BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Achieving sustainable cultivation of barley

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