combined marker-assisted selection

MAS and conventional phenotypic screening can be combined. Combined MAS is advantageous over phenotypic screening or MAS alone for the purpose of genetic gain maximization. This technique could be used when additional QTLs controlling a trait is not identified or a large number of QTLs need to be manipulated and when trait heritability is low.

In many instances, there is a low recombination level between a marker and QTL, unless markers flanking the QTL are used. However, a marker assay may not be a 100 % reliable. Nevertheless, using such markers for plant selection may remain helpful to plant breeders for selecting a subgroup of plants. The number of plants needed for phenotypical evaluation can be reduced. This has the advantages of reducing the cost especially for quality traits when the marker genotyping is less expensive than phenotypic screening.

1.6 Overview of cereal breeding using MAS

Plant breeding as we mentioned earlier is all about the selection of certain plants with desirable traits. The selection involves the evaluation of a breeding population for one or more traits in the field or in the glasshouse trials (e.g. agronomic traits, resistance to disease or tolerance to stresses), or with chemical tests (e.g. the quality of grain). Plant breeding works towards assembling of more desirable combinations of genes in new varieties.

Pedigree breeding is the most common used method for the selection of desirable plants. The method starts in early generations for higher heritability traits. However, the selection for low heritability traits starts when the lines become more homozygous in (F_5 or F_6) generations. Selecting of superior plants begins with visual assessment for agronomic traits or resistance to stresses, followed by chemical assays for quality or any other traits. At (F_5 or later) when the breeding lines become homozygous, they can be harvested in bulk and assessed in replicated field experiments. A long period of time (5–10 years is needed for superior lines identification),

The plant population size and composition in a breeding programme must be taken into consideration. The larger the number of segregating genes in a population, the larger the size of the population needed for specific gene combinations identification. Breeding programmes commonly grow hundreds or even thousands of populations, and several thousands or millions of individual plants. Having in mind the amount and needed selection complexity in breeding programmes and the number and size of the populations. One can easily welcome MAS as an advanced way for plant selection and justifies the high cost of its application.

Marker assisted selection has many advantages over conventional phenotypic selection because it is faster and requires fewer plants to be grown to maturity for phenotyping. Selection can be carried out at the seedling stage. Single plants could be selected, and all these advantages could be employed by plant breeders as the selection for target genotypes can be more effective, certain traits may be 'fast-tracked', and eventually faster line development and variety release. Markers can also replace phenotyping, allowing off-season selection in nurseries and enabling the growing of more generations per year.

The total number of lines needed for testing can be decreased and use of glasshouse and/or field space can be utilized efficiently. As many lines can be excluded after MAS breeding programme took place, this permits more efficient use of glasshouse and/or field space and only important breeding lines are saved for future development. MAS may not be practical or more

efficient for all traits, because phenotyping for many traits, is already available and its selection cost for large populations is lower. MAS is rarely applied when it is more expensive than phenotyping (Steele et al.,2018).

There are five major considerations for the application of DNA markers in MAS these are: reliability of the markers; quantity and quality of DNA required; technical procedure for marker assay; level of polymorphism, and cost (Collard and Mackill, 2008). Reliability. Less than 5cM genetic distance between a marker is preferred and a target locus. The application of flanking markers or intragenic markers.

A flanking marker is an identifiable location (i.e., polymorphism) based near a gene which can be used in linkage studies to track the gene of interest coinheritance. DNA quantity and quality. Some markers approaches require large quantities and high quality of DNA, which practically could be difficult to get, adding up to the total cost of the whole process. Technical procedure. Simplicity level and needed time for the technical procedure are critical points. Quick and simple procedures are highly desirable. Level of polymorphism. High polymorphism in breeding material (i.e. distinguishable genotypes) is highly preferred. Cost is a key determinant on the application viability of marker-assisted selection (MAS) as a breeding method. Despite its employment advantages.

1.6.1 Types of DNA markers used in cereals

Some markers use PCR for amplification of DNA fragments that differ in length. These include simple sequence repeats (SSRs) or microsatellites that represent the most used markers in cereals (Gupta et al., 1999; Abebaw et al., 2017; Drine et al., 2017) to date. SSRs are reliable, abundant, simple, low cost to use and highly polymorphic (Wang et al., 2010). Their disadvantage is the requirement for polyacrylamide gel electrophoresis and they produce data about one single locus per analysis, despite the possibility of numerous markers multiplexing. Furthermore they require substantial time and money investments to develop (Jo et al., 2017).

Other DNA markers used in cereals include restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), single nucleotide polymorphism (SNP) and inter-simple sequence repeat (ISSR) (Drine et al., 2017). Kompetitive allele-specific PCR (KASP) is a recently developed marker system. This system, produced by LGC Genomics, detects SNPs using PCR with florescent detection of alternate alleles. And is a cost-effective and flexible technique (Semagn et al. 2014; Patil et al. 2017). KASP is a single-step genotyping technology that reveals, via fluorescence, resonance energy transfer (FRET) (Steele et al., 2018). KASP has an advantageous capability to explore functional markers within genes of interest. And preforms faster than PCR markers (Rasheed et al. 2016). Reed et al. (2016) also found that they could speed up the crossing parents and progenies characterization for MAS and can integrate with the inflexible, high-density SNPs.

Lister et al. (2013) found that KASP out performed other sequence-based markers in ancient DNA (a DNA) samples genotyping (78% versus 61% success, respectively) making it ideally suited for this kind of analysis. They added that this aDNA analysis process is hampered by cross sample contamination and DNA degradation which could be avoided by KASP application. They observed that KASP could typically be applied for ancient landraces and modern plant materials genotyping. KASP genotyping service can be provided by LGC Genomics or KASP analysis kits can be ordered from the same company (Steele et al., 2018). This approach could be applied to a number of experimental designs, variable target loci and sampling sizes (He et al. 2014).

1.7 Naked barley breeding

Naked barley crop breeding objectives aim to enhance quality components such as spikes and grain morphology, grain nutritional quality, e.g., β–glucan and amylose content, improve environmental adaptability and meet farmers preferred plant height, crop uniformity, total yield and disease resistance (Steele et al., 2013). Naked barley genetic resources are enormous, and

they could be exploited through the use of marker-assisted selection (MAS) and plant breeding techniques. Nowadays,

DNA markers are extensively employed in genetic diversity studies (Gong et al., 2009). In their review Collard and Mackill (2008) stated that the enormous number of quantitative trait loci (QTLs) mapping studies on various crops have supplied plenty of DNA marker–trait associations. They added that plant breeding is all about selecting certain plants for specific traits preferred by end uses and users.

This selection process passes through the following steps: evaluation of a breeding population for one or more desirable traits in the field or glasshouse trials with an ultimate target of integrating more desirable alleles in new varieties. The selection for higher heritability traits starts at early generations; in higher heritability traits the genetic factors have a strong influence on the amount of variation. On the other hand, the selection for low heritability traits takes place when the lines become more homozygous in later generations (F_5 or F_6) (Veatch-Blohm, 2007), in low heritability traits the environmental factors strongly influence the amount of vitiation.

1.7.1 Deiniol (naked barley line)

Deiniol line is a recently produced breeding line at Bangor University, developed by crossing Skardu naked landraces and Static UK hulled barley and with adaptability to the UK environment (Steele *et al.,* 2013). A large bulk population was selected for UK adaptation.

Two approaches were employed (Figure 1.1)

(1) Bulk selection for naked barley and line selection from the bulk for UK adaptation.

(2) F_2 non-selected segregating recombinant inbred lines. Selection took place for four naked breeding lines based on their agronomical traits, β–glucan content and yield.

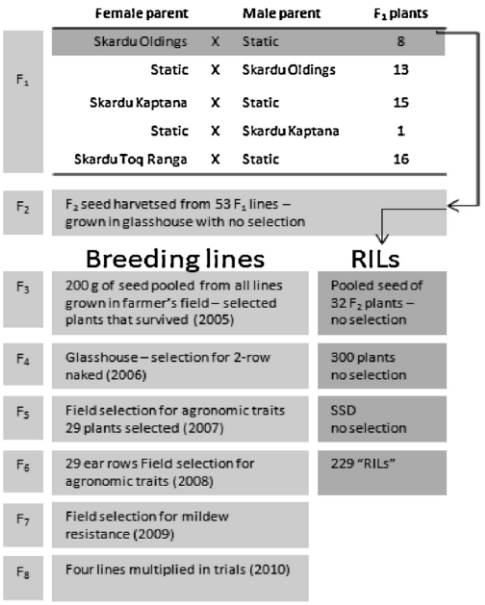

Figure 1.1: Schematic diagram showing population and line development for selected reciprocal crosses between Static and three Skardu landraces population from Skardu x Static. Source: (Steele et al, 2013).

1.8 Naked barley genetics

In naked barley varieties, a recessive naked caryopsis gene (*nud, nudum*) inhibits husks and caryopsis development. This feature is directly linked to barley usage and consumption (Duan et al., 2015). Naked barley needs no extra processing for human food production. However, naked barley kernels are prone to physical damage which hampers their germination (Dickin et al., 2011). Locally adapted, traditional varieties or landraces are defined as a cultivated, genetically heterogeneous variety that has evolved in a certain ecogeographical area and is therefore adapted to the edaphic and climatic conditions and to its traditional management and uses (Casañas et al., 2017). Such varieties are reported to have weak coleoptile development, poor crop growth, and high susceptibility to foliar diseases, lodging, and insignificant response to fungicide application (Dickin et al., 2010). However, the same authors found that some recent breeder lines had high seed emergence, stronger stems, and were vigorously resistant to diseases leading to a breakthrough in better crop germination and growth.

The *nud* gene present in naked barely correlates with short plant height, low plant density, lighter seed weight, and reduced yield (Choo et al., 2001). However, it has been shown that the *nud* gene lacks epistatic control on other traits, including seedling vigour , suggesting the possibility of breeding naked barley with enhanced seedling establishment (Capo-chichi et al., 2012). Choo et al., (2001) reported that rough-awned naked barley had more naked grains than smooth-awned barley. Extensive studies are needed to explore the effect of the *nud* gene on different traits, and to establish adequate selection strategies for naked barley breeding programmes.

1.8.1 Genetic diversity and genetic variability assessment studies

Genetic diversity or variation can be assessed through comparison of heritable variation in a species. Plant breeders can now use variation in the whole genome for efficient identification, quantification and characterization of genetic variation from widely available germplasm resources (Nadeem et al., 2018). Assessing genetic diversity for plant breeding programmes and gene banks helps to identify new sources of donor parents for breeding (Khajavi et al.,2014). High genetic variability is a key element for improving breeding programmes, as it facilitates selection of contrasting parents to produce effective crossings and increases the probability of success (Sayd et al., 2015). Variability is different from genetic diversity, which is the amount of variation seen in a particular population.

In their study Gong et al. (2009) tested 68 accessions of naked barley cultivars in China. These cultivars were assessed using sequence-related amplified polymorphism (SRAP) markers. They found that, 20 primers combinations. A primer is a molecule that serves as a starting point for a polymerization process. The primers produced a total of 350 clear bands with an average of 17.5 bands per primer pair, of which 153 bands (43.7%) were polymorphic. Polymorphism is the occurrence of several different forms or types of individuals among the members of a single species. 324 allelic phenotypes were amplified with an average of 16.2 alleles per primer pair.

Sayd et al. (2015) in their study entitled "genetic variability of irrigated naked barley" obtained 157 RAPD markers RAPD refers to random amplified polymorphic DNA: a technique in which random DNA segments are amplified by the Polymerase chain reaction using single Primers of arbitrary nucleotide sequence. They found that 89% of RAPD markers on the studied accessions were polymorphic. The studied accessions revealed that they are of a high genetic variability, which is due to the high percentages of polymorphic bands. Genetic distances among accessions based on molecular markers ranged from 0.131 to 0.484. They concluded that there is a highly genetic variability based on molecular and agronomic traits among naked barley varieties. Ethiopian varieties are more identical, and Brazilian varieties are more genetically distant. The two- and six-rowed barley varieties show divergent agronomic traits.

In their study Amabile et al. (2017) highlighted that it is more practical to pursue plant selection based on overall architecture, especially preserving higher yields, thicker and longer stems, shorter internodes and no lodging rather than selection based merely on plant height. In a different study examining covered barley in Algeria, Rahal Bouziane et al. (2015) assessed the genetic diversity of twenty nine barley landraces using twenty seven phenomorphological and agronomic traits with four controls.

1.9 Naked barley nutrient composition

The entire naked barley kernel contains approximately 65-68% starch, 10-17% protein, 4-9% β–glucans, 2-3% free lipids and 1.5-2.5% minerals. The architecture of barley grain varies depending on genetic makeup and environmental conditions prevailing throughout the growing season such as soil mineral content and water holding capacity, temperature, and daylight.

1.9.1 Starch, amylose, and amylopectin

Starch is a natural material found in many plants (Manelius et al., 2000). Starch fragments are built in the amyloplasts. Amyloplasts are non-pigmented organelles found in some plant cells. They are responsible for the synthesis and storage of starch granules. Barley's amyloplast is composed of a single granule (Oscarsson et al., 1998). Amylose and amylopectin are the main constituents of barley starch (Pérez and Bertoft, 2010). These two components, which are made of glucose subsets, confer to starch its specific characteristics. Amylose and amylopectin ratios vary between plants.

Generally, starch consists of 25% amylose and 75% amylopectin (Delcour and Hoseney, 2010). Barley varieties containing 100 % amylopectin are called "waxy barley" and other varieties that contain approximately 70% amylose are called "amylotypes". Other barley grain categories include high/low lysine content and high/low β-glucan content. Barley's stored starch represents an energy pool. It is consumed within dormant and re-emergence periods (Jobbing, 2004). Amylose is a linear or slightly branched molecule in which the sugar units are bound by α-1,4 links (Zeeman et al., 2010). Amylopectin, the most common constituent of starch, is a branched sugar molecule connected through α-1,4 links with branching taking place with α-1,6 glycosidic bonds (Hannah and Greene, 2009). Starch has an immense economic significance. Starch has recently gained importance as a beneficial, healthy food. The easily digested polysaccharides such as amylose and amylopectin contained in starch represent bioactive ingredients against that can protect against obesity, cardiovascular diseases and diabetes (Mann, 2007).

In addition to amylose and amylopectin starch contains low concentrations of lipids, phosphorus, protein and nitrogen (Delcour and Hoseney, 2010). The majority of starch is digested in the small intestine. However, starch degradation varies significantly depending on source, constituents and structure (Englyst et al., 1996).

1.9.2 β–glucan

β–glucans are linear polysaccharide mixed-linkage (1→3), (1→4) molecules consisting entirely of glucose. Mixed-linkage (1→3), (1→4) β–glucans are linearly cell walled homopolysaccharides of D-glucopyranose. D-Glucopyranose is the beta isoform which are similar functional proteins that have the same but not identical sequenced amino acid. D-glucopyranose is a synthetic simple monosaccharide energy source. It is oxidized in numerous tissues either under aerobic or anaerobic conditions through glucose glycolysis. Mixed-linkage (1→3), (1→4) - β-glucans are arranged as blocks of consecutive (1→4)-linked b-D-glucose residues (i.e., oligomeric; oligomeric refers to a polymer or polymer intermediate with relatively few structural units separated by single (1→3)-linkages. The resultant structure is a polysaccharide built mainly from b-(1→3)–linked trisaccharides with three glucose units, known as cellotriosyl (58–72%), and tetrasaccharides with four glucose units, known as cellotetraosyl (20–34%) (Staudte et al., 1983; Kiemle et al., 2014).

β–glucan in barley can be categorised as soluble or insoluble. Soluble β- glucan is characterised by (1→3)-linkages whereas cellulose only has (1→4) links (Sullivan et al., 2013). The β–glucan structure is similar in cereals regardless of its source (wheat, oat or barley) (Li et al., 2011). High-amylose and waxy naked barley typically contains 7-8% β-glucans, though its content can go up to 18% is some varieties (Andersson et al., 2004) , whereas covered barley contains 4.6% (Gao et al., 2009).

1.9.2.1 β–glucan molecular biology

Eight cellulose synthase-like genes (seven CslF and one CslH) control β–glucan production, and a competitor gene, UDP-glucose 4-epimerase (UGE), is also involved (Molina-Cano et al., 2007). B–glucan QTLs are present on chromosomes 1H, 2H, 5H and 7H. These QTLs were mapped, and their alleles have been specified and validated (Burton et al., 2011). Several other genes, such as CslF6 on chromosome arm 7HL and Glb1, respectively contribute

to β-glucan synthesis and deterioration (Taketa et al., 2011). CslF6 is about 11 cM above the *nud* loci determining hull formation or disappearance (Taketa et al., 2008) and are reported to be determined by different genes. However, Tonooka et al. (2009) concluded that CslF6 and *nud* might be connected to each other. The nud's emergence frequently coincides with high β–glucan content (Mezaka et al., 2011). This hypothesis was tested by Steele et al. (2013) who found that the genes were not linked.

1.9.2.2 Effect of environment and genetics in β–glucan content

β-glycan quantity is influenced by genotype x environment in sorghum (*Sorghum bicolor*) grain (Betts et al., 2015), in oats (Doehlert et al., 2001) and barley (Dickin et al., 2011). The viscosity for oat β-glucan is approximately 100-fold higher than barley β-glucan. The viscosity for both β–glucans is a direct consequence of β–glucan content regardless of the quantity and makeup of α-glucan impurities present (Mikkelsen et al., 2010). The solubility of β–glucans is related to the molecule structure, molecular weight and proportion of (1→4)-β- to (1→3)-β-links (Comino et al., 2013). B–glucan degradation was observed during oxidation with hydrogen hydroxide (Mäkelä et al., 2015).

Jansen et al. (2013) reported that ß-glucan concentration was not affected by growing conditions including N-fertilizing, fungicide dosage or the number of seeds used. The fungicide application in general reduced the β–glucan content. However, some varieties exhibited an increase in β–glucan (Dickin et al., 2010).

Heavy irrigation during barley maturity decreases β–glucan content in the kernels (Güler, 2003). B–glucan content varies depending on variety, location, year, and pre- and post-harvest conditions (Ehrenbergerová et al., 2008; Abdel-Aal and Choo. 2014). Naked barley cultivars cultivated at higher altitudes had higher bioactive β–glucan (Moza and Gujral, 2016).

1.9.2.3 β–glucan types

Naked barley β-glucans can be divided into soluble and non-soluble types, with their relative proportion's dependent on their source and extraction conditions. The soluble extracts of β-glucan increase from 50−70% as temperature increases from 40-65^{o}C (Comino et al., 2013). B–glucan primarily consists of two essential units cellotriose (DP3) and cellotetraose (DP4) linked by β 1-3 bonds (Wood, 2010). The ratio of DP3/DP4 is used to identify various types of cereal β–glucans and is linked to polymer solubility.

1.9.2.4 β–glucan extraction

Numerous industries strive to get economically feasible extracts of highly pure β–glucan from cereals for food production, and more research is needed in this regard (Güler, 2003; Hu et al., 2015). β–glucan extraction is dependent on temperature, pH, extraction time, molecular size, solvent used and crude material (Hu et al., 2015). The most recent β-glucan extraction method reviews were carried out by Gangopadhyay et al. (2015) and Maheshwari et al. (2017). A temperature of 55.7°C and a pH of 6.6 are reported to be optimal for β–glucan extraction Gangopadhyay et al. (2015). Higher levels of (1,3;1,4)-β–glucan in cereal kernels can be extracted and attained using specific amino acids residues and CSLF6 enzyme regions (Dimitroff et al., 2016). Nevertheless, β–glucan deterioration cannot be avoided regardless of extraction approach employed (Rimsten et al., 2003). Wet β-glucan extraction is expensive, limiting its application in food processing (Hu et al., 2015).

1.9.2.5 β-glucan and amylose analysis methods

Numerous approaches are used for β–glucan analysis including the Calcoflour method (Jørgensen and Aastrup, 1988), the enzyme assay of McCleary and Codd (1991), HPLC (Åman and Graham, 1987), HPAEC-PAD (Åman and Graham, 1987), MALDI-MS (Jiang and Vasanthan, 2000), and mid-IR (Kačuráková and Wilson, 2001). These detection methods are

associated with limitations including low sensitivity, long detection times, and high costs (Schmitt and Wise, 2009).

The enzymatic method (McCleary and Codd, 1991) described in Approved Method 32-23 AACC International (2000) is the most commonly used method for barley (and oat) β–glucan analysis. In that method the mixed-linkage, linear β–glucan polymer extracts obtained from barley flour. The monosaccharides are produced through subsequent enzyme reactions after that they are hydrolysed by (endo-(1→3, 1→4)-β-D-glucan 4-glucanohydrolase (Lichenase) followed by β-glucosidase) and ultimately adjoined for colour generation using glucose oxidase and a chromogenic substance. The equipment needed for this analysis is relatively simple and include: the principal reagents (available from Megazyme; www.megazyme.com), spectrophotometer, centrifuge, pipettes and water bath. Nevertheless, the enzymatic approach is laborious and costly. Hence, there is a need for an accurate, quick, non-destructive, and economically feasible method.

Ringsted et al. (2017) describe a method for analysing intact barley seed β-glucan content using a supercontinuum laser. They suggested that their method of assessing the β-glucan content of a single seed could be improved by instead analysing the average β-glucan content of each barley line. On the other hand, measuring the βglucan content for individual seeds has an advantage over bulk seed β-glucan analysis as diseased and insect damaged seeds cannot be detected and eliminated in the bulk analysis.

1.10 Naked barley food product development and research

Andersson et al. (2004) reported that dough production and fermentation time should be kept at short as possible while preparing bread from naked barley to preserve β-glucan high molecular weight. This is imperative for its cholesterol reducing effect. Naked barley flour requires more moisture as its water holding capacity is high. β–glucan release was higher after cooking, and lower after baking (Johansson, 2006). Naked barley noodles dough is less

compressed, and it is more prone to breaking into little chunks compared to hard wheat (Comino et al., 2016).

Naked barley is a potential substitute for most commonly used cereals, except in leavened wheat bread, and may lead to more diversified human food production (Kinner et al., 2011) and could be used in gluten-free bread (Ronda et al., 2015). Hussein et al. (2013) stated that whole barley meal could substitute wheat in the making of balady (flat unleavened local Egyptian bread), improving its β–glucan, protein, fat, fibre, ash, and mineral (Ca, P, K, and Fe) content. Sourdough, which is a blend of water and flour treated with lactic acid bacteria is reported to improve the flavour of barley bread (Mariotti et al., 2014).

1.11 Naked barley health benefits

It is claimed that β-glucan has a number of health benefits (Newman et al., 1998). The European Food Safety Authority (EFSA) reported that daily consumption of β-glucan enhances normal cholesterol levels in the blood (Tong et al., 2015; European Food Safety Authority (EFSA), 2011). β–glucan from barley dramatically suppresses the blood glucose increase experienced following eating, may lower the risk of heart disease, reduce the risk of type 2 diabetes development, increase blood cholesterol control, fortify the immune system and alleviate physical and mental stresses (Inglett, 2013). However, β-glucanase activity management is vital in realising these physiological advantages (Ronda et al., 2015).

Cereals made with superior quality naked barely, and naked oats, had a low glycemic index than any other cereals (Hussein et al., 2013). This qualifies naked barley and naked oats to be categorised as functional foods which positively enhances human health beyond basic diet. (Steele et al., 2013). Englyst et al. (2003) found that the glycemic index reflects the cumulative effects of different grain constituents on absorption and disposal of glucose from the blood. β–glucan increases Ca, Fe, Mg and P content in noodles (Aktaş et al., 2015).

1.11.1 Glycemic index (GI).

The glycemic index (GI) is a system developed three decades ago for categorizing foods based on their capacity to quickly raise blood sugar levels (Wolever et al., 1991; Yu et al., 2016). The GI approach could be usefully applied to fat and protein mixed meals (Wolever et al., 1986). The application of the GI system can help to avoid, and reduce the need for medication for chronic diseases such as diabetes (Jenkins et al., 2002) and its reliability is well–established (Scazzina et al., 2016; Wolever et al., 2017). Low GI meals for pregnant women with gestational diabetes mellitus can reduce blood glucose levels by 50 % and minimize glucose instability (Kizirian et al., 2017). High dietary GI is correlated with elevated cancer risk (Sieri and Krogh, 2016). The glycemic index can be calculated by AUC method which refers to the area under the two-hour blood glucose response curve following a 12-hour fast and ingestion of a food with a certain quantity of available carbohydrate (usually 50 g) , a defined blood sampling plan, with no less than two tests of the referenced food, and at least two subsets of $n = 10$ (Wolever et al., 2017).

1.11.2 Glycemic index calculations

The Glycemic index measurement is affected by food portion size, standard food used, testing repeats of the standard food, blood sampling frequency, and area calculation approach. Other related attributes include blood sampling method; demographic traits of participants such as age, sex, obesity status, glucose tolerance rating; dosage and insulin injection timing (Wolever et al., 1991).

The area under the glycemic response curve for each food is calculated as a percent of the mean response to the standard food taken by the same person, and the results are averaged to obtain the GI value for the food (Monro and Shaw, 2008).

1.11.3 Factors that affect the GI value attained

The Glycemic index is defined as 'the incremental area under the blood glucose response curve (IAUC) of a 50 g carbohydrate portion of a tested food expressed as a percent of the response to the same amount of carbohydrate from a reference food taken by the same participant (white bread or glucose), on a different day (Jenkins et al, 1981; Wolever et al., 2006).

1.11.4 Testing glycemic index in human.

The glycemic index is measured by feeding portions of test foods and white bread containing 50 g available carbohydrate to normal or diabetic persons in random order on separate occasions after an overnight fast. The standard food (white bread) should be repeated at least three times to minimize variability.

Finger blood samples are taken for normal fasting persons at 15, 30, 45, 60, 90, and 120 min after the start of the test meal, and at 30 minutes intervals for 3 hours. The normal dose of insulin or oral tablets if any, is taken after the fasting blood sample and 5-10 min before eating the test meal. Capillary blood is reported to provide a more precise blood glucose measurement than venous blood for the area calculation (Wolver et al., 2003). The subject characteristics, treatment, and degree of control of glucose content in the blood may have major effects on the glycemic response obtained, if they are standardized. They appear to influence the response to all foods similarly and so have only a small effect on the resulting GI value, usually by influencing the variability of glycemic responses. Other standard foods could be used but to allow comparison with the GI of glucose or white bread, the standard chosen should be compared carefully with white bread or glucose before doing other tests. Using low-GI foods rather than high GI foods have a little but efficient role in controlling diabetes in the medium term (Brand-Miller et al., 2003).

1.12 Traditional food barley worldwide

Jilal (2011) reported the following barley foods produced and consumed in different parts of the world: n Libya, *koubz* (bread), *bazin*, *zummeta*, *dshesha*, *harisa*, and *couscous*. In Tunisia: *kisra, malthouth, d'chich, mermez, bazine, assida, b'sissa, hail*, and *dardoura*. In Eretria, *Injera, Kisha, Geat*, and *Siwa*. In Ethiopia, *Besso, Zurbegonie, Chiko, genfo, kolo*, and *kinche*. In Yemen, *Zoam, Alaath, Maloog Nakia*. In Iran, barley soup and *ma-ol shaer*. In Nepal, roasted naked barley and barley porridge, whereas. In Ecuador, Colombia, Peru and Bolivia are *Machica* and cracked barley. In Tibet, *Tsangpa* and *Chang* are the principal naked barley food products. Naked barley has gained importance as a health food due to its high non-cellulosic polysaccharide β–glucan and arabinoxylan content (Behall et al. 2006;Barabaschi et al., 2012; Zheng et al., 2012 ;Abumweis et al., 2010; Zhu et al., 2016).

1.13 Objectives of this research:

This literature review has highlighted an increasing interest in naked barley worldwide. Previous breeding has produced a promising naked line (Deiniol) that has long stems, and there is a need to evaluate plant growth regulator effect on crop performance under UK conditions. Furthermore, screening different UK-adapted naked barley lines for shorter stem length, higher yield, and higher β-glucan, amylose, and amylopectin content can identify the lines best suited for end use as human food.

This research aims to:

1- Evaluate the effect of plant growth regulator on naked barley line as compared to hulled barley under UK conditions.
2- Screen naked barley lines for stem length, field performance, β-glucan, amylose, and amylopectin content and adaptation to UK conditions.
3- Develop a high β-glucan naked barley line with adaptability to UK growing conditions and calculate narrow and wide heritability for β-glucan.

Hypotheses tested:

- Application of plant growth regulator at a certain growth stage with a particular dosage will increase or decrease effects on naked barley stem length, yield and yield components under UK conditions.
- The different UK selected naked barley lines vary in their beta-glucan, amylose, and amylopectin and field performance.
- Crossing Deiniol naked barley with starch mutant Riso 13 may produce high β-glucan naked barley.

Chapter 2

General Materials and methods

This chapter describes general materials and methods commonly used in all field experiments. Specific methods are described with more details in their relevant chapters.

2.1 Management practices

2.1.1 Experimental site

This research was conducted at Bangor University's Research Centre (Henfaes), (henceforth "Henfaes") Abergwyngregyn, Gwynedd. Located at 53° 14′N, 4° 01′W on North Wales.

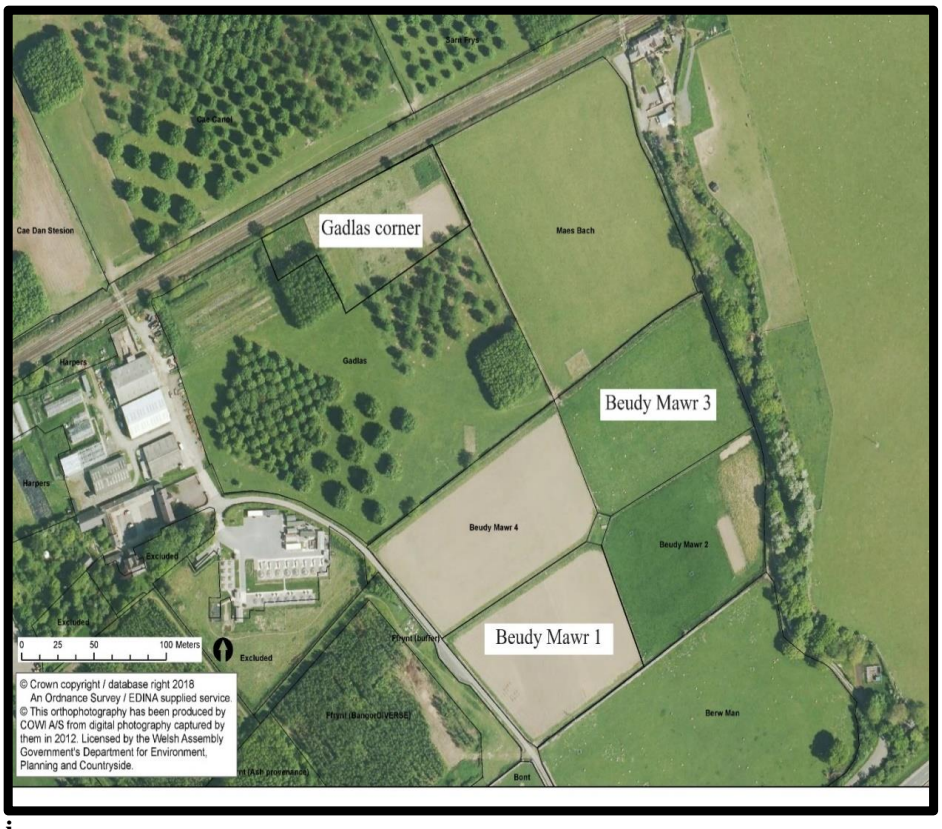

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Figure2-1: Experimental plots at Henfaes experimental station

The average annual precipitation is 1250 mm with a mild maritime climate (Millett et al., 2012). Field trials took places on the field named as Beudy Mawr 1, Beudy Mawr 3 and Gadlas corner (Figure2.1). During the spring seasons of 2015, 2016 and 2017 respectively. Table 2.1 shows soils analysis for pH, Phosphorus (P) and potassium (K) and magnesium (Mg) for different experimental fields.

Table 2.1: Selected analysis of the experimental site

Property	2015	2016	2017
pH	6.2	5.8	5.6
Available P (mg /l) and availability index	46.2(4)	35.4(3)	22.6(2)
Available K (mg /l) and availability index	157(-2)	103(1)	174(-2)
Available Mg (mg /l) and availability index	63(2)	54 (2)	82(2)

Figure 2.2 shows the monthly average temperature (°C) and total monthly rainfall (mm) during the period from the 25th of March to the 15th of September 2015-2017 in Henfaes research station.

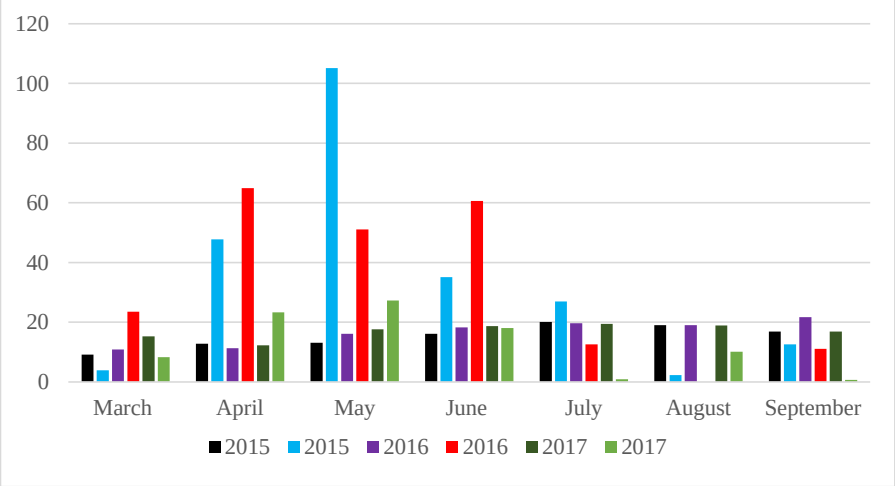

Figure 2.2: Temperature (°C; **black, purple, dark green) and rainfall (blue, red, light green) during the spring season 2015-2017**

2.1.2 Soil preparation

The land was ploughed and harrowed. A Wintersteiger drill was used for plots sowing, and fertilizer application, with 12 cm inter-row spacing, and plot size was as described in (Table 2.2). The seed rate was adjusted based on year, genotype, germination percentages, and 1000 grain weight to achieve the targeted number of seeds per square meter as described in (Table 2.2).

Table 2.2: Plot size, number of rows and seeding rate per square meter

Year	Plot size	Number of rows	Seed rate Seeds/m^2
2015	1.8 m x 10.0m	16	250
2016	1.8 m x 10.0 m	16	250
2017	1.2 m x 4.0 m*	10	280

*The drill width was adjusted in 2017 to the above mentioned from 1.8 m to 1.2 m.

Weed control was applied by a tractor mounted hydraulic nozzle sprayer.

PGR was applied by a tractor in 2015 and with a knapsack sprayer in 2016 at growth stage 32 and growth stage 37 respectively and with no PGR in 2017. Bamboo canes were used to mark out plots and treatments application. Deiniol received PGR on the 7th of June 2015 and Sanette on the 12th of the same month. Lime, Fertilizer granules, herbicides and PGR rates were applied as described in (Table 2.3).

A sub –set rows from all the plots were harvested by hand. Randomly chosen one meter long eight middle rows in 2015, Two randomly chosen rows, one meter long in 2016 (representing 0.25 m^2 of the plot in order to minimize the size of work needed in samples preparations for measurements) and five rows randomly chosen one meter long rows representing 0.5 m^2) from two sampling areas within each plot in 2017 (as the width of the plot was decreased from 1.8 m to 1.2 due to seed drill width adjustment during that season). The left-over crop was harvested later by the combine harvester.

Table 2.3: Field trials summary included in this research and their treatments

Year/ Chapter	Fertilizer	Herbicide	Lime	PGR Treatments
2015/ Chapter 3	50 kg/ha N Ammonium nitrate 130 kg/ha Phosphorus Triple superphosphate 130 kg/ha potassium Murate of potash	Glyphosate 6 l/ha + 2,4 D 1 l/ha	650 kg/ha	0.5 l/ha Moddus PGR
2016/ Chapter 3	The same as previous	The same as previous	The same as previous	0.0.25.0.5,0.75 and full strength

2.1.3 Plant material

Table 2.4 displays seed resources used in this research.

Table 2.4: Seed resources used in this research

Name	Hull type	Row type	Source	Usage	No of entries
Deiniol	Naked	2	Bangor University	Food	1
Sanette	Hulled	2	Syngenta / UK	Feed	1
Propino	Hulled	2	Syngenta / UK	Malting	1
Riso 13	Hulled	2	Scandinavian	Malting	1
Static	Hulled	2	UK	Malting	1
Westminster	Hulled	2	Nickerson /France	feed	1
Optic	Hulled	2	UK	Malting	1
Lawina	Naked	2	Germany	Food	1
Skardu	Naked	2	Pakistan	Food	1
ICARDA 93 X	Naked	2	ICARDA	Food	1
27 DUS	Naked	2	Bangor University	Food	1
Deiniol x Propino	Naked	2	Bangor University	Food	20
Static x Skardu	Naked	2	Bangor University	Food	4
Line 20 x Westminster	Naked	2	Bangor University	Food	8
Line 15 x Cocktail	Naked	2	Bangor University	Food	9
Line 15 x Westminster	Naked	2	Bangor University	Food	5
Line 15 x Optic	Naked	2	Bangor University	Food	2
Deiniol x Rios 13	Naked	2	Bangor University	Food	56
Skardu Oldings x Static RILs	Naked	2	Bangor University	Food	10
				Total	125

2.1.4 Grains for data collection and polysaccharide analysis

The spikes were oven dried at 80^{o} for 48 hours, weighed, threshed, chaff weighed, data were recorded and samples were prepared for consecutive β–glucan and amylose analysis.

The following formula was used to calculate Grain harvest index:

Grain harvest index = (Grain yield)/ (Grain + straw yield).

2.1.5 Lodging assessment

A visual percentage area estimation of lodging crop area for each plot before harvesting was carried out and the data were recorded.

2.1.6 Grain threshing

Minibatt rechargeable handheld seed thresher was used for seed threshing.

2.1.7 Thousand grain weight (TGW)

Numerical seed counter (Sinar technology company, UK) (www.sinar.co.uk) was used to count 1000-grain. The equipment was adjusted based on the manufacturer's instructions. A container feeder holds the seeds which are forced to the outlet through vibration. The falling grains pass through a photoelectronic counter. The grains after collection are used to calculate thousand grain weight (TGW).

2.1.8 Grain and straw moisture content

The fresh sample weight was recorded dried at 80 ℃ for 48 hours, and grain moisture content was calculated using the following formula:

GMC %= [(FW-DW)/FW] *100

Where the FW=Fresh weight of the sample and DW=Dry weight of the sample.

2.1.9 Megazyme β–glucan assay procedure

Megazyme assay kit (K-BGLU) which includes (Lichenase enzyme, β -Glucosidase suspension, GOPOD Reagent Buffer, GOPOD Reagent Enzymes, D-Glucose standard solution, and standardized barley flour control). This assay kit was used for β–glucan analysis with the following procedure. Barley grains were ground using an electrical mill (Cyclotech, Foss, UK). About 0.5 g barley flour of known moisture content was weighed in polypropylene tubes. 1.0 ml

ethanol (50% v/v) was added to each tube to enhance samples dispersion. 5.0 ml sodium phosphate buffer (20 mM, pH 6.5) was added and mixed on a vortex mixer. The tubes were incubated on a boiling water bath for about 2 minutes. After that, the tubes were removed and stirred on a vortex mixer. The tubes were heated for three more minutes in the boiling water bath.

The tubes were cooled to 40℃, and 0.2 ml of Lichenase was added to each tube. The tubes were capped, stirred and incubated at 40 ℃ for I hour. The volume of each tube was adjusted to 30.0 ml by distilled water addition. The contents were mixed, and an aliquot of 1.0 ml of each tube was transferred to 2.0 ml Eppendorf tubes centrifuged at 2000 g for 5 minutes. An aliquot of (0.1 ml) from each Eppendorf tube was transferred to the bottom of three test tubes. A 0.1ml of sodium acetate buffer (50 mM, pH 4.0) was added to one of the tubes (the reaction blank), while 0.1 ml B-glucosidase (0.2 U) (50 mM sodium acetate buffer, pH 4.0) was added to the two other tubes (the reaction). The tubes were incubated at 40oc for 15 minutes. 3.0 ml of GOPOD reagent (one of the reagents included in the assay kit) was added to each tube and incubated at 40oc for 20 minutes. The absorbance reading at 510 nm was read on microplate spectrophotometer (Bio-Tek Instruments, Luminar Technology Ltd., Waltham, Southampton, UK) for each reaction blank and reaction was recorded. Mega-Calc. (an Excel calculation sheet) provided by Megazyme Company was used to calculate the content of β–glucan in each sample.

2.1.10 Megazyme amylose /amylopectin assay procedure

The kit includes (K-AMYL) the following chemicals: Freeze dried Con A, Amyloglucosidase, GOPOD Reagent Buffer, GOPOD Reagent Enzymes, D-Glucose standard solution, Starch reference sample (with known amylose content).

2.1.10.1 A. Starch pre-treatment

Barley flour samples (25mg) were accurately weighed into a 10.0 ml screw capped Kimax sample tubes, and the weight was recorded. I.0 ml of Dimethyl sulphoxide (DMSO) was added. The tubes were stirred on a vortex mixer. The tubes were capped, heated in a boiling water bath so that the samples were entirely mixed. No gelatinous lumps were left over. The contents were mixed vigorously in a vortex mixer, and the tubes were placed in a boiling water bath and heated for 15 min with intermittent vigorous mixing on a vortex mixer. After that, the tubes were stored at room temperature for about 5 min, and 2.0 ml of 95 % (v/v) was added with continuous mixing. Additional 4.0 ml of ethanol was added. The tubes were capped and inverted to mix. The formed precipitated starch was left to stand for 15 min.

The tubes were centrifuged at 2000 g for 5 min. The supernatant was discarded, and the tubes were drained on tissue paper for 10 min. The formed pellet at the tube bottom was subsequently used to determine amylose and starch content. 2.0 ml of DMSO was added to the starch pellet while gently mixed. The tubes were placed in a boiling water bath for 15 min and were intermittently mixed. No gelatinous lumps were left to remain in the tubes. After the tubes were removed from the boiling water bath, 4.0 ml Con A solvent was added stirred vigorously and the content of the tubes was transfected to 25.0 ml volumetric flask by repeatedly rinsing with Con A solvent (included within the assay kit), The volume was diluted with Con A solvent (this was labelled as Solution 1). The solution was filtered through Whatman No one filter paper.

2.1.10.2 B. Con A Precipitation of amylopectin and amylose determination

1.0 ml of solution A was transferred to a 2.0 ml Eppendorf microfuge tube. 0.50 ml of Con A solution (bottle1**)** was added to the tubes gently mixed by repeated inversion. The tubes were left to stand for **one** h at room temperature. The tubes were centrifuged at 14000 g for 10 min at room temperature. I.0 ml of supernatant was transferred to a 15.0 ml centrifuge tube. 3.0 mL of 100 mM sodium acetate buffer, pH 4.5 was added mixed and heated in a boiling water

bath for 5 min to denature the Con A. The tubes were placed in a water bath at 40^{o} C and equilibration was allowed for 5 min. 0.1 ml of amyloglucosidase/α-amylase enzyme mixture was added and incubated at 40^{o} C for 30 min. The tubes were centrifuged at 2000 g for 5 min. 4.0 ml of GOPOD reagent (Reagent B) was added to 1.0 ml of supernatant aliquots. Incubated at 40^{o} C for 30 min and centrifuged for 20 min. The Reagent blank and the D-Glucose controls were incubated concurrently. The absorbance for each sample and the D-glucose controls were at 510 nm against the reagent blank.

2.2.10.3 C. Determination of Total starch

0.5 ml of Solution A was mixed with 4.0 ml of 100 mM sodium acetate buffer pH 4.5. 0.1 ml of amyloglucosidase/α-amylase solution was added, and the mixture was incubated at 40^{o}C for 10 min. 1.0 ml aliquots (in duplicate) of this solution was transferred to a glass test tube, and 4.0 ml of GOPOD Reagent (solution 4) was added and mixed. The tubes were incubated at 40^{o} C for 20 min. The incubation was concurrently done for samples and standards from section B above.

$$\text{Amylose \% (w/w)} = \frac{\text{Absorbance (Con A supernatant)}}{\text{Absorbance (Total Starch Aliquot)}} \times \frac{6.15}{9.2} \times \frac{100}{1}$$

$$\text{Amylose \% (w/w)} = \frac{\text{Absorbance (Con A supernatant)}}{\text{Absorbance (Total Starch Aliquot)}} \times 66.8$$

Where 6.15 and 9.2 are dilution factors for the Con A, and Total Starch extracts respectively.

2.2.10.4 Amylopectin calculation

The following formula was used to calculate amylopectin:

Amylopectin %= 100 (%) - amylose (%).

2.3 Statistics

2.3.1 Analysis of variance (ANOVA)

ANOVA was used to analyze data using IBM SPSS 24 software in all sections except section 3.3.4.1. General linear model (GLM) univariate analysis was used. Normal data distribution test, general analysis of variance probabilities were calculated to check if the overall means differences are significant or not at ($p \leq 0.05$) level .And then followed by post hoc test to explore multi-comparison differences among different means and variance was calculated.

2.3.2 Regression analysis

Regression analysis identifies the relationship between a dependent variable and one or more independent variables. A hypothesized model of the relationship is estimated and regression equation is developed. Scatter plot was built using Microsoft Excel 2016.

Chapter 3

Effect of plant growth regulator

3.1 Introduction

3.1.1 Stem shortening (2015 field trial)

With growing interest for naked barley as healthy food. There is a need to increase naked barley yield, higher harvest index and, improving its quality. In their review Berry et al., (2004) reported that breeding crops for shorter stems through genotype selection could be limited due to minimum stem length compatibility with higher yields. Boukerrou and Rasmusson (1990) found in 42 barley genotypes under investigation that semi-dwarf barley genotypes were 3.3% higher in harvest index than the taller genotypes.

Lodging which is defined as the displacing of small grain cereals from their vertical position (Knapp et al.,1987). It adversely affects grain moisture content, grain quality and complicates combine harvesting process. Lodging usually occurs mostly after spike or panicle emergence. It can adversely affect plants water, nutrient uptake and consequently minimizes grain filling (Berry et al., 2004). Lodging could contribute to 80 % loss of harvested grain, higher grain drying cost and slower mechanical harvest by combine harvesters (Berry et al., 2000) .Leading to low marketability and consequently a plunge in farmers' turnovers. Lodging is triggered by topography, type of soil, previous crop wind and rain. There two types of lodging stem lodging caused by 1) broken stem bases by wind and rain and 2) roots lodging caused by roots displacement in the soil (Matthan et al., 2016).

However, stem shortening, strengthening, yield improvement and higher harvest index could be achieved through PGR application and, consequently a significant scope for wide genetic variation exploitation for these plants' traits. Starting from mid-1960s three types of PGRs were introduced chlormequat chloride was the first. It was followed by ethephon and trinexapac-ethyl during late 1980s and 1990s respectively(Rademacher, 2015).

Moddus a plant growth regulator from Syngenta Company UK. Moddus curbs lodging by reducing stem length, and enhancing stem and root structure (Grijalva et al., 2012). Consequently, boosting and raising up crop yield in different barley varieties and other cereals. Moddus contains 250g/l (25.5% w/w) trinexapac-ethyl per litre. Moddus as most PGRs inhibits gibberellic acid production (Matysiak, 2006). The application of PGR should be avoided when rain or frost is expected and when the crop is wet. The crops under stress of waterlogging, pest attacks and disease should not be sprayed. Early PGR application on barley should take place prior to third node detectable stage (ZGS 32.). There is a need to study the effects of plant growth regulators on naked, hulled barley under UK conditions and their effects on β–glucan, amylose and amylopectin content.

Deiniol inherits long plant stem from its Pakistani ancestor (Steele et al., 2013). Making it more vulnerable to lodging and less attractive to UK farmers' preferences of short plants for heavier spikes and easier combine harvesting. Zeeman et al., (2010) reported that Sanette is a hulled spring malting barley variety. Sanette yield is one of the highest spring barley yield on the recommended HGCA list of recommended barley varieties for 2014. It is a promising barley variety all over the UK regions bred in the UK by Syngenta and developed in New Zealand. It has a great potential for malting and animal feeding. It is a well disease resistant with a reasonable straw strength and early-moderate maturity.

Maximizing economic revenues are the most important criteria to farmers than maximum yield. Therefore, scientists and researchers in UK exert substantial efforts aimed at profit maximization and reduction in chemical consumption in agricultural production. This research hypothesizes that smaller doses of manufacturer's recommended PGR rate may be as effective as the full dose on lodging control and yield improvement under low N inputs (50 kg ha^{-1}).

3.2 Objectives

This research aims to assess the effects of plant growth regulator (Moddus) on stem length, yield, beta-glycan, amylose and amylopectin content on Deiniol (naked) compared to Sanette (hulled).

- To study the effect of late time PGR application at ZGS (37) on yield, lodging and whether smaller doses of plant growth regulator could be used to minimize the cost on farmers and lower impact on the environment.

3.3 Materials and methods

3.3.1 Stem shortening trial

Deiniol from Bangor University previous crossings, previous season produce and Sanette the most recent hulled from Syngenta UK (www3. syngenta.com) were used in this experiment. Germination was tested on Whatman No. 1 filter paper, moistened with deionized water. The germination percentage was 85 % and 100% for Deiniol and Sanette respectively. The seed rate was adjusted based on germination test and thousand grain weight and the rate used as mentioned in table 2.1. Each plot was divided into two. Randomly chosen one half of each plot was treated with plant growth regulator (PGR) while the other part was not treated.

Split-plot design with four replicates was used in this section of chapter 3. The varieties were the main plots while PGR/ no PGR treatments were the subplots. The plots were sown on the 25th of March 2015. Fertilizers and PGR were applied as described in table 2.2. The trial was harvested on the 23rd of July 2015. In all plots stem length for five plants from different parts of each plot StL were measured and recorded. The length from the base of the stem to the bottom of ear was measured. Eight middle rows 1 m long sampling area was randomly selected from each plot. Data were recorded at the time of final harvest. The plants were pulled out of the soil and underground plant parts were cut off and discarded.

3.3.2 Late PGR application trial

Four different concentrations of PGR and one control was used in this experiment (Table 3.1). And Deiniol naked barley was the only variety used in all plots.

Table 3.1: List of PGR treatments

Treatment	Quantity (ml/ha)	Abbreviation
Control (no PGR)	0	C
Quarter dose	125	0.25
Half dose	250	0.5
Three quarter doses	375	0.75
Full dose	500	Full

Experimental design, seeding rate and plot size were as described in table 2.1 and table 2.2 (above) respectively. The experiment was sown on the 21st of April harvested on the 2nd of August 2016 by hand and the left-over crop was combine harvested. The following parameters in all plots were measured stem length (StL) for five randomly chosen plants from different parts of each plots, internode length for ten randomly chosen stems, straw dry weight, number of ears per meter square (EPSM), number of grains per ear (GPE) thousand grain weight (TGW), harvest index (HI), lodging and grain yield per meter square (GY).

3.3.3 Measurement of plant characters

Responses to PGR application were recorded by monitoring stem length (for five plants in different positions in each plot), visual lodging (%) of the plot , disease incidence, ears m^{-2} (EPSM), grains ear^{-1} (GPE), grain yield in gm^{-2} (GY), 1000-grain weight (TGW), total straw fresh per square meter (TsF) weight, fresh straw subsample (FSSW) , an oven dried at 80 ℃ for 48 hours subsample weights (DSW), and moisture content was calculated.

3.3.4 Statistical analysis

3.3.4.1 Stem shortening trial

Split-plot analysis of variance in Genstat was used to study significant responses of key treatments and their interactions. Data were analysed as for a split-plot design with varieties as the main plots and PGR as subplot. Probability level of 5% was used to compare and contrast between treatment means. Regression analysis was used to explore relationships between variables.

3.3.4.2 Late PGR application trial

The data for this part of the chapter were analysed as described in section 2.3.1.

3.4 Results

3.4.1 Stem shortening trial

3.4.1.1 Fungal infection

Few plants, in different plots, were infected with smut as the season was relatively a dry season. Those plants were taken out of all plots and destroyed.

3.4.1.2 Stem length (StL)

There was a significant effect for variety, PGR and their interaction on StL for naked and hulled varieties. The StL decreased with PGR application as compared to non-PGR treated plants (Table 3.2).

Table 3.2: PGR effect on stem length

	No PGR	PGR	Mean
Deiniol	118.2	75.5	96.9
Sanette	80.2	68.5	74.4
Mean	99.2	72.0	

SE variety means=2.1, p=0.002
SE Treatment means=3.71, $p < 0.001$.
SE interactions means=4.26 except for comparing within varieties (5.24), p=0.006.

The stem length for Deiniol decreased from 118.2 cm to 75.5 cm and from 80.2 cm to 68.5 cm for Sanette a 36% and 15% decrease for both varieties respectively.

3.4.1.3 Grain yield (GY) per m^2

A significant difference was observed in GY for both varieties, but no significant difference was seen for PGR and there was no significant difference was shown for variety x PGR interaction (Table 3.3).

Table 3.3: PGR effect on grain yield (g/m^2)

	No PGR	PGR	Mean
Deiniol	308.4	350.6	329.5
Sanette	644.7	788.4	716.6
Mean	476.5	569.5	

SE variety means=18.71, $p < 0.001$.
SE Treatment means=49.31, $p = 0.108$.
SE interactions means=52.74 except for comparing within varieties (69.73), p=0.343.

3.4.1.4 Straw dry weight per m^2

Straw dry weight recorded significant differences for PGR and variety x PGR interaction but it was not significant between varieties. The straw dry weight increased from 180.0 g to 595.6 g for Deiniol and from 373.6 g to 405.7 g for Sanette which is 2.31- and 0.16-folds increase in both varieties respectively (Table 3.4).

Table 3.4: PGR effect on straw dry weight (g/m^2)

	No PGR	PGR	Mean
Deiniol	180.0	595.0	387.5
Sanette	373.0	405.7	389.4
Mean	276.0	500.4	

SE variety means=56.63, p=0.976
SE Treatment means=44.86, p= 0.002.
SE interactions means=72.24 except for comparing within varieties (63.45) p= 0.005.

3.4.1.5 Harvest index (HI)

HI had significant means differences between varieties, treatments, and PGR x varieties interactions (Table 3.5). HI shown a significant decrease (p =0.028) from 0.53 to 0.32 for Deiniol as a response for PGR treatment. Sanette on the other hand had a significant increase from 0.60 to 0.62 as a response for the same treatment.

Table 3.5: PGR effect on Harvest index

	No PGR	PGR	Mean
Deiniol	0.53	0.32	0.43
Sanette	0.60	0.62	0.61
Mean	0.57	0.47	

SE variety means=0.047, p=0.028.
SE Treatment means=0.038, p= 0.044.
SE interactions means=0.060 except for comparing within varieties (0.053) p= 0.021.

3.4.1.6 Ears per square meter (EPSM)

Significant differences were observed between EPSM variety means at (p=0.026). no significant differences were seen between treatment means (at p=0.099), but no significant differences were recorded for variety x treatments means (Table 3.6).

Table 3.6: PGR effect on ears per square metre

	No PGR	PGR	Mean
Deiniol	372.0	508.0	440.0
Sanette	553.0	563.0	558.0
Mean	462.5	535.5	

SE variety means=28.7, p=0.026.
SE Treatment means=37.3, p= 0.099.
SE interactions means=47.1 except for comparing within varieties (52.8) p= 0.140.

3.4.1.7 Thousand grain weight (TGW)

Significant differences were observed between variety means for TGW at (p=0.010) and between treatments means (at p=0.034) and variety x treatments interactions (Table 3.7).

Table 3.7: PGR effect on thousand grain weight (g)

	No PGR	PGR	Mean
Deiniol	37.0	43.0	40.0
Sanette	48.0	51.0	49.9
Mean	42.5	47.4	

SE variety means=1.70, p=0.010.
SE Treatment means=1.75, p= 0.034.
SE interactions means=2.44 except for comparing within varieties (2.47) p= 0.534.

3.4.1.8 B–glucan content (g/100g)

There were significant differences between PGR treatments means for B–glucan content at (p=0.010) but no significant differences were seen between varieties and PGR x variety interaction (Table 3.8).

Table 3.8: PGR effect on β–glucan content (g/100g)

	No PGR	PGR	Mean
Deiniol	3.37	5.37	4.37
Sanette	3.05	4.80	3.93
Mean	3.21	5.09	

SE variety means=0.501, p=0.064
SE Treatment means=0.157, p= 0.010.
SE interactions means=0.525 except for comparing within varieties (0.708) p= 0.811.

3.4.1.9 Amylose and amylopectin content (%)

Amylose and amylopectin means were not significantly different neither for variety nor for PGR treatments and their interaction (data not shown).

3.4.1.10 Lodging (%)

No signs of complete lodging were visually observed in all plots. However, severe necking (bending of the stem right under the spike) was noted on untreated Deiniol plots.

3.4.1.11 Regression

The regression analysis at p≤ 0.01 showed a significant negative relationship between stem length vs. yield (Figure 3.1). And a significant positive relationship between stem length vs harvest index (Figure 3.2).

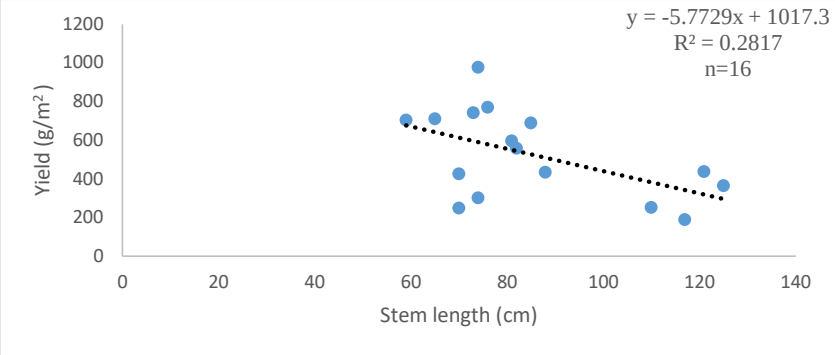

Figure 3.1. Scatter plot of stem length vs yield

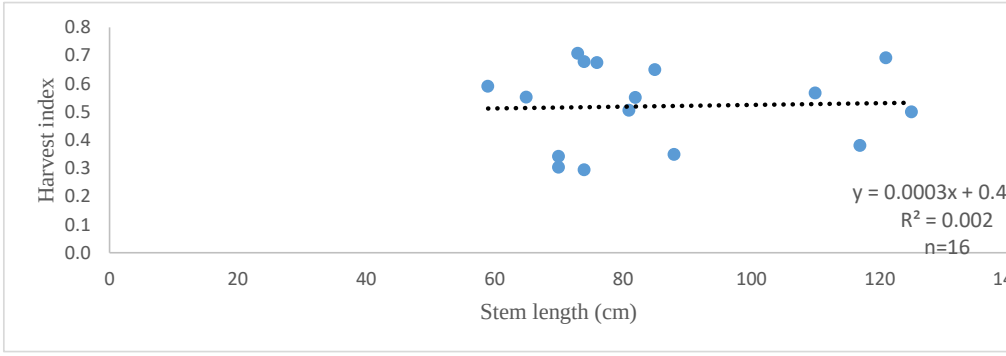

Figure 3.2. Scatter plot of stem length vs harvest index

3.4.2 Late PGR application time

Please refer to appendix 1.1 for tests of normality, frequencies and ANOVA table.

3.4.2.1 Stem length (StL)

There were no significant differences in stem length at $p \leq 0.05$. StL was not affected by different PGR concentrations (Figure 3.3).

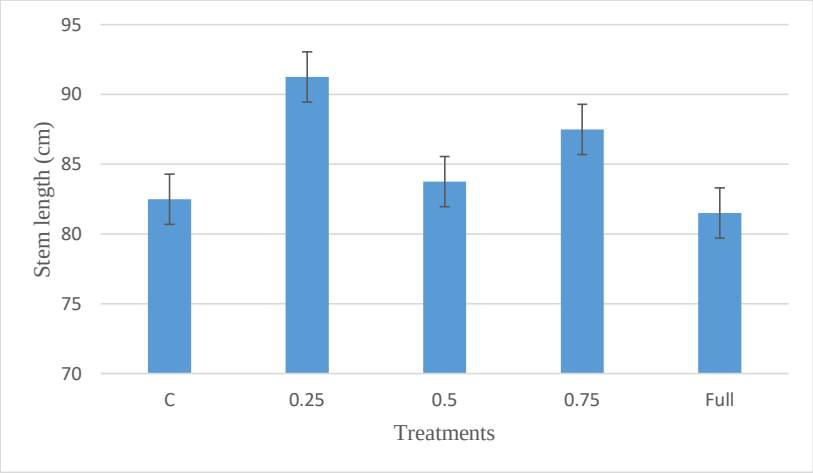

Figure 3.3: Effect of PGR on stem length
Bars on columns represent standard error of differences of means

3.4.2.2 Grain yield (GY) per m^2

No significant differences were observed between different treatments in GY as shown in Figure 3.4.

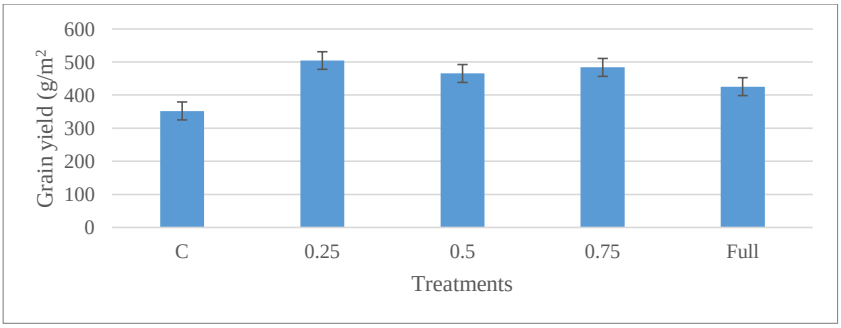

Figure 3.4: Effect of PGR on grain yield
Bars on columns represent standard error of differences of means

3. 4.2.3 Straw dry weight per m^2

Straw dry weight was not significantly different between different treatments at $p \leq 0.05$ (Figure 3.5).

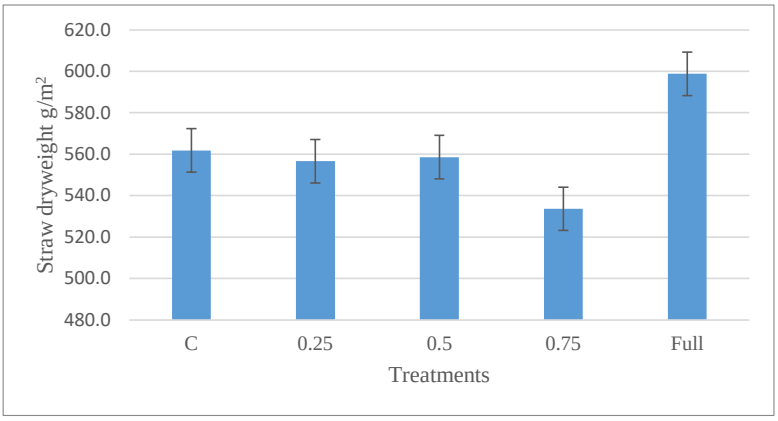

Figure 3.5: Effect of PGR on straw dry weight
Bars on columns represent standard error of differences of means

3.4.2.4 Harvest Index (HI)

No significant differences were seen between different treatments in HI at $p \leq 0.05$. (Figure 3.6).

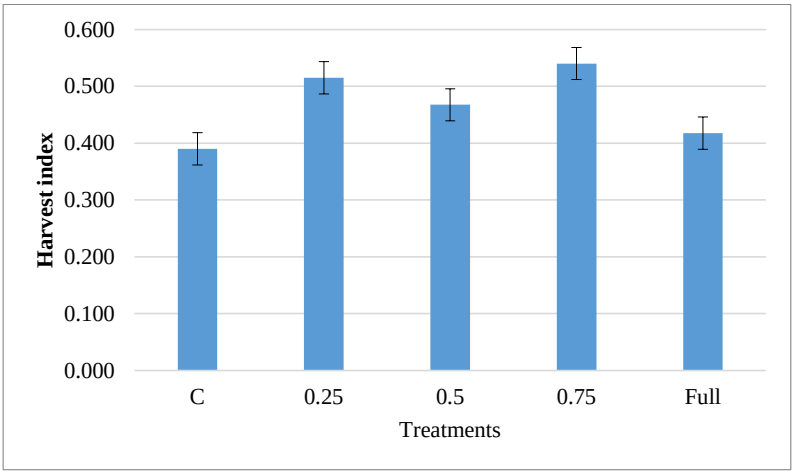

Figure 3.6: Effect of PGR on Harvest index
Bars on columns represent standard error of differences of means

3.4.2.5 Ears per square meter (EPSM)

No significant differences were observed between different treatments in EPSM (Figure 3.7).

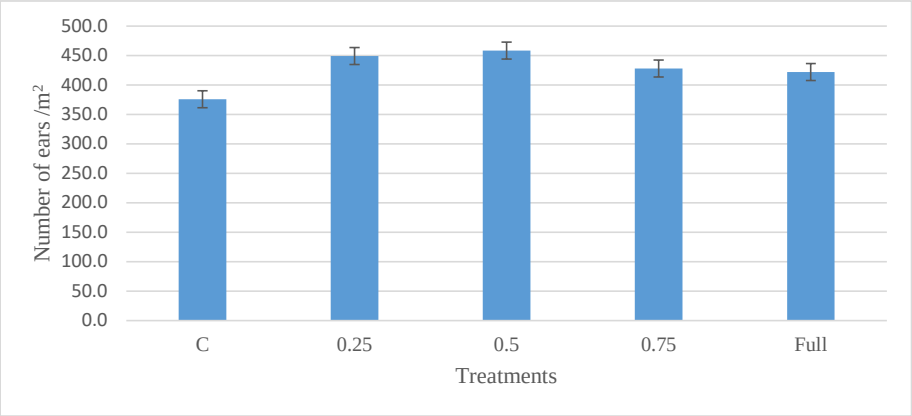

Figure 3.7: Effect of PGR on Number of ears per square meter (EPSM)
Bars on columns represent standard error of differences of means

3.4.2.6 Thousand grain weight (TGW) (g)

TGW was not significantly different at $p \leq 0.05$. (Figure 3.8).

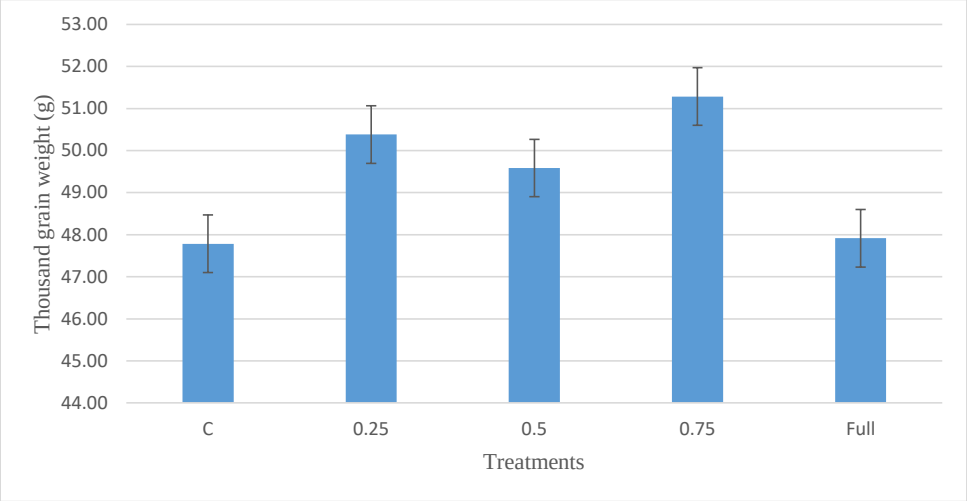

Figure 3.8: Effect of PGR on thousand grain weight
Bars on columns represent standard error of differences of means

3.4.2.7 Lodging (%)

No significant differences were observed between different treatments in visually estimated lodging percentage (Table 3.9)

Table 3.9: Lodging percentage

Treatment	Mean lodging (%)
C	14.4
0.25	21.3
0.50	8.5
0.75	15
Full	10

3.4.2.8 Internode length

No significant differences were shown between different treatments at $p \leq 0.05$. (Figure 3.9).

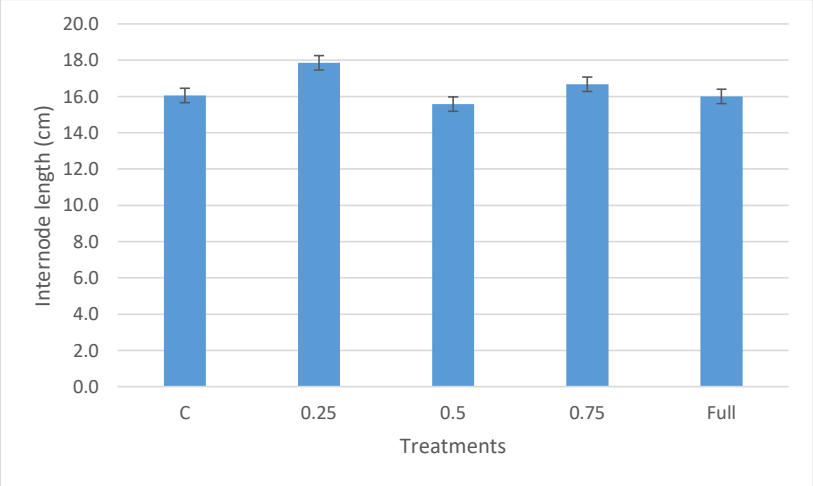

Figure 3.9: Effect of PGR on internode length
Bars on columns represent standard error of differences of means

3.4.2.9 Regression

A significant positive relationship $p \leq 0.01$ was observed between numbers of grain per ear vs. thousand grain weight as can be seen in (Figure 3.10). The same relationship was also observed between numbers of grain per ear and yield (Figure 3.11)

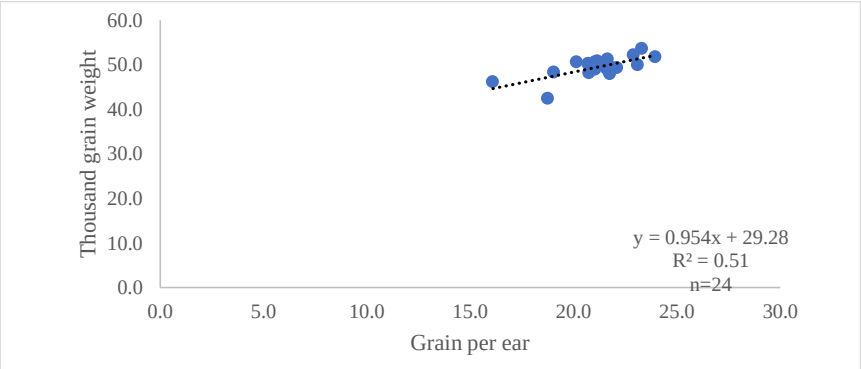

Figure 3.10: Scatterplot of number of grains per ear vs. thousand grain weight.

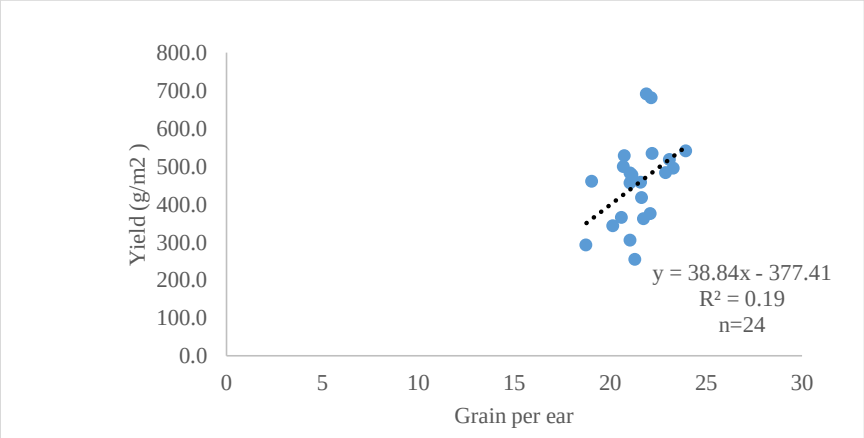

Figure 3.11: Scatterplot of number of grains per ear vs. yield.

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3.5 Discussion

3.5.1 Stem shortening trial (2015)

3.5.1.1 Effect of PGR on stem length (StL)

Deiniol and Sanette stem length was reduced as a positive effect of PGR application. The stem length for Deiniol decreased from 118.2 cm to 75.5 cm and from 80.2 cm to 68.5 cm for Sanette a 36% and 15% decrease for both varieties respectively. The effect was sgnificant as compared to non-treated plots, these findings are in agreement with (Rajala and Peltonen-Sainio, 2002) who reported that the early PGR application on barley at early growth stages of ZGS13-14 significantly reduced the stem length at $p < 0.001$ by7.59 %, 8.22 % and 6.96 % for CCC, Ethephon and trinexapac-ethyl after 14 days of PGR application respectively.

Of 53 barley varieties treated with chlormequat PGR, 77.0 % of the varieties exhibited different degrees of stem length decrease 9.4 % of the varieties were significantly reduced, 24.5 % of the total number were reduced by 13.2 % of their original stem length and 22.6 % grew with longer stems (Clark and Fedak, 1977). The PGR Modus applied at ZGS 31-32 and the PGR Cerone applied at ZGS 39-43 reduced the stem length from 66.0 cm to 58.0 cm for barley spring variety Henni and from 74.1 to 63.5 for the spring barley variety luoke (Supronienė et al., 2006).

The application of trinexapac-ethyl mixed with ammonium sulphate and citric acid on spring barley. reduces the spray liquid pH without lowering its effectiveness. Th e highest stem length reduction was obtained with trinexapac ethyl full dose application and its reduced dose mixed with citric acid or ammonium sulphate. The reduction was from 5.6 to 16.5% with differences between years (Miziniak et al.,2016).

Generally speaking StL reduction by shortening internode and StL depends upon PGR type used (Rajala and Peltonen-Sainio, 2001; Rajala and Peltonen-Sainio, 2002), crop growth stage at the time of PGR application (Ramburan and Greenfield, 2007) and genotype (Tripathi *et al.*, 2003).

The PGR application was more efficient on Deiniol StL reduction than Sanette. That could be related to the fact that Sanette is a short stem selected variety whereas Deiniol was not selected on a short stem basis and it had not passed through rigorus selection criterion for StL. Reduction in Deiniol StL is a significant finding. It enhances lodging resistance and meets UK farmer's preferences of shorter plants with more capabilities of holding heavier ears and facilitated combine harvesting.

3.5.1.2 Effect of PGR on lodging

Our results of early PGR application confirms its potential in controlling lodging. Furthermore, outcome in this part of this trial would suggest that lodging resistance may not be contributed by by StL alone. Lodging is a multi-factor-controlled trait influenced by many factors such as stem thickness (Zuber et al., 1999), ear weight (Tripathi *et al.*, 2003) and, seeding rate (Berry *et al*., 2000) and it is not just influenced by a single factor.

Lodging adversely affects cereal crops growth during different growth stages and results in lower yield and diminished grain quality (White, 1991). It has been reported that PGR application during early plant growth stages ZGS (31-32) strengthen plant stem and root structure (Sanvicente et al., 1999;Rajala and Peltonen-Sainio, 2002).

PGR application contributes efficiently to lodging control alongside with genotype, appropriate sowing date and improved crop husbandry (Berry et al., 2004). Barley crop yield and yield components responses to PGR application are not consistent as their effect in stem shortening (Ramburan and Greenfield, 2007). Lodging can-reduce crop productivity by up to 80 %, and numerous aspects could be experienced such as diminished crop quality, higher drying costs, slow and complicated harvesting (Berry et al., 2004).

It was found that PGRs can not eradicate lodging entirely in highly susceptible crops. The stem cell wall components and structure play a crucial role in cereal crops breeding for lodging resistance (Wang et al., 2006). Lodging severity differs from season to another, and it is a factor

of rain, wind speed, and the soil type. Crop plants responses to PGRs and plant hormonal-based signals are rich research areas and future targets for plant breeders (Wilkinson et al., 2012).

3.5.1.3 Effect of PGR on grain yield (GY)

At early PGR application Deiniol GY was not significantly different between treated and non treated crop. However, Sanette yield increaed significantly between treated and untreated crop. That is in agreement with (Simmons et al., 1988) who found that Ethephon PGR application oftenly significantly increases GY by 13 % or decreases GY by 9 % depending on prevaling weather conditions and varieties grown. They also found that when lodging does not occur Ethephon application reduces yields.

3.5.1.4 Effect of PGR on harvest index (HI)

Higher HI is one of the primary factors enhancing genotype yield improvements in barley, wheat, and oats. HI reflects high yielding when different varieties are compared (Ramburan and Greenfield, 2007). The most considerable input to promising yield potential of cereal crops came from HI increase. There was a quantitative increase in HI through the introduction of dwarf genes into the new cultivars since the green revolution (Cassman, 1999).

Sanette GY was significantly increased as an effect of PGR application that is related to the fact sanette is a semi-dwarf barley variety, and its absorbed nutrients were translocated to the grains. However, HI decreased significantly between treated and untreated plots. That decrease could be attributed to the fact that early PGR application at stage 32 was not efficient to trigger responses on the reproductive crop stage and yield incremnet.

3.5.1.5 Effect of PGR on ears per square meter (EPSM)

Early PGR application did not trigger the initiation of new ears in our early PGR application trial. That was in agreement with (Ma and Smith, 1991) who found that early PGR application at ZGS 30 does not increase the EPSM. While (Rajala and Peltonen-Sainio, 2001)

found that PGRs enhanced EPSM production in a rate that does not compensate the stem length reduction attained. However, we observed significant differences between hulled and naked barleys.

3.5.2 Late PGR application time trial

3.5.2.1 Effect on stem length

PGRs are efficient in reducing StL of Deiniol naked barley as was found in our previous trial section 3.5.1.2. However, these effects depend on the PGR type used (Rajala, 2004), time of application (Caldwell et al.,1988). In this experiment, no effect for late Moddus PGR application on stem shortening was seen on Deiniol. That is in agreement with the findings of (Caldwell et al., 1988) in that late PGR application at the (ZGS 37) has no effect on stem shortening and uneven crop maturity which was also in agreement with (Lauer, 1991).

3.5.2.2 Effect on grain yield (GY)

At late PGR application no, significant differences were observed between different treatments in GY. That was in agreement with (Lauer,1991) who found no significant differences were observed in GY at ZGS 39. However, Caldwell et al.,1988 found that Ethephon application without mixing with any other PGR at ZGS 37 resulted in significant GY loss. chlormequat chloride (CCC) or mepiquat ctrloride (DPC) counteracted against the detrimental ethephon effects on the crop.

3.5.2.3 Effect of PGR on ears per square meter (EPSM)

No significant differences where observed for EPSM between control and other treatments at late PGR application. That is in disagreement with the findings of (Lauer, 1991; Foster et al.,1991) who found that late PGR application increases EPSM by increasing the total number of green tillers which are not preferred by farmers. Furthermore, he found that green

tillers number was 8% of the total spikes, but the weight of the grains was lighter than early emerging grains and of lower quality.

3.5.2.4 Effect of PGR on straw dry weight

Straw dry weight was not affected by late Moddus PGR application in this is in agreement with (Jordan and Stinchombe, 1986).

3.5.2.5 Effect of PGR on internode length

The Significant differences observed between control and (0.25, 0.5 and full treatment) in internode length is the main effect of the application of Moddus PGR at a late growth stage on Deiniol in this trial. In our trial, we found that late PGR application had significantly increased the total stem length despite that we did not distinguish between early emerging internodes and late emerging internodes in our study.

3.5.2.7 Effect of PGR on grains per ear (GPE)

A significant positive relationship between grains per ear and thousand grain weight, and yield. The inconsistency of results in the effects of late PGR application in this experiment could be attributed to inappropriate dry weather for several days followed by wet weather conditions prevailing at and after the application growth stage. Therefore, farmers should take all necessary steps to ensure PGR application at optimum weather conditions and crop growth stage preferably using tractor mounted spraying machines to ensure an even spray and distribution of the PGR across the growing area.

3.5.2.8 Early PGR vs. late PGR application

Without a direct comparison between early and late application of PGR in the same trial, no firm conclusions can be drawn of the effect of a late application.

Chapter 4

Screening naked barley breeding lines for field performance

4.1 Introduction

Amezrou et al. (2017) stated that plant breeders are concerned with utilizing diversified genotypes in hybridization to develop beneficial traits. They added that molecular and morphological diversity promotes the success of selective breeding. Substantial data have been produced from genetic diversity screening in naked and hulled barley over the last decade (Muñoz-Amatriaín et al., 2014).

The employment of elite US and UK breeding germplasm has allowed the discovery of beneficial agronomical and/or kernel qualitative traits (Wang et al., 2012). Wang et al. (2012) added that a large number of quantitative traits (QTLs) have been detected in the last two decades, but few of them have been used in breeding programmes. They attribute this to (1) the QTL allele of interest being already fixed in the breeding programme, (2) the linkage drag which is related to the transfer of undesirable traits alongside the gene of interest, (3) the possibility of phenotypical trait scoring. Landraces and wild populations play an important role in donating genes for target traits. Molecular markers can help identify beneficial traits (Zhu et al., 2008).

4.1.1 Traits selection

Selection for short plant stems plays a crucial role in enhancing productivity. when utilising dwarfing or semi-dwarfing alleles in breeding programmes, it is important that shortened stem length doesn't simultaneously result in reduced yield (Wang et al., 2014). Wang et al. (2014) also stated that numerous genes influence yield indices such as ears per square meter (EPSM), grains per ear (GPE), and thousand grain weight (TGW), Breeding plants for shorter stems can improve yield by controlling lodging and increasing the harvest index (Bezant et al., 1996). The main objectives of plant breeders are to develop new cultivars with higher grain yield (GY) and higher adaptability to a wide range of soils and climates.

4.1.2 **Food barley selection**

In the health food sector, numerous nutritional aspects are prioritized by food processors and targeted by plant breeders including β-glucan content, grain hardness, solvent retention capacity and nakedness. Soluble β-glucan quantity in barley is higher in dry, growing conditions. Tiwari and Cummins (2008) found that genotype is more important in determining β-glucan content than agronomical factors. Chutimanitsakun et al. (2013) reported that all modern US barley cultivars with high β-glucan content are also have a high waxy starch content. Kam et al. (2016) argue that there is potential to develop crops with the capacity to treat chronic diseases such as diabetes. They added that more research is required into the production process of crops such as millet and their potential role in the human diet. They argue that more research exploring the health benefits of such crops is required.

4.1.3 **Registered food naked barley lines**

In Turkey, the cultivar Ozen was the first registered naked barley cultivar in the country. It is derived from a cross between ICB-101086 x Tokak 157/37. Ozen was produced using a bulk selection modification method. The selection was based on grain yield, grain quality and resistance to diseases. Ozen was produced to be cultivated under irrigation. The cultivar produced a grain yield of 3.784 kg/ha, a thousand grain weight of 32.5 g and a stem length of 83.9 cm (Ergun et al., 2017). In Latvia, Bleidere et al. (2013) found that the grain yield of registered spring naked barley cultivar Kornelija increased significantly (from 2.73 to 3.50 t/ha) when nitrogen application was increased from 80 to 120 kg/ha. Kornelija produced 53.5 and 56.3 g /kg of β-glucan during the 2011 and 2012 spring seasons, respectively. Two of the most recently developed naked barley cultivars are BARLEYMax, developed in Australia, and Transit, developed in the USA (Steele et al., 2013).

4.1.4 Barley breeding programmes in the UK

About £40 is the added value produced from £1 invested in plant breeding in the UK (Webb, 2010). Successful barley breeding programmes have increased yields by 1% per year in the UK. Conventional line selection is more resource efficient than marker-assisted selection. However, marker-assisted selection can be used for the selection of main–gene targets. UK barley breeding programmes compete with similar programmes in 20 European countries. 35-40 winter barley lines have been entered into the national recommended trial list since 1993. The hulled spring barley cultivar Optic has been recommended for more than ten years as a spring barley line (Rae et al., 2007).

Naked barley has received growing interest in recent years due to its bioactive constituents,- especially β-glucan-(Chutimanitsakun et al., 2013). Naked food barley can be sold for premium prices (Dickin et al., 2011). Almost all commercial covered UK barley have been bred for low (<3%) β-glucan content, and is used as animal feed. Low naked barley yields can be attributed to poor adaptation to UK growing conditions and the absence of breeding programmes (Steele et al., 2013). Keerio et al. (2011) reported that coleoptile length is significantly correlated with field establishment in naked barley varieties. The development of naked barley varieties highly adapted to UK conditions is a priority.

4.1.5 Bangor University naked barley breeding programme

In recent years Bangor University has undertaken a naked barley improvement programme (Steele et al., 2013). A large number of genotypes from around the world were collected and assessed (Dickin et al., 2011). At the beginning of the programme it was found that exotic naked barleys were not performing well under UK conditions. Crosses between naked x UK hulled barley were carried out. The lines were examined to explore their genetics. Well

adapted naked barley lines with ≥65% genetic similarity to UK hulled barley, sturdy straw naked barleys and highly disease resistant lines were selected (Steele et al., 2013).

The Bangor University programme Dickin et al. (2011) reported that β-glucan concentration was lower during the rainy summer of 2007 compared with the dry summer seasons of 2006 or 2009. However, β-glucan concentration was not found to be affected by higher yields. They also found that some naked Himalayan x UK crosses and their progenies had outstanding crop establishment in the field. A considerable range of β-glucan contents (3.0 - 7.0 g/100g; dry weight basis) was found between different locations was found in these segregating lines. In the same programme, 44 naked barley varieties and four hulled UK varieties were grown in Henfaes Research station in 2008 and 2009. B-glucan content and growing conditions were investigated. Poorly-performing lines were taken out of the programme. The Deiniol naked line described in section 1.1.8.1.3 was developed as one of the outcomes of this research.

B-glucan QTLs were found on chromosomes 1H, 2H, 5H and 7H. The cellulose synthase-like *CslF* enzyme mediates β-glucan synthesis (Burton et al., 2011). Amylose and amylopectin are two constituents of starch. Barley can be categorized according to amylose content; normal amylose (25-27% amylose), waxy type (below 5% amylose) and high amylose (>35% amylose).

The UK hulled barley varieties used in this research were selected to be crossed with Deiniol naked barley for the following reasons: (1) Propino is a high yield malt barley. It is a cross between Quench x NFC Tipple. It has an excellent foliar resistance to fungal diseases, good treated and untreated yields, medium stem length and suitable straw strength. It is expected to be in high demand for many years. (2) Westminster is a high yield feed spring barley variety with a tall, strong straw, and excellent disease resistant and seed quality. Westminster is highly resistant to mildew and Rhynchosporium. (3) Optic accounts for 50-60 per cent of the barley market. It was developed during the 1990s. Optic is characterized by stronger short stems,

resistance to diseases and a late maturing crop compared to other barley varieties. It is known as the master of malt. (4) Cocktail a high yield malting variety with very good disease resistance.

4.2 Objectives

This research aims to

1- Assess different naked barley lines for short plant stem length, and higher beta-glucan, amylose, and amylopectin content.
2- Identify pre-breeding priorities, agronomic requirements and investigate the field performance of these lines as compared to Deiniol as a control.
3- Diversify the UK gene pool, identify germplasm suitability for future development and suggest a potential alternative to Deiniol.
4- Suggest optimum agronomic practices for the UK production of naked barley.
5- Provide an understanding of G x E interaction for naked barley bioactive components.
6- Explore the suitability of naked barley lines for different end users and food producers.

4.3 Materials and methods

4.3.1 Plant material

Naked barley lines selected from Skardu (Pakistan) x Static cross population, namely line 15, line 20 and Deiniol, were crossed with hulled UK barley lines (Propino, Westminster, Cocktail, and Optic). Selected naked barley F_4- F_6 generation seeds from the glasshouse crosses were sown in the field in 2013.The plots were coded as MA01-MA20 lines as shown in Table 4.1. The F_6 seeds were selected for their adaptation to UK conditions based on their nakedness, straw strength and disease tolerance. The plants were hand harvested. The selected lines from the field trial were planted in the glasshouse on the 19th of September 2013 in black plastic pots, in a completely randomized design (CRD) with three replicates for each line.

Table 4.1: Naked line x hulled barley and their corresponding ID

No	Cross	Code	No of entries
1	Deiniol x Propino (F3 Bulk)	DE X PRO	1
2	Line 20 x Westminster	MA01	5
3	Line 20 x Westminster	MA03	
4	Line 20 x Westminster	MA04	
5	Line 20 x Westminster	MA05	
6	Line 20 x Westminster	MA06	
7	Line 15 x Westminster	MA07	5
8	Line 15 x Westminster	MA08	
9	Line 15 x Westminster	MA09	
10	Line 15 x Westminster	MA10	
11	Line 15 x Westminster	MA11	
12	Line 15 x Cocktail	MA12 rep1	9
13	Line 15 x Cocktail	MA12 rep2	
14	Line 15 x Cocktail	MA13	
15	Line 15 x Cocktail	MA14	
16	Line 15 x Cocktail	MA15 rep1	
17	Line 15 x Cocktail	MA15 rep2	
18	Line 15 x Cocktail	MA16	
19	Line 15 x Cocktail	MA16 rep2	
20	Line 15 x Cocktail	MA17	
21	Line 15 x Optic	MA18	2
22	Line 15 x Optic	MA19	
23	Line 20 x Westminster	MA20	3
24	Line 20 x Westminster	MA20 rep1	
25	Line 20 x Westminster	MA20 rep2	
26	Deiniol	Deiniol	1
		Total	26

4.3.2 Experimental design

The survived previously head rows field grown plants in 2013 were selected based on their resistance to powdery mildew and grain yield. The crop was grown in the field on the 25th of March 2015 in a non-replicated trial due to a shortage of seeds. The crop was harvested on the 2nd of August 2015. 26 plots containing the progenies of the crosses described in Table 4.1 were grown with the aim of screening for higher β-glucan content, shorter stems and amylose content

in spring season of 2015. In the 2017 spring season, field performance, components, β-glucan and amylose content were further investigated in a replicated trial.

4.3.3 Crop management

The plot size, seeding rate, and fertilizers applied are described in Table 2.1 and Table 2.2 above.

4.3.4 Grains for data collection and chemical analysis

At harvest the stem length for 5 plants in different positions in each plot were measured. All spikes from different plots were hand harvested, grains were threshed and grain subsamples were taken from all the plots. The subsamples were oven dried at 80℃ for 48 hours. Grain weight for each plot were recorded. Later, grains were prepared for β-glucan and amylose analysis as described in sections 2.1.9 and 2.1.10 above.

4.3.5 Grains for re-planting in 2017

The remaining spikes were air dried at 40℃ for 48 hours in order to keep the seed alive and viable for growing during a later season.

4.4 Results

4.4.1 Lodging (%)

Based on visual assessment of different plots, there was no evidence of lodging.

4.4.2 Disease incidence

No severe disease was observed in any of the plots except for three loose smut-infected plants in MA6 (one of the line 20 x Westminster progenies). The infected plants were removed by hand.

4.4.3 β-glucan content

The results of the β-glucan content analysis are shown in Figure 4.1.

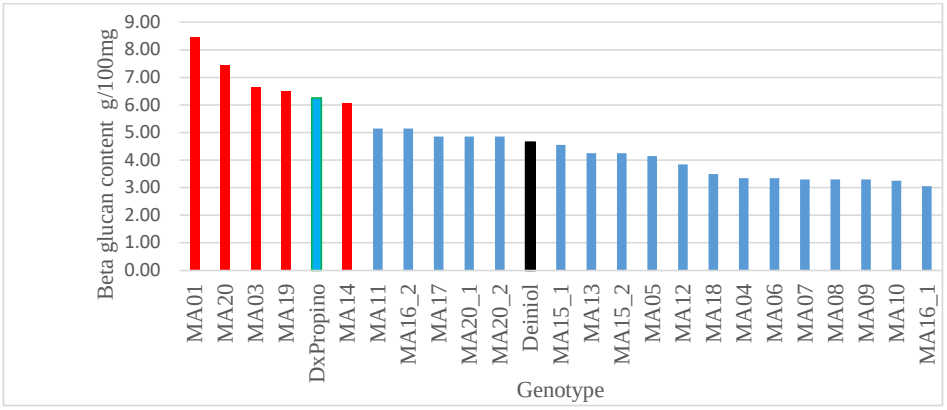


Figure 4.1: β-glucan content of naked barley lines, ranked by mean.

MA01 had the highest β-glucan content followed by MA20, Deiniol x Propino (bulk), MA03, MA19, and MA14.

4.4.4 Stem length (StL)

Figure 4.2 shows the stem length of different naked barley lines under investigation. Deiniol had the highest stem length, while MA18 measured the shortest. The Deiniol x Propino cross was not selected despite its high β-glucan due to its long stem. MA18 was not selected despite it having the shortest stem, due to its low β-glucan content.

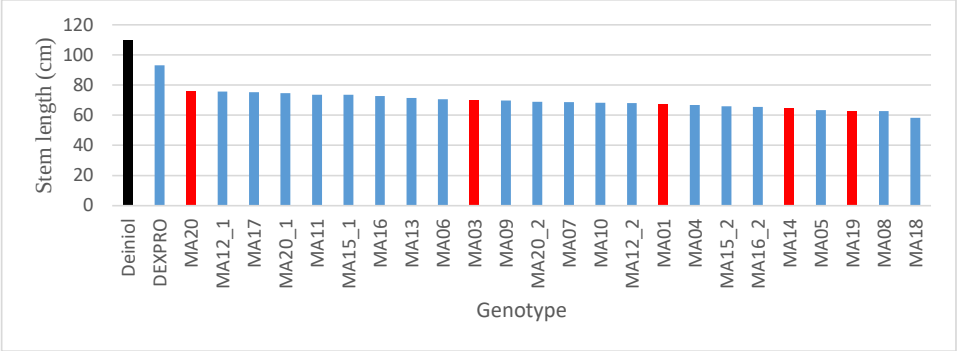
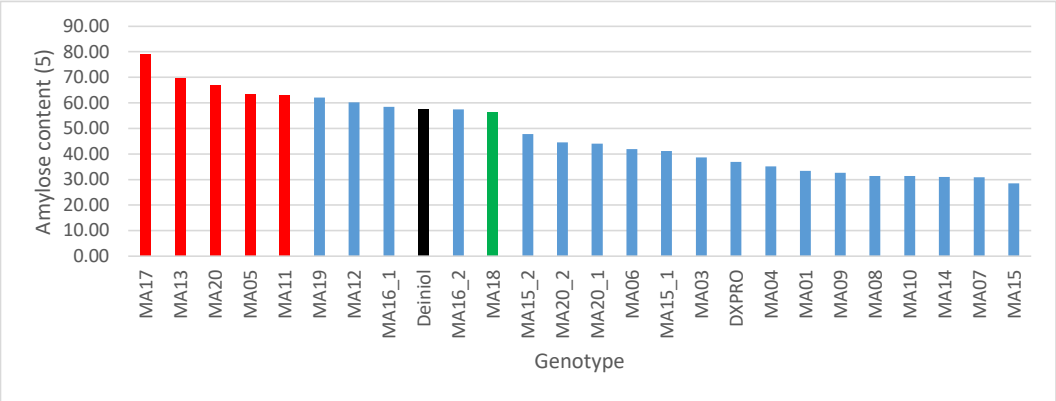


Figure 4.2: Stem length for different naked lines

4.4.5 Amylose content (%)

Figure 4.3. shows the amylose content (%) of the different crosses under investigation. MA17 had the highest amylose content while MA15 had the lowest amylose content



1.27 Figure 4.3: Amylose content of different naked barley lines

4.4.6 Amylopectin content

The amylopectin content is the opposite of the amylose results graph.

4.5 Selection of lines for further testing in the 2017 field performance trial

The five highest ranking β-glucan lines with the shortest stems (Table 4.2) were selected based on this study and their field performance assessed in spring 2017.

The selected lines were chosen on the following basis:

1) The highest-ranking β-glucan content naked barley lines MA01, MA20, MA03, MA19, and MA14. 2) The shortest stems were selected to be evaluated under field condition during the spring season of 2017.

4.6 2017 Field trial - evaluation of selected crosses under field conditions

4.6.1 Materials and methods

The five selected naked barley lines (Table 4.2) screened in 2015 were used in this experiment. A seed rate of 280 seeds/m^2 was used. A randomized complete block design (RCBD) with three replications alongside Deiniol as a control was used. Fertilizers were applied as described in Table 2.2 above. The plots were sown on the 21st of April 2017 and harvested on 29th of August 2018 using the same method described in section 2.2.

Table 4.2: Selected lines with highest β-glucan content and shortest stems

Code	Cross
MA 1	(Line 20 x Westminster)
MA 3	(Line 20 x Westminster)
MA 14	(Line 15 x Cocktail)
MA 19	(Line 15 x Optic)
MA 20	(Line 20 x Westminster)

4.6.2 Management practices

Management practices and parameters measured are described in section 2.2 above.

4.6.3 Statistical analysis

Analysis of variance (ANOVA) for a randomized complete block (RCBD) design was carried out using SPSS 24 software. Regression analysis was carried out using MS Excel.

4.6.4 Results

Please refer to appendix 1.2 for tests of normality, frequencies and ANOVA table.

4.6.4.1 Stem length

Analysis of variance revealed significant differences in stem length between different lines at ≤ 0.05. Significant differences were observed between Deiniol and MA1, MA3, MA14, MA19 and MA20 at (p=0.01, p=0.007, p=0.02, p=000 and p=0.003, respectively). Deiniol had the longest stem while MA 19 had the shortest. A significant difference was observed between MA14 and MA 1, and MA14 and MA 19 at p=0.02 and p=00,3 respectively.

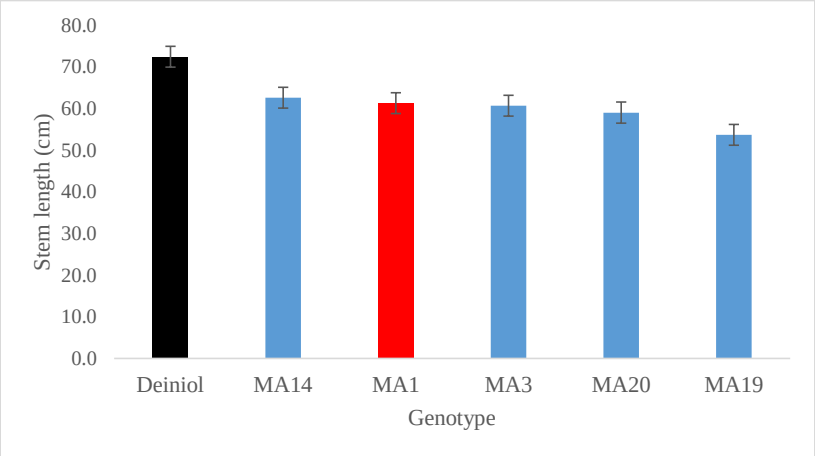


Figure 4.4: Stem length for different naked barley lines
Error bars on represent the standard error of the mean

4.6.4.2 Grain yield (GY)

A significant difference was observed in GY (g/m^2) between MA 1 and MA 19 at p=0.035. Significant differences were observed between MA19 and MA20 (p=0.004), and between MA19 and MA3 (p=0.03). MA 20 had the highest GY, while MA 19 had the lowest (Figure 4.5).

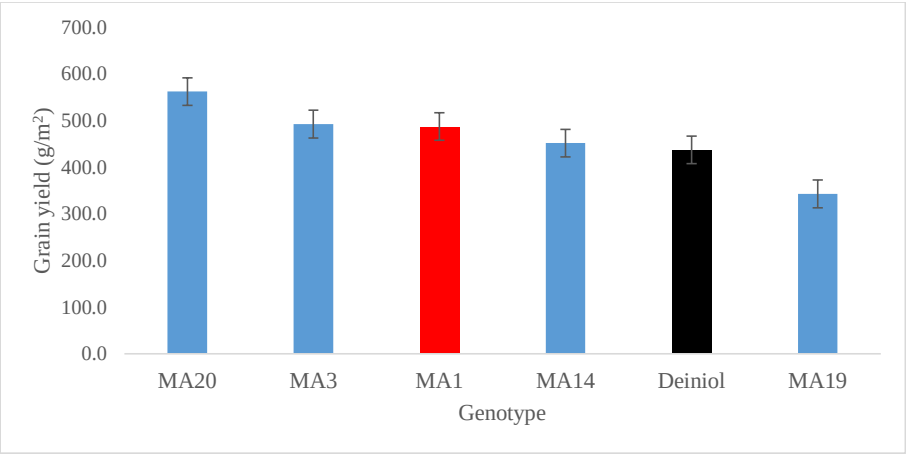


Figure 4.5: Grain yield for different naked barley lines
Error bars on represent the standard error of the mean

4.6.4.3 B-glucan content (g/100 g; dry weight basis)

The following significant differences in β-glucan content were observed between lines: between Deiniol and all other lines at $p \leq 0.001$; between MA1 and MA14, MA1 and MA20 ($p < 0.001$); between MA3 and MA14, MA3 and MA20 ($p < 0.001$).; between MA14 and MA 19 at p= 0.01 and MA14 and MA20 at ($p < 0.001$). Deiniol had the highest β-glucan content while MA14 had the lowest.

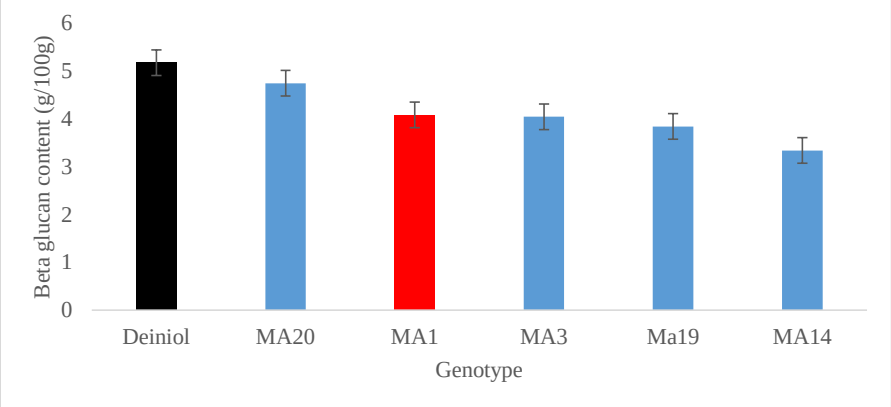


Figure 4.6: B-glucan content of naked barley lines
Error bars on represent the standard error of the mean

4.6.4.4 Amylose content (%)

The following significant differences in amylose content were observed between lines: between Deiniol and MA20, and Deiniol and MA19 at p= 0.035 and p=0.01, respectively; between MA1 and MA19, and MA1 and MA20 at p=0.014 and p=0.048, respectively. Deiniol had the highest amylose content, while MA19 had the lowest amylose content.

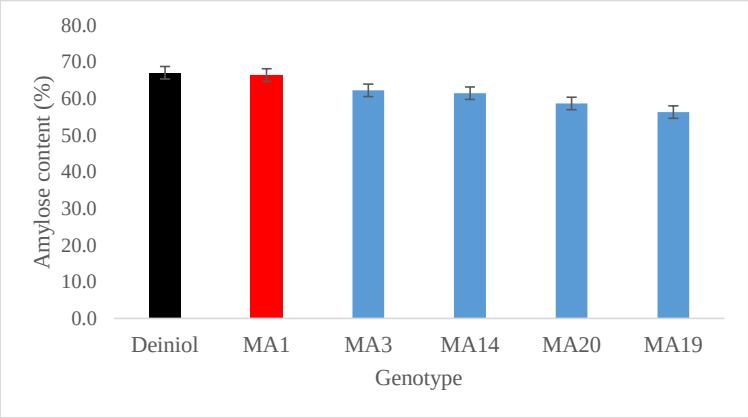


Figure 4.7: Amylose content for different naked barley lines
Error bars on represent the standard error of the mean

4.6.4.5 Amylopectin content (%)

The lines in order of decreasing amylopectin content are simply the opposite of the amylose results graph.

4.6.4.6 Harvest index (HI)

Deiniol had the highest HI, MA 14 and MA19 had the lowest HI. No significant differences were observed between different lines (Figure 4.8).

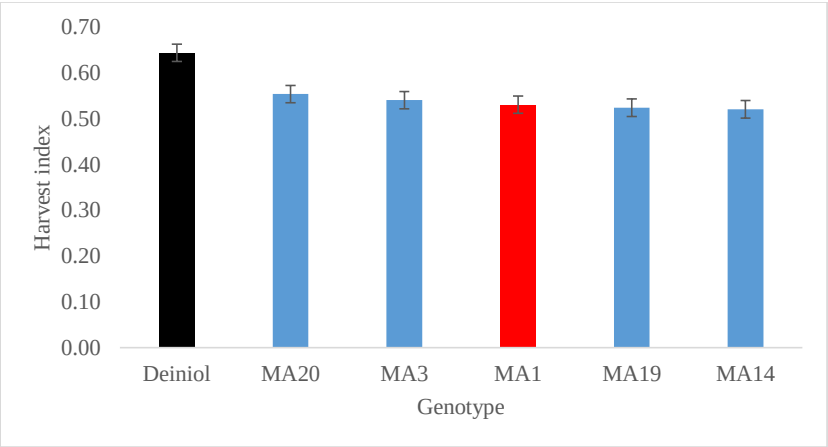


Figure 4.8: Harvest index for different naked barley lines
Error bars on represent the standard error of the mean

4.6.4.7 Ears per square meter (EPSM)

No significant differences were observed between Deiniol and other lines in terms of ears per square meter (EPSM) (Figure 4.9). MA20 had the highest number of EPSM while MA14 had the lowest.

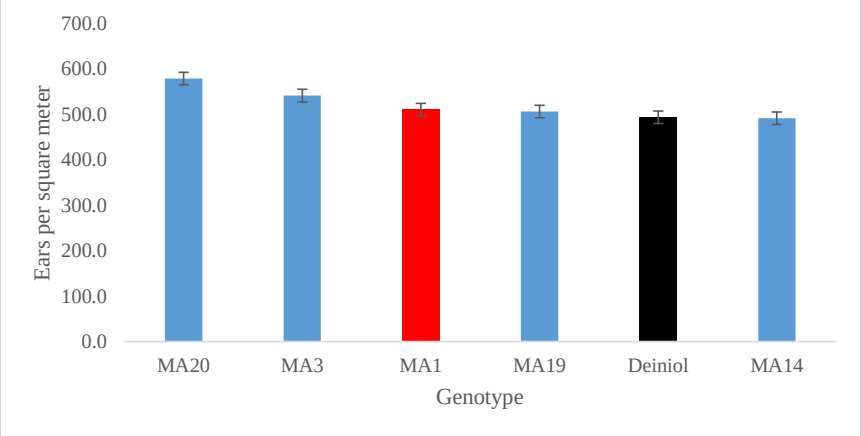


Figure 4.9: Number of ears per square meter for different naked barley lines
Error bars on represent the standard error of the mean

4.6.4.8 Thousand grain weight (TGW)

The following significant differences in TGW were observed between lines; between Deiniol and MA19 at p=0.01; between MA1 and MA19 at p=0.004; between MA20 and MA19 at p=0.037. MA1 had the highest TGW while MA19 had the lowest (Figure 4.10).

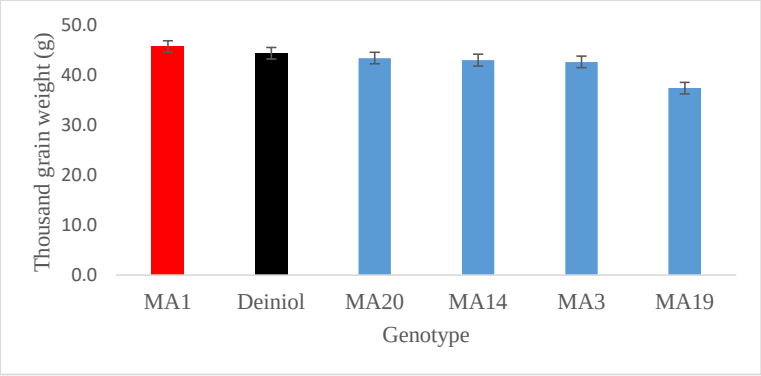


Figure 4.10: Thousand grain weight for different naked barley lines
Error bars on represent the standard error of the mean

4.6.4.9 Straw dry weight (g/m^2)

Significant differences in straw dry weight were observed between Deiniol and MA20, MA1, MA3, and MA14 at p=0.006, p=0.015, p= 0002 and p=0.03, respectively. No significant difference was observed between Deiniol and MA19. Naked barley line MA20 had the highest straw dry weight while Deiniol had the lowest (Figure 4.11).

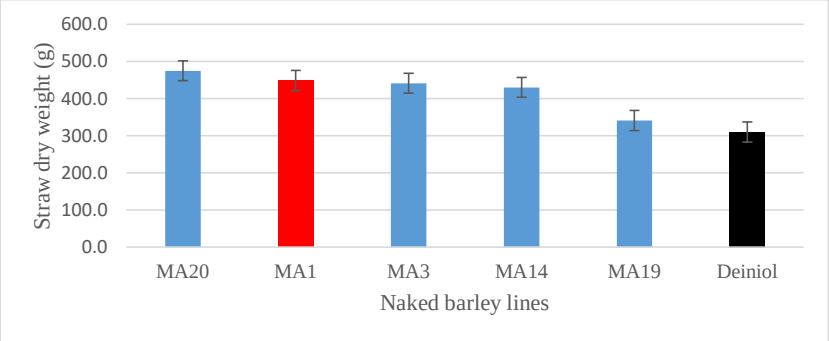


Figure 4.11: Straw dry weight for different naked barley lines
Error bars on represent the standard error of the mean

4.6.4.10 Chaff weight (g/m^2)

Significant differences in chaff weight were observed between Deiniol and MA14 and MA1 at p=0.035 and p=0.028, respectively. No significant differences were observed between any other lines. MA14 had the highest chaff weight while Deiniol had the lowest (Figure 4.12).

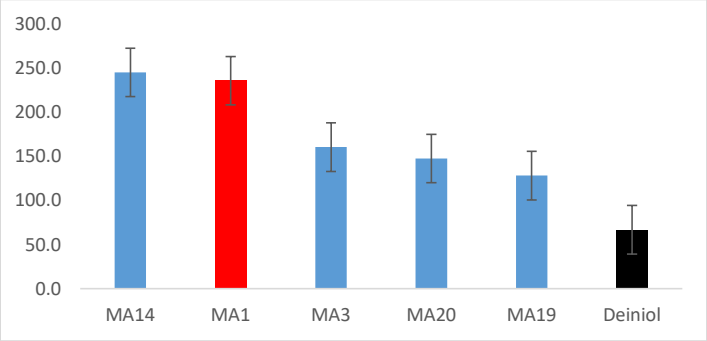


Figure 4.12: Chaff weight for different naked barley lines
Error bars on represent the standard error of the mean

4.6.4.11 Grain per ear (GPE)

A Significant difference in GPE was observed between MA20 and MA19 at p=0.037. No significant differences were seen between other naked barley lines in this experiment. MA20 had the highest GPE while MA19 the lowest (Figure 4.13).

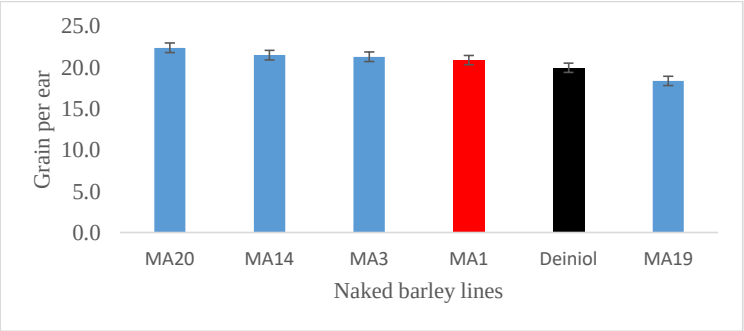


Figure 4.13: Number of grains per ear for different naked barley lines
Error bars on represent the standard error of the mean (n=?)

4.6.4.12 Regression

Significant positive correlations were observed between the number of ears/m^2 and ears dry weight ($p \leq 0.001$, n=18, r2 = 0.57; Figure 4.14A), ears dry weight and grain yield ($p \leq 0.001$) and ears dry weight and GPE ($p \leq 0.05$)

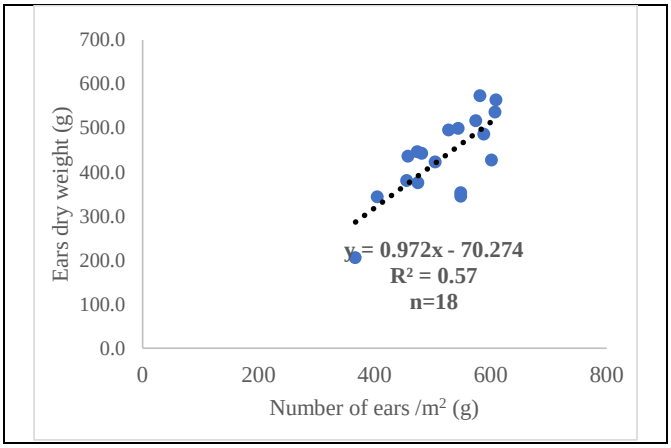


Figure 4.14: Scatter plot showing relationship between number of ears/$m^{2\ and}$ ears dry weight.

4.7 Discussion

4.7.1 2015 naked lines screening.

4.7.1.1 Lodging

As described earlier, lodging is a multifactorial trait. No lodging was observed in the present study., This can be attributed to the favourable weather conditions which prevailed during the growing season.

4.7.1.2 Disease resistance

Only three diseased plants were found (in the MA6 plot). These were infected by loose smut and once identified were removed by hand.

4.7.1.3 Stem length

In 2015 screening trial the selected naked barley lines were found to be comparable to the UK hulled barley in terms of stem length. Deiniol recorded the longest stem length and Deiniol x Propino (bulk) had the second longest stem. The long stem in Deiniol is thought to be inherited from its Pakistani Skardu ancestors, as shown in Figure 4.4. The remaining naked lines inherited shorter stems from their parents as hulled UK barleys were developed through barley breeding programmes in the country.

4.7.1.4 β-glucan, amylose, and amylopectin

Breeding for stable β-glucan content across different growing seasons may not be achievable (Steele et al., 2013). In the present study, naked barley β-glucan content ranged from 3.0? to 8.5 g/100 g; dry weight basis. This finding is in agreement with previous Bangor University naked barley research (Dickin et al., 2011; Henry, 1987). Islamovic et al. (2012) found that QTLs in the chromosomes 3H, 4H, 5H, 6H, and 7H control high β-glucan content in field grown naked barley. Nevertheless, environmental factors also have an effect on final grain composition (Baik and Ullrich, 2008). Weather conditions during early growth stages until

flowering, which is the most crucial period for soil nutrient uptake, is also important in determining β-glucan content (Leistrumaite, and Paplauskiene, 2005; Bleidere et al., 2013).

Based on the end use food application intended, the highest ranking β-glucan naked genotypes and short stems were selected (Figure 4.1 and Figure 4.2, respectively). MA20 had the second highest β-glucan content after Deiniol. Significant differences in β-glucan content were observed between Deiniol and MA20, and other naked barley lines. This qualifies MA20 to be a potential alternative to Deiniol, especially given its other desirable traits (shown in Table 5.3). Regression analysis showed no significant relationships either between β-glucan and amylose content or between β-glucan and amylopectin content. This is not in agreement with the results reported by Shu and Rasmussen (2014).

4.8 2017 naked barley lines field assessment

4.8.1 Stem length

Of the naked barley lines chosen for the 2017 field trial (Table 4.2), Deiniol, as expected based on the 2015 screening, had the longest stem, with significant differences found between Deiniol and all the other naked barley lines in this respect. MA20 had a significantly shorter stem than Deiniol. Shorter stemmed plants are much desirable in modern agriculture as they facilitate mechanical harvesting using combine harvesters.

4.8.2 Grain yield (GY)

Barabaschi et al. (2012) found there was significant potential to increase naked barley GY up to levels observed for covered barley. Ears per square meter (EPSM) and grain per ear (GPE) are the most important determinants of GY (Bingham et al., 2007)**.** The size of the grain is influenced by prevailing conditions pre- and post-anthesis (Calderini et al., 2001). Thomason et al. (2009) reported that naked barley GY was 3725 kg/ha with a seeding rate was 371 seeds/m^2 compared with 5700 kg/ha for covered barley. Nevertheless, they found that at a seed rate of 649

seeds/m^2 naked barley produced 25% less grain than hulled barley. In our trial, MA20 had significantly higher grain yield than Deiniol.

4.8.3 Harvest index (HI) and ears per square meter (EPSM)

Deiniol had the highest HI though this was not significantly different from other lines. MA20 was the next highest HI. Despite this difference, MA20 GY was significantly higher than Deiniol. This could be attributed to the higher straw dry weight of MA20 compared to Deiniol. High straw weight may be an effect of more assimilates being used in building cell walls than being transferred to the grain sink in the endosperm of the kernel.

4.8.4 Thousand grain weight (TGW)

The size of the grain was significantly different between MA19 and all other except MA 14 and MA3.

4.8.5 Potential alternative naked barley lines for the UK

The lines associated with significantly higher grain yield and shorter straw than Deiniol could be potential alternatives to Deiniol for UK cultivation. 2

selected as a potential alternative naked barley line for the UK based on the discussion above.

Chapter 5

Heritability

5.1. Introduction

5.1.1. Heritability

The growing interest in investigating natural variation in genes to improve adaptability and quantitative and qualitative traits had lead on advances in the application of metabolic, phenotypic and genetic traits in an integrated view for the plant breeders (Fernie *et al.*, 2006). Heritability is the magnitude of the phenotypic variability between individuals in a population that can be attributed to the effects of genes. Heritability analysis is an estimation of the relative contribution of genetic and non-genetic variance factors in a population. Narrow sense heritability' (h^2) refers to the portion of variation that is caused by additive genetic factors. Additive genetic effects: A mechanism of quantitative inheritance referring to combined effects of genetic alleles at two or more gene loci that are equal to the sum of their individual effects**.** Conversely, cumulative effects refer to the association between several genetic variants. Broad sense heritability (H^2) refers to the ratio of total genotypic variance to total phenotypic variance.

5.1.2 Semi dwarfing in barley

With the green revolution during the 1950s and 1960s bringing intensified crop cultivation, Spikes on high yielding cereals could not be supported by long stems especially with extreme weather conditions of rain and wind (Rademacher, 2015). Severe lodging tends to occur in one out of three or four years in the UK (Baker et al., 2014). Berry et al. (2004) reported that breeding of shorter stem cultivars has helped to reduce lodging in high yielding varieties. However, they added that shorter stems under UK conditions may have a negative effect on light interception, foliar disease outbreaks and ease of harvesting. Furthermore, the potential to minimize stem length in cereals is limited as there is a minimum crop height that is compatible with high yields (Berry et al., 2004). They added that resistance to lodging could be achieved by

investigating the wide genetic diversity in these plant characters and through the application of crop innovative management decisions. Early lodging (shortly after anthesis) has more impact on grain yield and quality compared with lodging close to harvesting (Hoffmann, 1992).

A large number of genes control most economic and agronomic traits in barley with some of these traits also influenced by the environment (Wang et al., 2014). Since the green revolution, semi-dwarf genes have played a vital role in increasing yields with their higher harvest index and, in cereal crops, a resistance to lodging (Milach and Federizzi, 2001). More than 30 types of dwarfing genes were extensively investigated in barley but few of them were successfully used in breeding programmes. The selection for shorter stems was used by plant breeders to increase yield by decreasing lodging and increasing yield potential and harvest index (Kuczyńska et al., 2013). Since 1950 more than 350 dwarf and semi-dwarf cultivars and lines have been developed , producing up to a 4.7-fold yield improvement compare to landraces and old cultivars (Jing and Wanxia, 2003).

The most widely known classes of mutants are the *ert* (short for erectoides) mutants. These are distinguished by a combination of compact, dense ears and an upright growth habit, with many also. characterized by a short and sturdy stem (Franckowiak and Lundqvist, 2012). Other mutant classifications include *brh* (short for brachytic; with the principal trait of shortened internodes), *ari* (short for breviaristatum; characterized by shorter awns), *dsp* (dense spike), uzu (semi-brachytic), *dwf* (dwarf), *sdw* (semi-dwarf), or *sld* (slender dwarf) (Franckowiak and Lundqvist, 2012).

Stem length is controlled by dwarfing and semi dwarfing genes. Semi dwarfing genes in barley include semi-brachytic 1 (*uzu1*; Nomura et al., 1999), semi-dwarf 1 (*sdw1* or *denso*; Jia et al., 2015), *arie* (Agricultura, 2004) and *hcm1* (Thomas et al., 1984). *Uzu* genes were brought to naked barley breeding in Japan (Nomura et al., 1999). The *uzu1* and *sdw1* genes are both located on chromosome 3H (3HL) on its long arm (Barua et al., 1993). Nowadays, cultivars with the

sdw1/denso gene are in the pedigree of most modern barley cultivars worldwide (Dahleen et al., 2005). To date two alleles of the *sdw1* gene *sdw1.d and sdw1.a* have been identified in modern barley lines and cultivars (Xu et al., 2017).Xu et al. (2017) also found that the gibberellin 20-oxidase gene (*HvGA20ox2*) is the candidate gene of *sdw1* mutant barley. Furthermore, their results provided proof that partially or totally lost *HvGA20ox2* gene function could be compensated by improved expression of its homologs *HvGA20ox1* and *HvGA20ox3*. Thomas et al. (1991) found that the *denso* gene was associated with later heading, lower seed weight, and higher β-glucan content.

Ert-k.32 was produced by an X-ray-induced mutant in Bonus barley and released as a Pallas *ari-e.GP* cultivar (Gustafsson and Züchter, 1967). was produced in 1956 from a γ-ray mutagenized Maythorpe cultivar and released with the name Golden Promise (Forster, 2001). *sdw4.ba* was produced in the late 1960s from γ-ray treatment of the Chinese cultivar Zhenongguangmangerleng and released as Zhepi 1 (Zhang et al., 2006). Many short stem barley mutants are available in various germplasm depositories [e.g. IPK Gatersleben, Germany (www.ipk-gatersleben. de/en/resources/genebank-information-system/) and (the Nordic Genetic Resource Centre, Alnarp, Sweden (www.nordgen. org)] (Dockter and Hansson, 2015).

Double-haploid lines (referring to the formation of genotype when haploid cells undergo chromosome doubling) have been developed by crossing a *brh1* mutant (*brachytic1* semi dwarf gene) and the European malting cultivar Quench, resulting in acceptable malting quality but a decreased yield. The decrease in yield is relate to the activities of the starch-degrading enzyme β-amylase (Braumann et al., 2018b). Teplyakova et al. (2017) estimated the functional polymorphism effect of *HvGA20ox2* (the candidate gene for *sdw1/denso* locus) on the variation of agronomically important barley traits such as stem length, flowering time, thousand grain weight, and grain starch. They found that the 7-bp deletion (a mutation where a piece of chromosome or a DNA sequence is lost) in the *HvGA20ox2* gene decreased stem length by

approximately 13 cm and delayed flowering time by 3–5 days. This in turn was found to improve lodging resistance and hence produced higher yields under certain conditions.

Investigating the mutated genes preserved in gene banks would significantly improve our understanding of their role in controlling stem length in cereals. This would enhance the diversified gene pools available for plant breeders and help in developing new mutants capable of producing higher grain yields and of tolerating harsher weather conditions (Dockter and Hansson, 2015).

5.1.3 Grain yield (GY)

Grain yield is influenced by numerous major and minor genes controlling quantitative trait loci (QTLs) such as thousand grain weight. These QTLs are present in all barley chromosomes. However, their numbers, positions and their overall effects depend on the population and the type of markers used (von Korff et al., 2006). For example, 3 QTLs were identified for barley thousand grain weight (Peighambari et al., 2005), demonstrating that thousand grain weight is affected by several QTLs rather than a single major gene, as is the case for the dwarfing trait, which is controlled by a single major gene (ref).

5.1.4 Tillering

Tillering is considered one of the principal grain yield components in barley (Hussien et al., 2014), with higher yield associated with a larger number of tillers (Evers and Vos, 2013). Variability in tillering is related to genetic and environmental factors (Alqudah and Schnurbusch, 2014; Borràs et al., 2009).

5.1.5 Recombinant Inbred Lines

A recombinant inbred line (RIL) is a member of a plant population developed by using single seed descent from the F_2 generation, derived from a cross between two inbred lines. Each RIL is homozygous (or almost homozygous) and the resultant set of homogenous RILs can be

used in replicated experiments and for QTL mapping. This is because each RIL has inherited a different combination of alleles from the original two inbred line parents.

5.1.6 Variation in starch composition

Starch is an alpha-glucan composed of two glucose polymers, amylose and amylopectin. Significant variation in starch structure and composition occurs between barley seeds but this variability has not yet been fully exploited in breeding food barley (Howard et al., 2014). A number of divergent starch mutants have been identified (e.g. Patron et al., 2004). Exploiting this variability to develop cultivars with specific starch characteristics may enable the development of new products with health benefits (Howard et al., 2014).

Riso 13 is a mutant barley line defective in starch biosynthesis that is reported to have a higher β-glucan content and a lower starch content and grain weight. The *lys5* locus in Riso 13 contributed to lower (by as much as 30 %) starch content, higher (by as much as 15–20%) β-glucan content, higher (by approximately 1.5%) dry matter content, lower water content, and preserving polysaccharides production 50–55% in normal barley (Jordan et al., 2012).

Barley grain amylose content is one of the most critical qualitative traits (Fan et al., 2017). Lafiandra et al. (2014) investigated strategies for modifying the composition of kernel polysaccharides to improve their health benefits, exploiting their natural diversity and utilizing mutagenesis and transgenesis to achieve further alterations. They aimed to develop cereal cultivars and products to meet the food production and health challenges of the 21st century. They added that traditional plant breeding depends on manipulating natural variation, which is achieved by recombination of genotypes through crossing of variant genetic backgrounds. The range of diversity varies from trait to trait. Furthermore, they added that mutation breeding, involves the use of radiation or chemical compounds to induce mutations, facilitating the development of novel genetic variation in major traits that could be employed in breeding programmes.

5.2 Objectives

This chapter aimed to:

1- Compare the lines produced in the current study with lines developed previously in terms of yield, thousand grain weight, β-glucan, and amylose (Study 1).

2- Assess the Deiniol x Propino lines in terms of grain yield, short plants and screening for β-glucan, amylose, amylopectin (under glasshouse condition) (Study 2).

3- Develop a higher β-glucan content naked barley from a Deiniol x Riso 13 mutant (study 3)

5.3 Study 1

5.3.1 Materials and methods

Ten RILs were selected from the seeds of a Skardu x Static F_6 cross developed in a previous study at Bangor University (Table 5.1) based on the two extremes of β-glucan content. In addition to the 10 RILs, naked breeding lines (Table 5.2) and the Static and Skardu parent lines were planted in a completely randomized design (CRD), with three replicates, in the glass house at Henfaes research station.

The plants were grown in two litre black plastic pots on the 1st of April 2015. John Innes no. 2 compost was used for planting. Starting from the third week after germination, NPK fertilizer with a concentration of 1 g per 2L was used to feed all the plants on a weekly basis. The seeds were harvested on the 25th of July 2015. The grains for polysaccharide analysis were dried and prepared as described in section 2.1.9 and 2.1.10.

Table 5.1. The RILs used in this trial

RIL	RIL
131	195
99	22
16	106
101	37
85	57

Table 5.2 barley cultivars (Released) and breeding lines (Unreleased)

Barley genotype	Hull type	Released/Unreleased
27 DUS	N	Unreleased
ICARDA 93 X Tripple	N	Unreleased
Lawina	N	Released
Line 4	N	Unreleased
Line 15	N	Unreleased
Line 20	N	Unreleased
Deiniol	N	Unreleased
Static	H	Released
Skardu	N	Unreleased

5.3.2 Data collected

After harvest the grains from the three replications was bulked together (as the main objective was practice for β-glucan content investigation as mentioned above). Total dry grain yield (GY) per pot was recorded as well as thousand grain weight (TGW).

5.3.3 Polysaccharide analysis

Two replications from each RIL and breeding line barley flour were analysed for β-glucan and amylose content. 0.5 g and 0.25 g samples were used for β-glucan and amylose analyses, respectively, following the procedures described in sections 2.1.9 and 2.1.10 Amylopectin content was also calculated.

5.3.4 Statistical analysis

Descriptive statistics were used to compare GY and TGW data. Analysis of variance (ANOVA) was used to compare β-glucan and amylose results using SPSS 24 software.

5.3.5 Results

5.3.5.1 Grain yield (GY) and thousand grain weight (TGW)

Descriptive statistics revealed that mean GY was 13.63±1.74 (g) for the three pots, while mean TGW was 52. 0 ± 1.41 g. Naked RIL 99 had the lowest in GY and Static (a hulled barley line) had the highest GY. Figure 5.1 shows the GY for different RILs and breeding lines in this experiment. RIL 99 has the highest TGW index and RIL 101 had the lowest in TGW index (Figure 5.2).

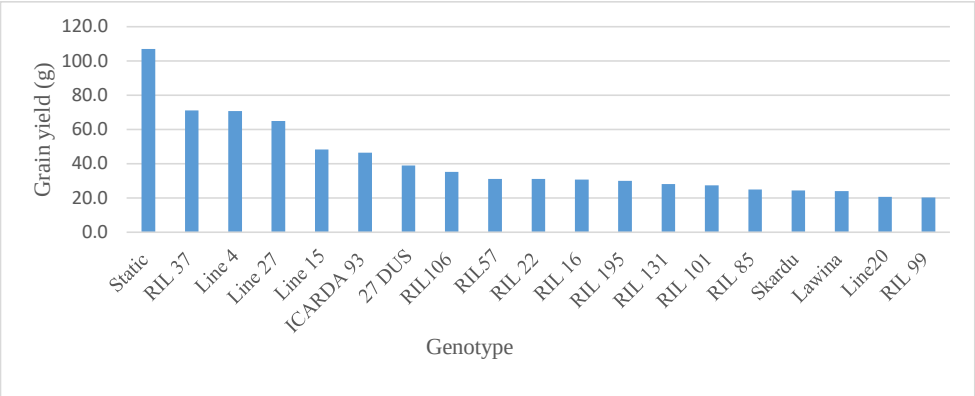


Figure 5.1: Grain yield for RILs and breeding lines

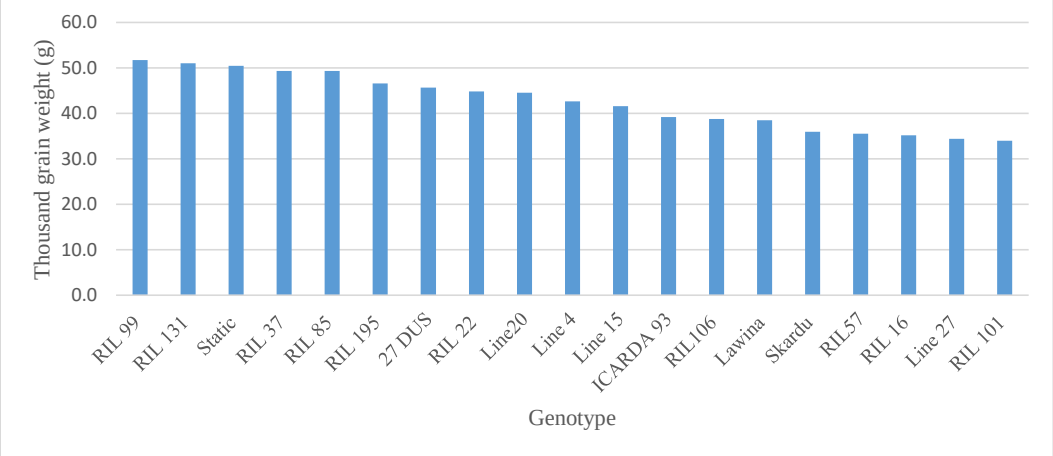


Figure 5.2: Thousand grain weight for RILs and breeding lines.

5.3.5.2 B-glucan content (%) for RILs and breeding lines

Significant differences were observed between various RILs and breeding lines in β-glucan content at p≤ 0.05 (Figure 5.3).

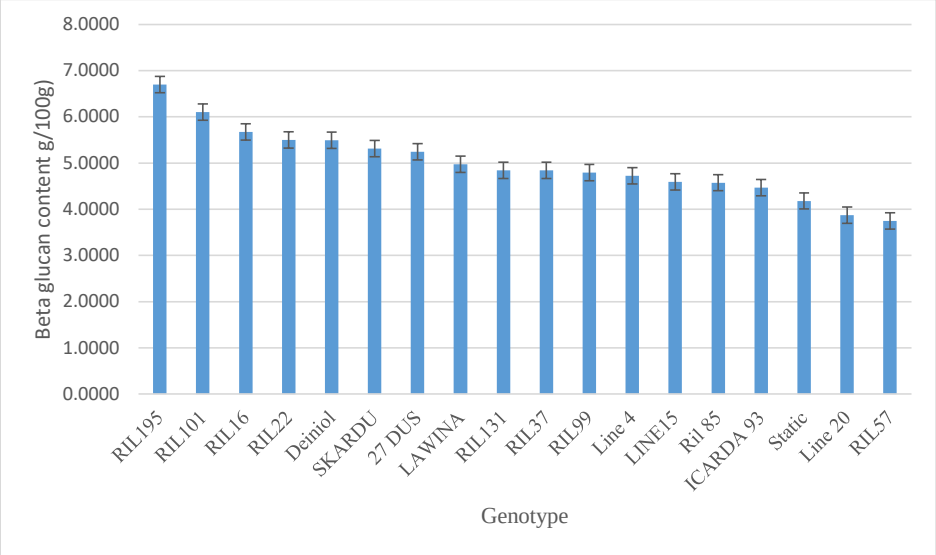


Figure 5.3. Ranked β-glucan content of RILs and breeding lines.
Bars on columns represent standard error of differences of means.

RIL 195 had the highest β-glucan content and RIL 57 had the lowest β-glucan content.

5.3.5.3 Amylose and amylopectin content (%) for RILs and breeding lines

Significant differences (at p ≤ 0.05) were found between various different lines and RILs in amylose content. RIL 85 had the highest in amylose content while line 20 was had lowest in amylose content (Figure 5.4).

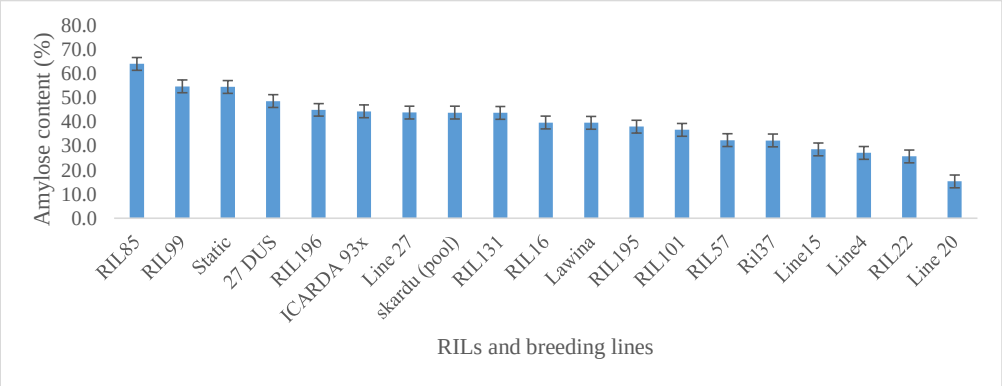


Figure 5.4. Ranked amylose content (%) of RILs and breeding lines
Bars on columns represent standard error of differences of means.

The same pairs were significantly different for amylopectin content but in the opposite direction (highest amylose lines had lowest amylopectin).

5.4 Study 2: Deiniol × Propino population

5.4.1 Materials and methods

Nineteen naked F_5 seeds were selected from the Deiniol x Propino population (derived from F_2 seed kindly developed by Dr E. Dickin, Harper Adams University) alongside with their parental lines were planted in a completely randomized design (CRD) in the glasshouse. This was a factorial experiment with 6 replicates from each line and parent. Four seeds from each line were seeded in each pot and the plants were thinned after emergence to one plant per pot for this experiment. Two litre black plastic pots filled with John Innes No. 2 compost were used for planting. The seeds were sown on the 21st of April 2016 and the plants were harvested on the 28th of July 2016.

5.4.2 Data collected

The following parameters were measured at harvest for each plant: stem length, number of fertile ears, GY and TGW.

5.4.3 Polysaccharide analysis

B-glucan, amylose and amylopectin were assayed using the methods described in sections 2.1.9 and 2.1.10 Two replicates (6 samples in total) from each line were combined due to small sample weights for some samples. 0.5 g and 0.25 g samples were used for β-glucan and amylose analyses, respectively.

5.4.4 Statistical analysis

Analysis of variance (ANOVA) was used to analyse data as described in section 2.3.1.

5.4.5 Calculating narrow sense heritability

The following formula was used to calculate narrow sense heritability (h^2) from the variance (V) components of the populations:

$$h^{2=}\frac{Vgenetic}{V\ additive}=\frac{Vadditive-V\ environmental}{V\ additive}.$$

where V additive = $(VF_2 + VF_3)/2$

The following formula was used to calculate the broad sense heritability (H^2) from the variance (V) components of the populations.

H^2 = (V additive + V dominant)/ (V additive +V dominant +V environmental)

where V dominant = V additive- V environmental

and environmental = P2*

*Because variance of P1 for Deiniol is so large it gives odd results, the calculations were carried out using only P2 variance which is Riso 13 variance for stem length, yield, grain number and thousand grain weight. The β-glucan heritability analysis was done using P1 and P2 variances as these two variances were low for this trait. The heritability values calculated are only estimates; (since variations are likely to occur between generations grown on different seasons.

5.4.6 Results

Please refer to appendix 1.3 for normality, frequencies and ANOVA results.

5.4.6.1 Stem length (StL)

Significant differences were observed in stem length between different lines ($p \leq 0.01$).Line 3 was the line with longest stems, while line 10 had the shortest (Figure 5.3).

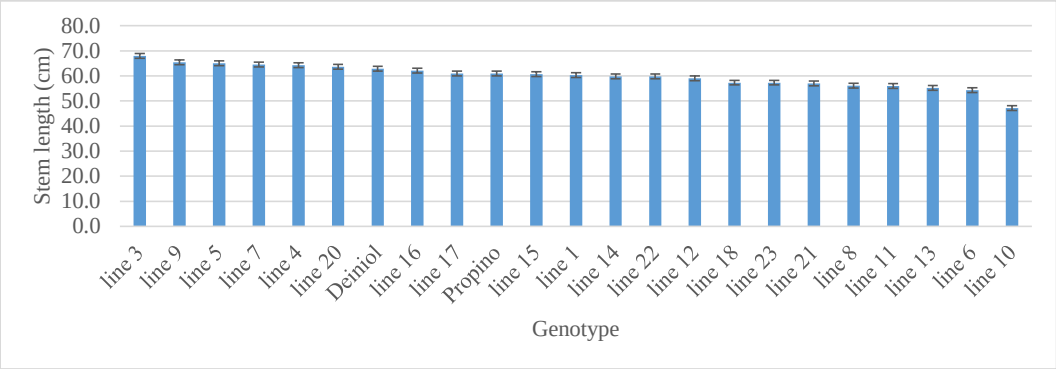


Figure 5.5. Stem length for Deiniol x Propino
Error bars represent the standard error of the mean

5.4.6.2 Grain yield (GY) per pot.

ANOVA revealed significant differences in GY between different lines ($p \leq 0.001$). No significant differences were found between Deiniol and line 9, line 20, line 16, and Propino in GY (Figure 5.6). Deiniol had the highest GY, while line 10 was the lowest in GY

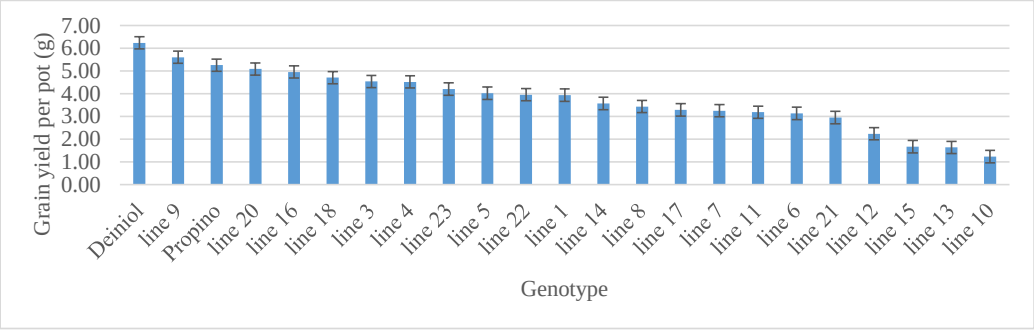


Figure 5.6. Ranked grain yield per pot for Deiniol x Propino population
Error bars represent the standard error of the mean

5.4.6.3 Number of fertile ears

Significant differences were observed in the number of fertile ears between different lines ($p \leq 0.001$) (Figure 5.7). Line 9 had the highest number of fertile ears, while line 10 had the lowest number of fertile ears.

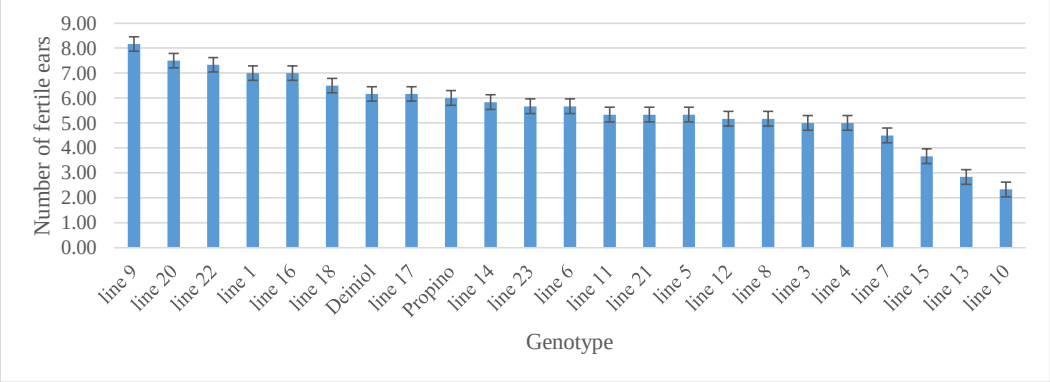


Figure 5.7. Ranked number of fertile ears per pot of Deiniol x Propino population
Error bars represent the standard error of the mean

5.4.6.4 Thousand grain weight (TGW)

Analysis of variance revealed no significant differences in TGW between the different lines at $p \leq 0.05$ (Figure 5.8).

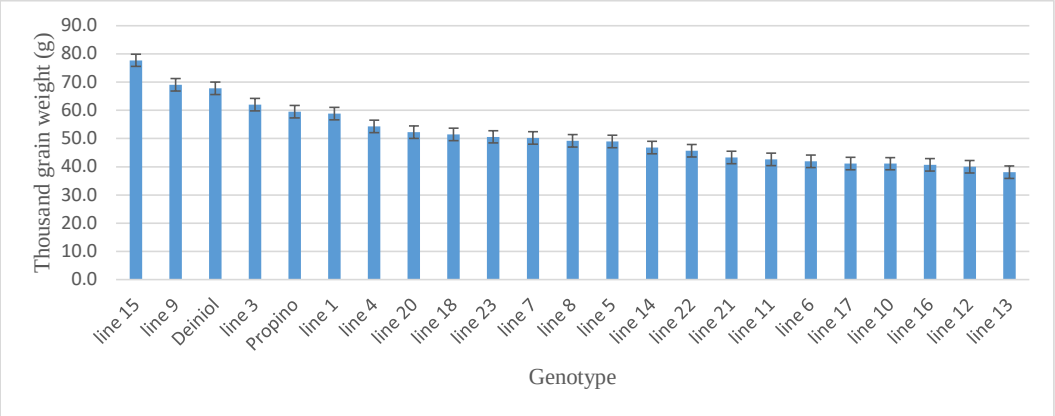


Figure 5.8. Ranked thousand grain weight (g)
Error bars represent the standard error of the mean

5.4.6.5 Grain per ear (GPE)

Significant differences were observed between different lines in GPE at $p \leq 0.01$ (Figure 5.9).

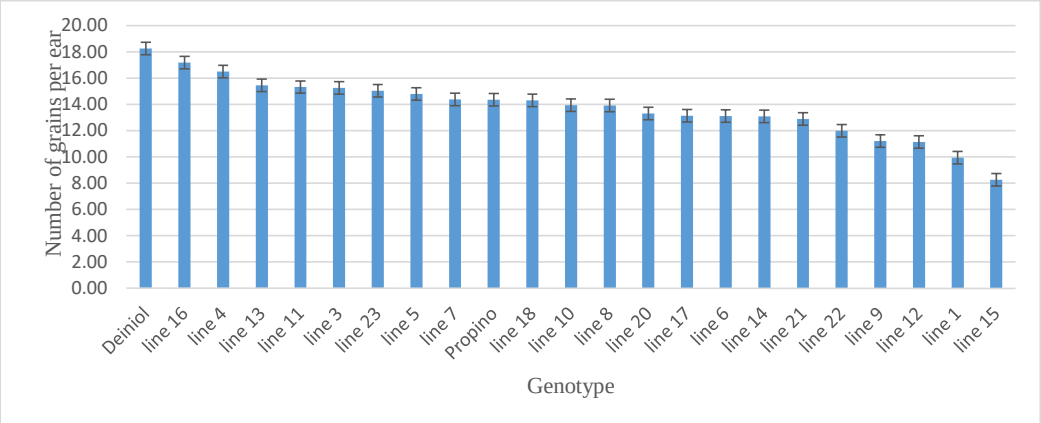


Figure 5.9. Ranked number of grains per ear
Error bars represent the standard error of the mean

5.4.6.6 B-glucan content for Deiniol x Propino (%)

Significant differences were observed between the different lines for β-glucan content at $p \leq 0.05$ (Figure 5.10). Line 1 was the highest in β-glucan content, while line 15 was the lowest in β-glucan content.

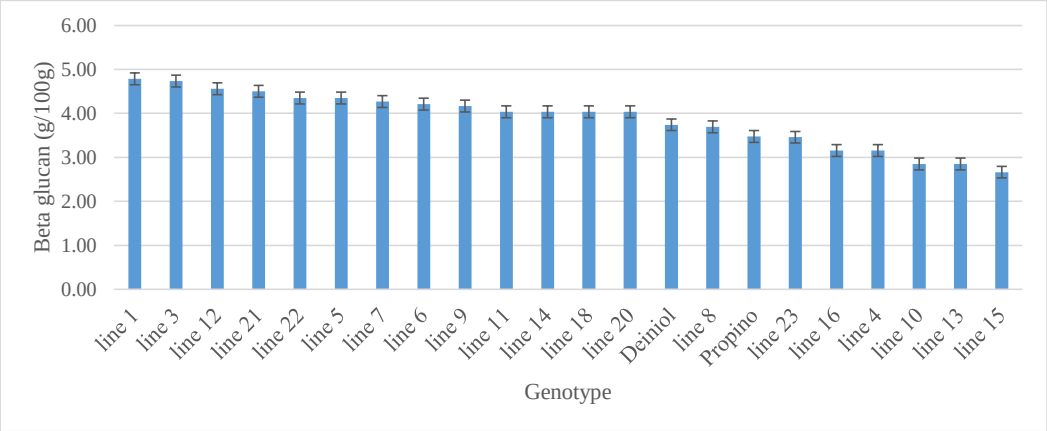


Figure 5.10. Ranked β-glucan content of Deiniol x Propino population
Error bars represent the standard error of the mean

5.4.6.7 Amylose and amylopectin content for Deiniol x Propino (%)

Significant differences were observed between different lines in terms of amylose content at $p \leq 0.01$ (Figure 5.11). The line 23 was the highest in amylose content while line 13 was the lowest. The same pairs were significantly different for amylopectin but in the opposite direction (highest amylose lines had lowest amylopectin),

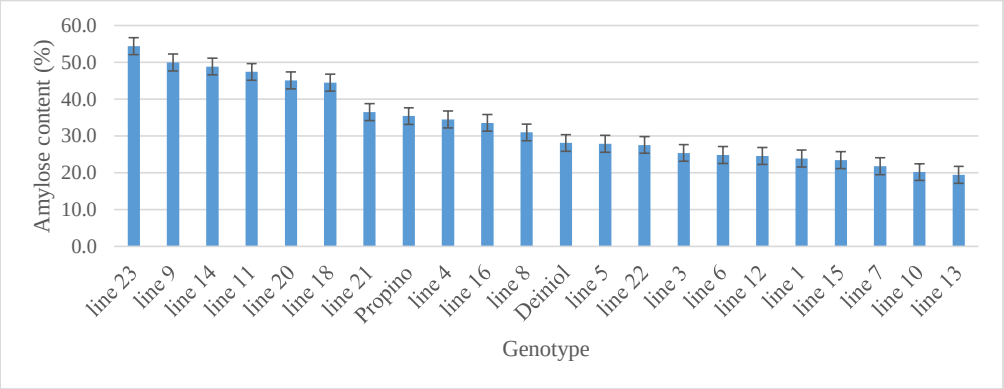


Figure 5.11. Ranked amylose content of Deiniol x Propino population
Error bars columns represent the standard error of the mean

5.4.6.8 Regression analysis

A significant positive correlation at $p \leq 0.01$ was observed between stem length and grain yield for the Deiniol x Propino population (r^2= 0.15, n=138) (Figure 5.10). R^2=0.15, n=138.

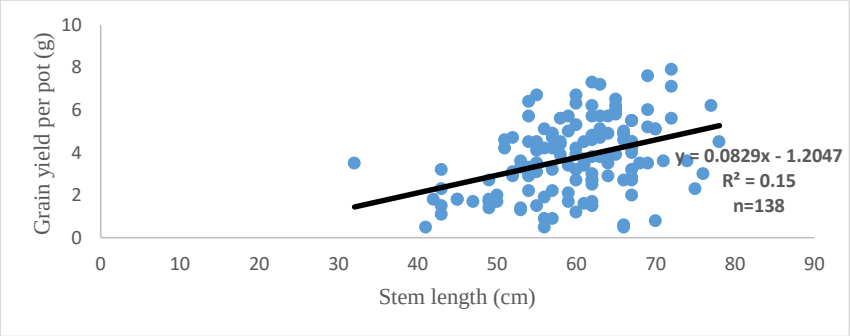


Figure 5.12: Scatter plot of stem length vs grain yield per pot for Deiniol x Propino

5.5 Study 3: Deiniol x Riso 13.

5.5.1 Materials and methods

5.5.1.1 Barley crossing

This experiment took place in the glasshouse and was carried out with the assistance of a technician. Pots were arranged according to variety and sowing date and labels were assigned for each pot. Seeds from each parental line were planted on staggered dates once every ten days

during October and November 2015 to secure a perfect match between anthers and female flower parts maturity time between the two varieties. Deiniol was the female parent while Riso 13 was the male parent.

Female parent ears were selected for crossing prior to pollen shedding. The ears were clipped off close to the tip of the uppermost spikelet. The awn upper parts and the flag leaf were removed. Spikelets over the anthers were also clipped. At this stage the anthers could be seen. The three anthers inside each spikelet were removed (emasculated) using tweezers. The spikelets were re-checked to make sure the emasculation was done perfectly. The female spike was covered with a glassine bag and labelled with the date of emasculation. Prior to pollination the spikelets were checked to see if they had already set seeds. Any formed seeds were removed.

After 2-4 days emasculated spikelets which had opened were pollinated with pollen from the male parent. Pale yellow anthers from the male parent plant were chosen to pollinate female plants. Unshed pollen spikes which were still at the bottom of the spikelet were used. Spikelets above the anthers were clipped. A few minutes after clipping the anthers puffs up, pollen sheds and this was used for crossing the emasculated female flowers. Glassine bags were returned back to cover the female spike after pollination. The formation of hulled seeds was used as a validation criterion for the successful naked barley cross.

5.5.1.2 Data collected

As all produced grains were hulled in F_1, no data other than the overall number of produced lines were collected from that generation. The following parameters were measured for F_2 and F_3 produced grains: stem length, number of fertile spikes per pot, grain yield per pot, number of grains per pot and thousand grain weight.

5.5.1.4 F1 generation

One litre black plastic pots were used for growing during this part of this trial. A completely randomized design was used. The number of treatments and sowing dates are shown on table 5.3. The pots were filled with John Innes no. 2 compost. The parent lines were planted along with progeny lines on the same day. After emergence, the plants were thinned to one plant per pot. At the 3rd week after sowing plants, fertilisation using an NPK fertiliser with a concentration of 1 g per two litres once a week. Plant feeding continued until crop maturity. The stem length was measured. The spikes were harvested and air dried in the oven at 40°C for 48 hours. The grains were threshed and counted, and thousand grain weight was calculated.

Table 5.3: Summary of Deiniol x Riso 13 crossing activities

Generation	Number of treatments		Reps	Seeds Produced	Progenies Hull type		Sowing Date	Harvest Date
					Hulled lines	Naked Lines		
Parent lines crossing	-		No. reps	F1	All hulled	No	Different sowing dates	28-7-2016
F1 seeds	31 Hulled		4	F2	69	18	1-8-2016	21-11-2016
F2 seeds	69 Lines Hulled	18 Lines Naked	3	F3	193 discarded	38	16-12-2016	25-4-2017

5.5.1.5 F_2 generation

F_1 seeds with the number of treatments and replications described in Table 5.3 were planted in a complete randomized design (CRD). The seeds were sown in one litre black plastic pots. F_2 seeds were segregated into hulled and naked barleys at a 3:1 ratio, respectively (Table 5.2).

5.5.2.6 F_3 generation

15 plugin plastic trays each with removable 18 pots of 200 ml on each tray were used. The pots were filled with John Innes no 2 compost. A completely randomized design (CRD) was

used in this trial. The pots were irrigated daily and fertilised as described above. At harvest the stem lengths were measured and the spikes and grains were oven dried at 80℃ for 48 hours.

5.5.2.7 Polysaccharide analysis

F_3 grains were the only grains that were analysed for β-glucan and amylose. Two replicates from the combined three reps of each progeny breeding line and their parents were used. A sample of 0.3 g each for β-glucan analysis and two replications for each line with 0.25 g each for amylose analysis and amylopectin content was calculated. Barley flour was prepared as described in sections 2.1.9 and 2.1.10 for β-glucan and amylose analyses, respectively, and amylopectin content was calculated as described in section 2.1.10.4.

5.5.2.8 Statistical analysis

Descriptive statistics were used to analyse the data in F_2 and F_3 generations and standard deviation was used as the differences between reps were large. Analysis of variance (ANOVA) was used to analyse β-glucan, amylose and amylopectin content.

5.5.3 Results

5.5.3.1 Deiniol x Riso 13 F_1 and F_2 generations

All F_1 generation produced grains were hulled because *nud* is a recessive gene. In the F_2 generation the grains showed a 3 hulled to 1 naked barley ratio as hull presence is controlled by a dominant gene unlike the naked gene. Table 5.4 shows F2 descriptive statistics for 18 naked barley lines and Deiniol.

Table 5.4: F_2 generation descriptive statistics

Parameter	N	Range	Min.	Max.	Mean	Std. Deviation
Stem length (cm)	19	29.0	45.0	74.0	56.6	± 7.8
Grain yield (g)	19	13.9	0.61	14.4	5.3	± 3.5
No. of grains per pot	19	269.0	11.0	280.0	113.0	± 64.5
No. of fertile spikes	19	24.0	1.0	25.0	11.7	± 6.8
Thousand grain weight (g)	19	84.8	23.5	108.3	45.6	±16.8

Yield per plant ranged between 0.6g and 14.4g with an average of 5.3g and standard deviation of ± 3.5. The stem length ranged between 45 and 74 cm while the average was 56.6 cm. Number of grains ranged between 11 and 280 while the mean was 113.0 ± 64.5. The minimum number of fertile spikes was 1 spike while the maximum was 25 ± 6.8 spikes. The TGW ranged between 23.5 g and 108.3 ± 16.8 g.

5.5.3.2. Phenotypic variation in the F_3 generation - stem length

Significant differences in stem length were observed between different lines at $p \leq 0.05$. Line 5_3 was the longest while the line 10_4 was the shortest (Figure 5.13). Please refer to appendix 1.4 for frequencies and ANOVA table.

5.5.3.3. Phenotypic variation in the F_3 generation - Number of spikes

Significant differences in the number of spikes were observed between different lines at $p \leq 0.05$ (Figure 5.14). Line 25_3 had the highest number of spikes while line 10_4 had the lowest in the number of spikes.

5.5.3.4. Phenotypic variation in the F_3 generation - thousand grain weight (TGW)

Significant differences were observed in TGW between different lines at $p \leq 0.05$ (Figure 5.15). Line 19_2 had the highest TGW while line 9_4 had the lowest in TGW.

5.5.3.5 Phenotypic variation in the F_3 generation - Grain yield (GY)

Significant differences were observed in GY between different lines at $p \leq 0.05$ (Figure 5.16). No grains were formed and harvested from Riso 13 plants during this season. Line 11_4 had the highest GY while line 10_4 was the lowest in GY.

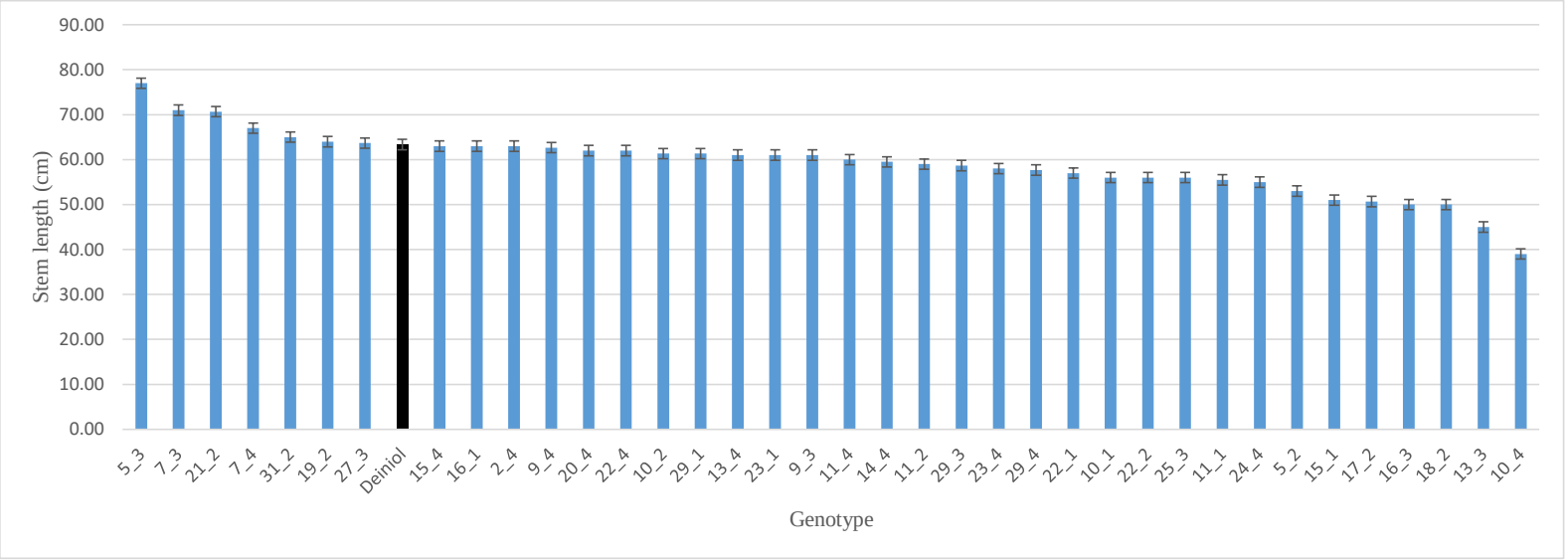


Figure 5.13: Ranked stem length of Deiniol x Riso 13 population
Error bars represent the standard error of the mean

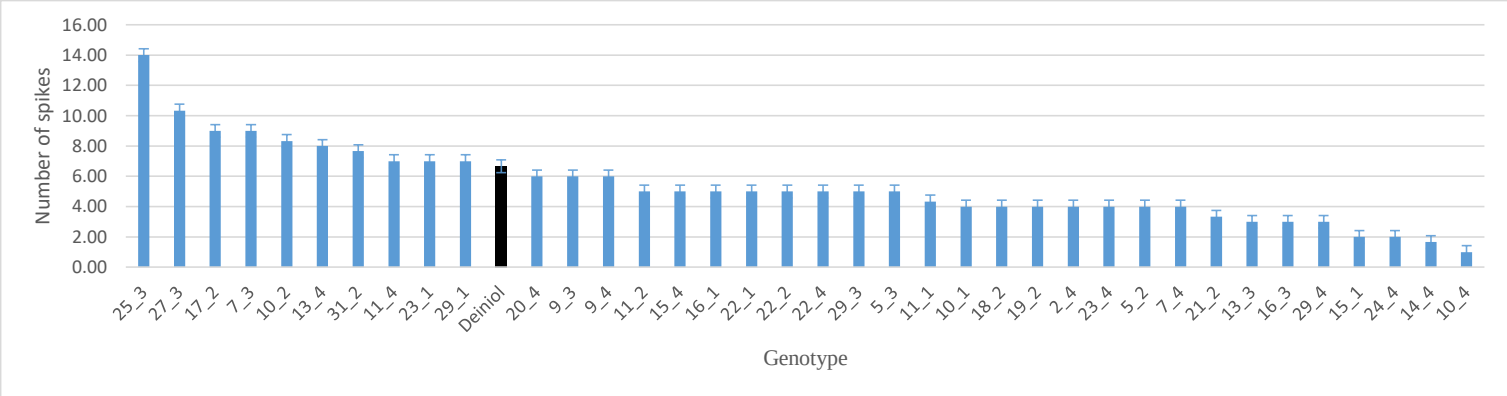


Figure 5.14: Ranked number of spikes of Deiniol x Riso 13 population
Error bars represent the standard error of the mean

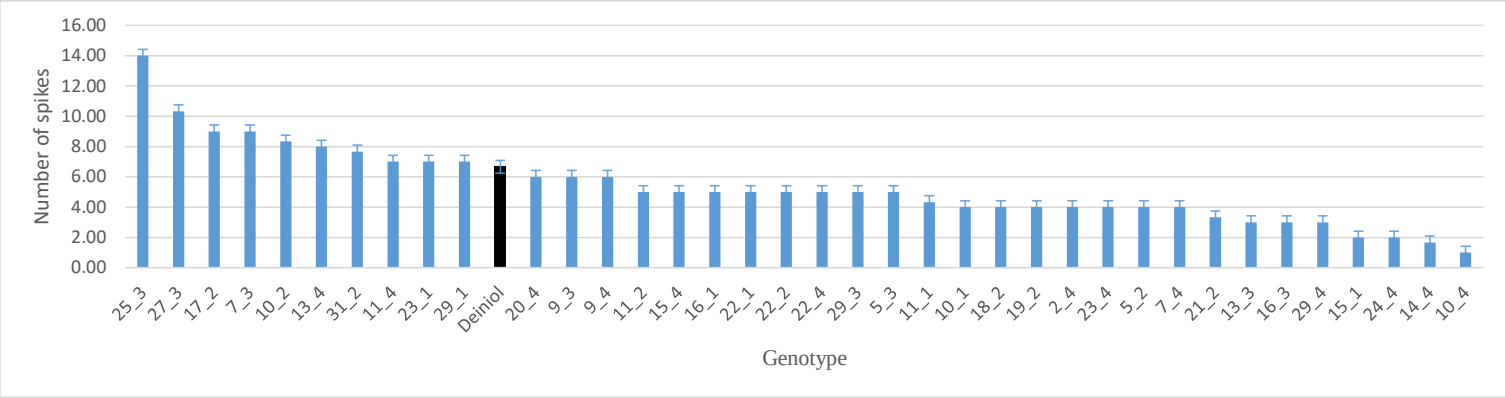


Figure 5.15: Ranked thousand grain weight of Deiniol x Riso 13 population
Error bars represent the standard error of the mean

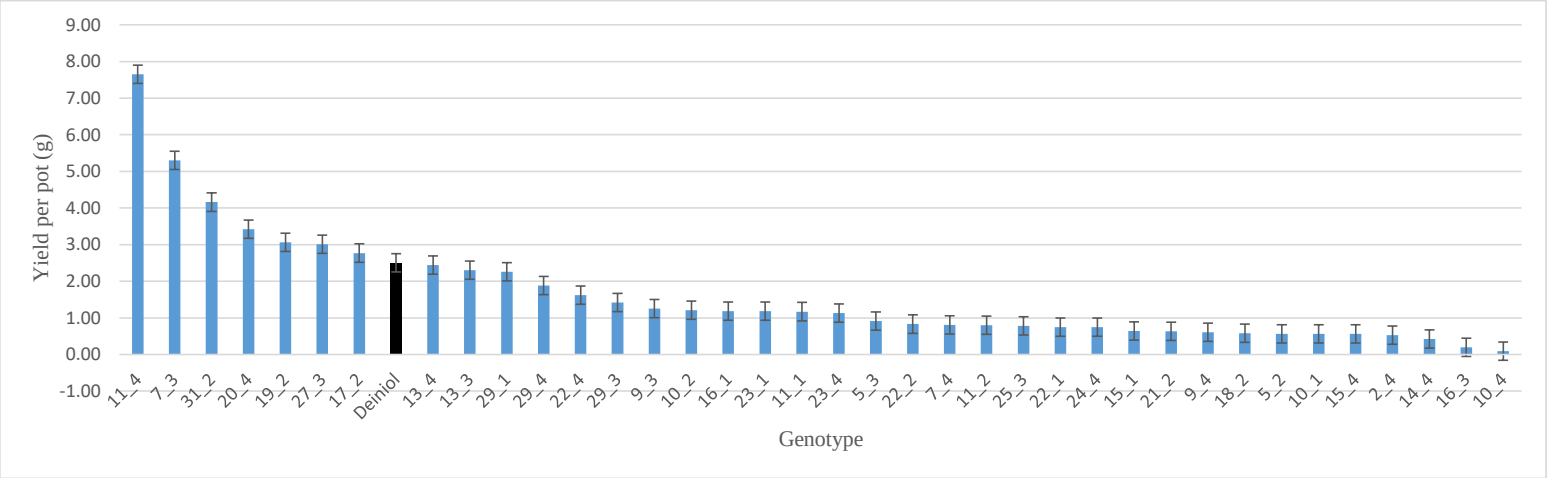


Figure 5.16: Ranked grain yield of Deiniol x Riso 13 F3 population
Error bars represent the standard error of the mean

5.5.3.6. Phenotypic variation in the F_3 generation - Bioactive components

Significant differences were observed in β-glucan content between different lines at $p \leq 0.05$ (Figure 5.17). Line 31_2 had the highest β-glucan content while line 7_3 had the lowest. Please refer to appendix 1.4.3 for frequencies and ANOVA table.

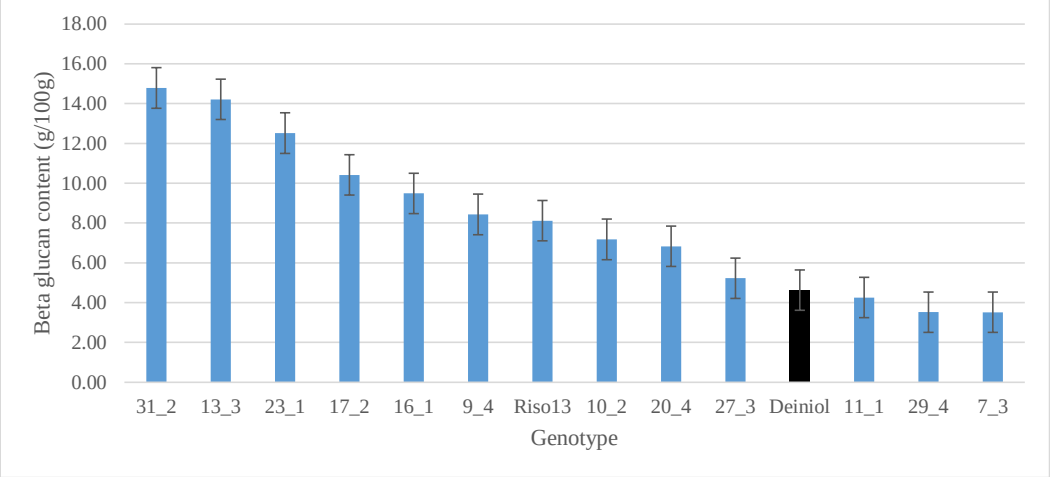


Figure 5.17: B-glucan content of Deiniol x Riso 13
Error bars represent the standard error of the mean

Significant differences were observed in amylose content between different lines at $p \leq 0.05$ (Figure 5.18). Deiniol had the highest amylose content while Riso 13 had the lowest. Please refer to appendix 1.4 for mean values for all lines and parents.

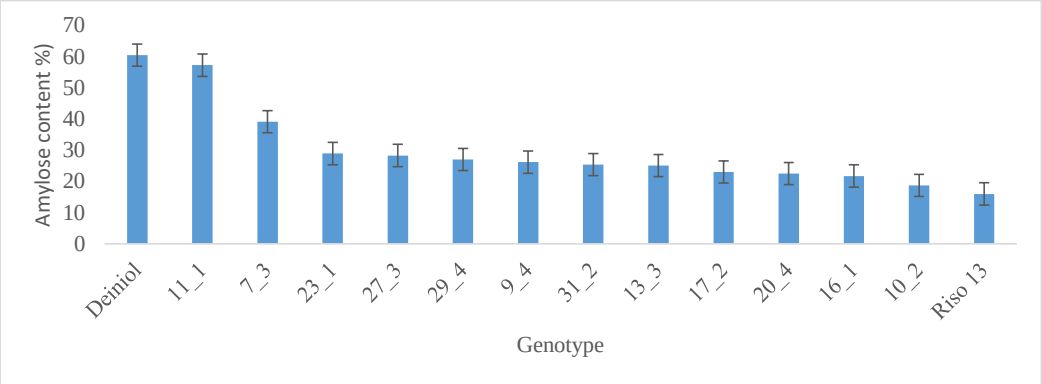


Figure 5.18: Ranked amylose content Deiniol x Riso 13 (%)
Error bars represent the standard error of the mean

The amylopectin content is just the opposite of the amylose results graph.

5.5.3.7 Heritability estimates

Table 5.5 shows narrow sense heritability (h^2) and broad sense heritability values for the traits studied in Deiniol x Riso 13 F2 and F3 populations. As the P1 variances were large the calculations were done using only P2 estimates of environmental variance.

Table 5.5: Narrow sense and broad sense heritability for different traits

Trait	h^2	H^2
Stem length	0.76	0.88
Grain yield	0.80	0.90
Grain Number	0.69	0.85
TGW	0.54	0.77

B-glucan heritability calculations were done using P_1 and P_2 environmental variance (Table 5.6).

Table 5.6: Broad sense heritability

Trait	h^2	H^2
B-glucan	1.0	1.0

5.5.4 Discussion

5.5.4.1 Stem length

The stem length was not recorded for the RILs and the breeding lines (study 1). This was only recorded for the progenies of the two crosses (Deiniol x Propino; Deiniol x Riso 13). Deiniol stem length was the highest, while line 10 was the lowest in Deiniol x Propino (Figure 5.3). In the F_3 population Deiniol x Riso 13 stem length ranged from 39.0 to 79.0 cm with a mean of 59.2 $\pm$ 7.95 (SD) cm. Jia et al (2015) found that the stem length for Bomi and semi dwarf mutant Riso 9265 were 83.2 ± 1.1 cm and 63.5 ± 2.4 cm, respectively. Mikołajczak et al. (2017) reported that the semi dwarfness associated with the *sdw1/denso* locus affects the high yielding magnitude of the modern barley varieties. They concluded that the high productivity of modern barley cultivars is mainly dependant on sources of semi dwarfness combined with the *sdw1/denso* locus.

5.5.4.2 Grain yield (GY) and thousand grain weight (TGW) for RILs, breeding lines and Deiniol x Propino

As expected the hulled UK barley Static (Released) was higher in GY than naked barley breeding lines (Unreleased) (Figure 5.1), which is in agreement with Choo et al. (2001), Dickin et al. (2011) and Dickin et al. (2012) who also reported naked barley yields that were lower than that of the hulled barley in our 1st study of RILs and breeding lines. In our 2nd study, Deiniol had the highest grain yield per pot and line 10 had the lowest grain yield. Dickin et al. (2012) also reported substantial differences in yield between autumn and spring sown crops.

5.5.4.3 Grain yield (GY) and thousand grain weight (TGW) for Deiniol x Riso 13

The yield for F3 in the Deiniol x Riso 13 cross ranged between 0.1 and 7.65 g per pot with a mean of 1.6 g ± 1.64 g (SD). Line 14_4 had the lowest line GY and line 11_4 had the highest. The lowest recorded TGW was 13.1 g for line 9_3 and the highest was 112.5 g for the

line 19_2, with a mean of 35.9 ± 20.57 g (SD). The TGY of Line 19_2 was 2.7 g and the number of grains was 28. Barley lines bearing the Riso mutant genes are most likely to be low grain yielding regardless of their genetic makeup (Howard et al., 2014).

5.5.4.4 β-glucan content in RILs, breeding lines and Deiniol x Propino

In our first glasshouse study, significant differences were observed between β-glucan content in different Static x Skardu RILs and breeding lines with the lowest value recorded for RIL 57 and the highest value for RIL 195. There was a slightly lower range in the second glasshouse study, where Deiniol x Propino F_5 lines ranged from 2.66 to 4.78 g in β-glucan content. This is in agreement with the findings of Cramer et al. (2005), Papageorgiou et al. (2005), Dickin et al. (2011), Wirkijowska et al. (2012) and Lin et al. (2018) who found that β-glucan content in their studies ranged from 3.0 to 7.0 g/100 g. Genotypic variation is the primary factor in determining the final β-glucan content of barley (Izydorczyk et al., 2000; Tiwari and Cummins, 2008).

Izydorczyk et al. (2000) found that differences in total β-glucan content is mainly due to genetic variation between different naked barley lines rather than the size of the grain endosperm. They also stated that the soluble portion of β-glucan is mainly responsible for delivering β-glucan health benefits rather than total β-glucan content. Harvesting the crop at early physiological maturity (ZGS 92) may reduce β-glucan content by about 32.6% in naked barley and β-glucan content may be increased by 20.1% if storage time is minimised (Tiwari and Cummins, 2008). Significantly higher total β-glucan content, ranging from 5.80% to 6.29%, was observed in coloured naked barley cultivars grown in India at an altitude of more than 4000 m as compared to naked barley cultivars grown at an altitude of 97 to 3500 m (Moza and Gujral, 2016).

The environment, and geographical location significantly affects naked barley β-glucan content (Zhang, et al., 2002; Cory et al., 2017;Moza and Gujral, 2017). B-glucan is an

intermediate compound rather than a final product. This makes it difficult to identify the effect of environmental on β-glucan content at the harvest time (Dickin et al., 2011). Dickin et al. (2011) also added that delayed harvesting could lead to diminished β-glucan health benefits for humans.

Izydorczyk et al. (2000) reported that thermal, enzymic, and physical treatments of the barley kernel can modify the extractability and physical characteristics of barley β-glucan. They added that hydrothermal barley treatments greatly affect β-glucan molecular weight and viscosity which in turn may have a positive effect on physiological responses to barley β-glucan in human. Furthermore, they found that enzymic and physical treatments may increase soluble dietary β-glucan extractability and hence activate desirable physiological effects in human. A positive correlation has been reported between β-glucan, DP3, DP4 and DP3 + DP4, with no preference for either subunit in higher or lower β-glucan lines (Cory et al., 2017).

5.5.4.5 β-glucan content in Deiniol x Riso 13

In our third study the β-glucan content in Deiniol x Rios 13 ranged between 14.80 (line 31_2) and 3.52 (line 7_3), respectively. The 31_2, 13_3, 23_1, 17_2 and 16_1 line had the highest β-glucan content of any UK naked barley lines recorded up to now, to the best of our knowledge. Breeding for a high barley β-glucan content is highly desirable (Hu, 2014). Natural β-glucan in low β-glucan content barley is insufficient to decrease metabolic responses (Liljeberg et al.,1996). Therefore, β-glucan enrichment is needed or the use of high β-glucan content barely as an alternative (Tappy et al., 1996). A high β-glucan content barley cultivar Prowashonupana was reported by Liljeberg et al. (1996).

Topping et al. (2003) developed a mutagenized barley cultivar, Himalaya 292, with 10g/ 100g β-glucan content. The very high β-glucan levels of the *lys5* locus in the Riso 13 mutant compensate the decrease in starch levels fully or partially (Munck et al., 2004). They also found that 19.8% β-glucan was produced by extreme gene lys5f (Riso 29 mutant) as compared to 13.3 % for lys5g gene (Riso 13 mutant). Furthermore, the trade-off between high β-glucan content

and starch results in approximately a 1.5% increase in dry matter and lower water content. They elaborated that this is due to more water being needed to bind starch in the amyloplasts compared to that required to bind β-glucan in the endosperm cell walls.

The establishment of a food barley industry based using naked barley cultivars with consistent β-glucan levels is necessary (Dickin et al., 2011). The distribution of β-glucan particles in the grain is extremely important in determining their retention in food products (Wirkijowska et al., 2012). Wirkijowska et al. (2012) also reported that the aleurone layer in high β-glucan lines does not show any intensive reaction with the fluorescent agent. Furthermore, they added that the cell walls in the sub aleurone and endosperm layers in those samples were thicker and brighter than in the low β-glucan barley.

Cory et al. (2012) found an association between high β-glucan content and the TR251 *HvCslF6* haplotype and that the QTL for β-glucan is located on chromosome 7H. Shu and Rasmussen (2014) found that amylose and β-glucan content had a negative correlations (R= −0.62, $P < 0.01$) as did amylopectin and β-glucan ($R = -0.487$, $P < 0.01$). Islamovic et al (2012) found high β-glucan controlling QTLs are located on chromosomes 3H, 4H, 5H, 6H and 7H.

5.5.4.6 Amylose content (%)

In Deiniol x Rios 13 amylose content ranged from 60.5 to 16.0, putting them in the range of normal amylose to high amylose content barley categories. That is in agreement with Topping et al. (2003) who found that amylose content was 7.84 and 22.43% in Azhul and Falcon naked barley cultivars, respectively. And that is also in agreement with the same author for the mutagenized barley cultivar Himalaya 292 developed with (71 %) amylose content. Islamovic et al. (2012) found that amylose-contributing QTLs are located on chromosomes 1H, 5H and 7H. They also report that QTLs stimulating both β-glucan and amylose components are located on chromosomes 1H and 7H. Patron et al. (2005) found that the starch granules in Riso 13 are abnormally shaped and smaller in size than normal barley.

5.5.4.7 Amylopectin content for Deiniol x Riso 13 (%)

Significant differences were observed between RILs and breeding lines in the three studies included in this chapter. No waxy naked barley lines were identified in any of our experiments.

Chapter 6

General discussion, conclusions and future work

6.1. Effect of effect of using early growth regulator application on naked barley

One of the aims of this project was to identify the optimum conditions and practices including the potential of PGR. Deiniol is a long-stemmed plant, inheriting this trait from its Skardu parents. Investigating the effect of PGR application at an early growth stage (ZGS 32) we found that the stem length of naked and hulled barley was significantly reduced (Table 3.1). This reduction was very important in the taller Deiniol line where PGR application at an early growth stage enhanced lodging resistance and consequently enhanced the crop yield by facilitating mechanized harvesting and improving the harvest quality. This finding agrees with other published results which suggests that PGRs improve the root growth and thereby improve anchorage and absorption of water and nutrients (Rademacher, 2018).

Significant differences were observed with early PGR application between naked and covered barley in terms of grain yield (Table 3.2), in agreement with previous studies e.g. Dickin et al. (2011). Covered barley had more grain yield per m^2 than naked barley. PGR application significantly increased straw dry weight as assimilates would have been otherwise utilised in lengthening stems were used to increase stem diameter (Table 3.3). However, this also resulted in a lower harvest index for Deiniol compared with hulled barely because of the inverse relationship between straw weight and harvest index (Table 3.4).

Thousand grain weight increased significantly in both genotypes as a result of early PGR application (Table 3.6). Another important finding in our study was the significant increase in germplasm β-glucan content in both barley varieties following PGR application (Table 3.6). However, no change in amylose or amylopectin content was observed.

6.1.1 Effect of late PGR application

Our study also investigated the effect of late PGR application. Analysis of variance (ANOVA) revealed no significant differences in grain yield and number of ears per square meter following late PGR application at (ZGS 37) between control and different PGR treatments (Figures 3.1-3.7). These findings are in agreement with those of Rajala and Peltonen-Sainio (2002), Rajala (2004) and Lauer (1991). However, since early PGR application and late PGR application didn't take place on the same trial no firm conclusion can be drawn.

6.2 Can genetic selection improve performance and yield in naked barley genotypes?

Deiniol barley was the best new naked barley line available at the start of this project, but it is not well adapted to UK production, because it is low yielding and has a tall stem. This project trialled various crosses to produce an alternative naked barley line with shorter stems, better adapted to UK conditions than Deiniol. In our research we used Optic (*sdw1*) and Westminster (*sdw1*) cultivars which are semi-dwarf genotypes (White et al., 2009) as gene donors for crossing with naked barley genotypes. The development of successful dwarfing varieties involves achieving a more robust structure whilst minimising reductions in yield (Wang et al., 2014). The same authors added that semi-dwarfing genes in barley may be preferable to dwarfing genes which may undermine vigour and grain yield. The *sdw1* mutations were initially selected for their reduced stem length and increased grain yield, as well as their effects on root traits including length and weight (White et al., 2009).

Stem length is controlled by quantitative trail loci (QTLs), qualitative genes and other plant height genes (Ji-Hua et al., 2007; Braumann et al., 2018a). *Uzu*-type barley was one of the first short-stem mutants to be used. Its distribution includes Japan, the Korean Peninsula and China (Saisho et al., 2004). *sdw1* and *denso* were used as semi-dwarfing genes in North America and Europe (Xu et al. 2017).

Chapter 4 described the evaluation of a range of novel naked barley genotypes (MA lines) for shorter stem length, and higher β-glucan, amylose and amylopectin content. Excluding the two entries with the longest stems (Deiniol and Deiniol x Propino bulk) the results suggested that there were several potentially useful short stemmed lines which could be further introgressed with semi-dwarf cultivars to produce high yielding and high β-glucan naked barley lines.

As well as genetics, cultural practices such as seeding rate, seed drilling depth and nitrogen fertilization should be taken into account when developing the most appropriate varieties for end users.

Chapter 5 considers heritability and plant genetics. Narrow sense heritability and broad sense heritability were both 1.0 for β-glucan content. This suggests that plant breeders' efforts should be focused on improving nutritional traits rather than merely environmental resilience.

6.2 Can genetic selection improve bioactive components in naked barley genotypes?

6.2.1 B-glucan content (%)

Chapter 5 concluded that line 31_2 was the most favourable of the UK-adapted lines developed, since it had the highest β-glucan content (Figure 5.17).

6.2.2 Amylose content (%)

Negative correlations were reported between amylose and β-glucan ($R = -0.62$, $P < 0.01$) and between amylopectin and β-glucan ($R = -0.487$, $P < 0.01$) (Shu and Rasmussen, 2014). Results presented in chapter 5 show that Deiniol had the highest amylose content compared to the progenies of Deiniol x Riso 13 and line 10_2 had the lowest amylose content (Figure 5.16).

6.2.3 Starch

Our findings of high β-glucan naked barley lines and their low amylose content lines are in agreement with (Sagnelli et al., 2016) who found that the genetic mutants with modified starches provide numerous potential applications for the food and other industries. Our results

are also in agreement with (Patron et al., 2005) who found that the starch content was reduced in lys5 mutations by up to 70 % . Furthermore our results are in agreement with (Johnson et al., 2003) who found that barley mutants such as Riso 13 and Riso 16 synthesize less starch than normal (Howard et al., 2014), their endosperm is shrunk and their grain weight is low (Patron et al., 2005).

Hybridization between UK hulled barley and naked barley lines represent an important tool for varieties release and adaptability enhancement. Previous studies targeted limited naked barley germplasm for UK adaptation. The study described in this thesis aimed to broaden the collection under investigation and consider a number of agronomic and food quality aspects. The significant collection included in this study confirms the importance of this germplasm resource (greater genotype effect) in relation to traits such as β-glucan, amylose and amylopectin content.

Food barley products with high β-glucan, amylose and amylopectin content and diverse viscosity could be directly developed out of accessions investigated. The evaluation of large numbers of complex traits is required for commercial production. These traits are time and money consuming therefore studying indirectly many traits through correlation could reduce the cost and avoid germplasm discard at early generation breeding. The association of advanced throughput genotyping platforms and the phenotyped data produced in this study will enable successful association of genetics approaches to be used for improvement and utilisation of gene pools in the future.

6.3 Recommendations for future breeding of naked barley for UK agriculture

Based on the present study, we recommend the following:

- Conducting a field experiment to explore the effect of different PGR doses on yield and yield components of naked barley.
- Studying the field performance of the highest β-glucan content lines.

- Crossing selected MA 1 line with the 31_2 high β-glucan content barely with the aim of developing a high β-glucan breeding line with higher yield and harvest index.
- Developing a high β-glucan barley line with low phosphorus content by crossing one of our high β-glucan content lines with low phosphorus content barley.
- Studying the effect of different dwarfing genes on naked barley yield, yield components and nutrition under UK growing conditions.

7. References

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8 Appendices

1.1 Appendix

1.1.1 Late PGR application trial (2016 field experiment) Teats of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
EPSM	.102	24	.200*	.977	24	.845
Straw dry weight (g)	.193	24	.021	.914	24	.043
Grain yield (g)	.168	24	.080	.959	24	.413
Hi	.314	24	.000	.798	24	.000
TGW	.160	24	.113	.908	24	.032
Stl	.195	24	.019	.853	24	.002
Lodging index	.254	24	.000	.749	24	.000
SWCM	.135	24	.200*	.946	24	.223
Stem diameter	.139	24	.200*	.975	24	.782
Total EPSM	.153	24	.155	.964	24	.520

1.1.2 Late PGR application trial Frequencies

	EPSM	Straw dry weight	Grain yield (g)	Hi	TGW	Stem length	Inter-node length
N	24	24	24	24	24	24	24
Mean	422.5	559.4	442.4	0.46	49.27	492.04	16.35
Std. Error of Mean	19.27199	19.08948	25.5	0.02	0.49	28.23	0.29
Variance	8913.826	8745.799	15593.4	0.01	5.78	19925.2	1.97
Minimum	248	414.3	138	0.1	42.4	0	13.6

1.1.3 Late PGR application trial ANOVA table

		Sum of Squares	df	Mean Square	F	Sig.
EPSM * Treatment	Between Groups	19262	5	3852.4	0.373	0.86
	Within Groups	185756	18	10319.78		
	Total	205018	23			
Straw dry weight * Treatment	Between Groups	51031.92	5	10206.38	1.224	0.338
	Within Groups	150121.46	18	8340.08		
	Total	201153.38	23			
Grain yield (g) * Treatment	Between Groups	50301.635	5	10060.327	0.587	0.71
	Within Groups	308347.688	18	17130.43		
	Total	358649.322	23			
Hi * Treatment	Between Groups	0.098	5	0.02	1.967	0.133
	Within Groups	0.18	18	0.01		
	Total	0.278	23			
TGW * Treatment	Between Groups	47.337	5	9.467	1.99	0.129
	Within Groups	85.633	18	4.757		
	Total	132.97	23			
Stl * Treatment	Between Groups	248132.5	6	41355.41	3.235	0.025
	Within Groups	230072.5	18	12781.81		
	Total	478205.0	24			
Internode length * Treatment	Between Groups	19.075	5	3.815	2.624	0.06
	Within Groups	26.165	18	1.454		
	Total	45.24	23			

1.2 Appendix

1.2.1 2015 MA lines

Beta glucan content Frequencies

Statistics

		BG
N	Valid	50
	Missing	0
Mean		4.73
Std. Error of Mean		.223
Variance		2.493
Minimum		2
Maximum		9

1.2.2 2017 field trial Evaluation of selected crosses under field conditions

1.2.2.1Tests of Normality

	Treatment	Shapiro-Wilk		
		Statistic	df	Sig.
Stem length	Deiniol	0.981	3	0.736
	MA1	0.921	3	0.455
	MA14	0.832	3	0.194
	MA19	0.75	3	0
	MA20	0.832	3	0.194
	MA3	1	3	0.981
Straw dry weight (g)	Deiniol	0.812	3	0.143
	MA1	0.967	3	0.65
	MA14	0.999	3	0.952
	MA19	0.769	3	0.042
	MA20	0.984	3	0.76
	MA3	0.842	3	0.218
Ears dry weight (g)	Deiniol	0.87	3	0.296
	MA1	0.788	3	0.085
	MA14	0.992	3	0.825
	MA19	0.979	3	0.72
	MA20	1	3	0.988
	MA3	0.844	3	0.226

EPSM	Deiniol	0.896	3	0.372
	MA1	0.809	3	0.136
	MA14	0.928	3	0.481
	MA19	0.909	3	0.414
	MA20	0.99	3	0.808
	MA3	0.936	3	0.512

2017 field trial Evaluation of selected crosses under field conditions (Normality tests - continued)

Yield (g)	Deiniol	0.984	3	0.755
	MA1	0.911	3	0.423
	MA14	0.958	3	0.604
	MA19	0.995	3	0.865
	MA20	0.915	3	0.434
	MA3	0.955	3	0.591
HI	Deiniol	0.936	3	0.51
	MA1	0.942	3	0.537
	MA14	0.976	3	0.702
	MA19	0.848	3	0.235
	MA20	0.964	3	0.637
	MA3	0.942	3	0.537
Chaff weight	Deiniol	0.934	3	0.503
	MA1	0.825	3	0.175
	MA14	0.776	3	0.059
	MA19	0.772	3	0.05
	MA20	0.997	3	0.891
	MA3	0.981	3	0.735
TGW	Deiniol	0.996	3	0.878
	MA1	0.861	3	0.271
	MA14	0.953	3	0.583
	MA19	0.998	3	0.925
	MA20	0.982	3	0.742
	MA3	0.93	3	0.49
GPE	Deiniol	0.999	3	0.933
	MA1	0.803	3	0.122
	MA14	0.99	3	0.811
	MA19	1	3	0.988
	MA20	0.947	3	0.554

2017 field trial Evaluation of selected crosses under field conditions (Normality tests - continued)

	MA3	0.284	3	.	0.934	3	0.503
Amylose content	Deiniol	0.263	3	.	0.955	3	0.593
	MA1	0.247	3	.	0.969	3	0.663
	MA14	0.229	3	.	0.982	3	0.74
	MA19	0.327	3	.	0.872	3	0.302
	MA20	0.191	3	.	0.997	3	0.9
	MA3	0.177	3	.	1	3	0.964
B–glucan content	Deiniol	0.31	3	.	0.899	3	0.382
	MA1	0.26	3	.	0.959	3	0.609
	MA14	0.235	3	.	0.978	3	0.716
	MA19	0.208	3	.	0.992	3	0.826
	MA20	0.307	3	.	0.903	3	0.394
	MA3	0.385	3	.	0.75	3	0

1.2.2.2 2017 field trial Evaluation of selected crosses under field conditions Frequencies

Statistics

		Stem length	Straw dry weight (g)	EPSM	Yield (g)	HI	Chaff weight(g)
N	Valid	18	18	18	18	18	18
Mean		61.7	407.9	519.9	461.6	0.55	164.2
Std. Error of Mean		1.62	18.8	16.85	21.8	0.02	22.8
Variance		47.2	6382.2	5114.9	8571.2	0.007	9395.1
Maximum		77.8	521	610	595.8	0.77	332.1

1.2.2.2 2017 field trial Evaluation of selected crosses under field conditions Frequencies (continued)

Statistics

		TGW	GPE	Amylose content	BG
N	Valid	18	18	18	18
Mean		42.7	20.7	62.1	4.20
Std. Error of Mean		0.84	0.52	1.27	0.15
Variance		12.7	4.86	28.9	0.41
Maximum		48.5	25.2	71.6	5.54

1.2.2.3 2017 field trial Evaluation of selected crosses under field conditions ANOVA table

			Sum of Squares	df	Mean Square	F	Sig.
Stem length * Treatment	Between Groups	(Combined)	564.96	5	112.992	5.726	0.006
	Within Groups		236.8	12	19.733		
	Total		801.76	17			
Straw dry wt. * Treatment	Between Groups	(Combined)	65498.08	5	13099.62	3.656	0.031
	Within Groups		42999.78	12	3583.315		
	Total		108497.9	17			
Ears dry weight * Treatment	Between Groups	(Combined)	86185.18	5	17237.04	3.496	0.035
	Within Groups		59170.4	12	4930.867		
	Total		145355.6	17			
EPSM * Treatment	Between Groups	(Combined)	17014.28	5	3402.856	0.584	0.712
	Within Groups		69938.67	12	5828.222		
	Total		86952.94	17			
Yield * Treatment	Between Groups	(Combined)	79381.4	5	15876.28	2.872	0.062
	Within Groups		66330.51	12	5527.543		
	Total		145711.9	17			
HI * Treatment	Between Groups	(Combined)	0.032	5	0.006	0.988	0.464
	Within Groups		0.079	12	0.007		
	Total		0.111	17			

2017 field trial Evaluation of selected crosses under field conditions ANOVA table (Continued)

			Sum of Squares	Df	Mean Square	F	Sig.
Chaff weight (g) * Treatment	Between Groups	(Combined)	68425.56	5	13685.11	1.799	0.188
	Within Groups		91291.79	12	7607.649		
	Total		159717.3	17			
TGW * Treatment	Between Groups	(Combined)	121.312	5	24.262	3.064	0.052
	Within Groups		95.013	12	7.918		
	Total		216.325	17			
GPE * Treatment	Between Groups	(Combined)	29.544	5	5.909	1.336	0.314
	Within Groups		53.07	12	4.422		
	Total		82.614	17			
Amylose content * Treatment	Between Groups	(Combined)	267.716	5	53.543	2.869	0.063
	Within Groups		223.94	12	18.662		
	Total		491.656	17			
BG * Treatment	Between Groups	(Combined)	6.45	5	1.29	33.725	0
	Within Groups		0.459	12	0.038		
	Total		6.909	17			

1.3 Appendix

1.3.1 Deiniol X Propino Frequencies

		Total grain	Number of fertile ears	Number of grains	TGW	Stem length cm	GPE
N	Valid	138	138	138	138	138	138
	Missing	0	0	0	0	0	0
Std. Error of Mean		.145	.164	2.70	1.86	.684	.316
Variance		2.912	3.75	1003.80	475.25	64.53	13.79
Minimum		.50	1.00	3.00	4.80	32.00	3.00
Maximum		7.90	11.00	167.00	200.00	78.00	21.60

1.3.2 Deiniol X Propino ANOVA table

			Sum of Squares	df	Mean Square	F	Sig.
Total grain * ID	Between Groups	(Combined)	221.414	22	10.064	6.490	.000
	Within Groups		178.330	115	1.551		
	Total		399.744	137			
Number of ears * ID	Between Groups	(Combined)	260.275	22	11.831	5.378	.000
	Within Groups		253.000	115	2.200		
	Total		513.275	137			
Number of grains * ID	Between Groups	(Combined)	65791.493	22	2990.522	4.795	.000
	Within Groups		71729.500	115	623.735		
	Total		137520.993	137			
TGW * ID	Between Groups	(Combined)	14675.209	22	667.055	1.521	.080
	Within Groups		50434.545	115	438.561		
	Total		65109.754	137			
Stem length * ID	Between Groups	(Combined)	2771.957	22	125.998	2.388	.002
	Within Groups		6068.167	115	52.767		
	Total		8840.123	137			
GPE * ID	Between Groups	(Combined)	683.829	22	31.083	2.965	.000
	Within Groups		1205.606	115	10.484		
	Total		1889.435	137			

1.4 Appendix

1.4.1 Deiniol X Riso 13 Phenotypic variations in F_3 Frequencies

		Stem length	Number of fertile spikes	Yield	TGW
N	Valid	114	114	114	114
	Missing	0	0	0	0
Mean		59.21	5.35	1.63	35.92
Std. Error of Mean		.745	.277	.153	1.923
Variance		63.243	8.725	2.681	423.23
Minimum		39.00	1.00	.09	13.10
Maximum		79.00	17.00	7.65	112.50

1.4.2 Deiniol X Riso 13 Phenotypic variations in F_3 ANOVA table

			Sum of Squares	df	Mean Square	F	Sig.
Stem length * ID	Between Groups	(Combined)	5583.45	37	150.904	7.338	0
	Within Groups		1563	76	20.566		
	Total		7146.45	113			
Number of fertile spikes * ID	Between Groups	(Combined)	747.298	37	20.197	6.432	0
	Within Groups		238.667	76	3.14		
	Total		985.965	113			
Yield * ID	Between Groups	(Combined)	262.731	37	7.101	13.413	0
	Within Groups		40.235	76	0.529		
	Total		302.966	113			
TGW * ID	Between Groups	(Combined)	42475.5	37	1147.99	16.31	0
	Within Groups		5349.19	76	70.384		
	Total		47824.7	113			

1.4.3 Deiniol X Riso 13 β–glucan and amylose content Frequencies

		Amylose	BG
N	Valid	36	28
	Missing	0	0
Std. Error of Mean		1.87	0.71
Variance		125.90	14.03
Minimum		16	3.16
Maximum		64	14.88

1.4.4 Deiniol X Riso 13 β–glucan and amylose content ANOVA table

			Sum of Squares	df	Mean Square	F	Sig.
BG * ID	Between Groups	(Combined)	351.600	11	31.964	220.502	.000
	Within Groups		1.740	12	.145		
	Total		353.340	23			
Amylose * ID	Between Groups	(Combined)	4652.555	13	357.889	5.648	.001
	Within Groups		887.187	14	63.371		
	Total		5539.742	27			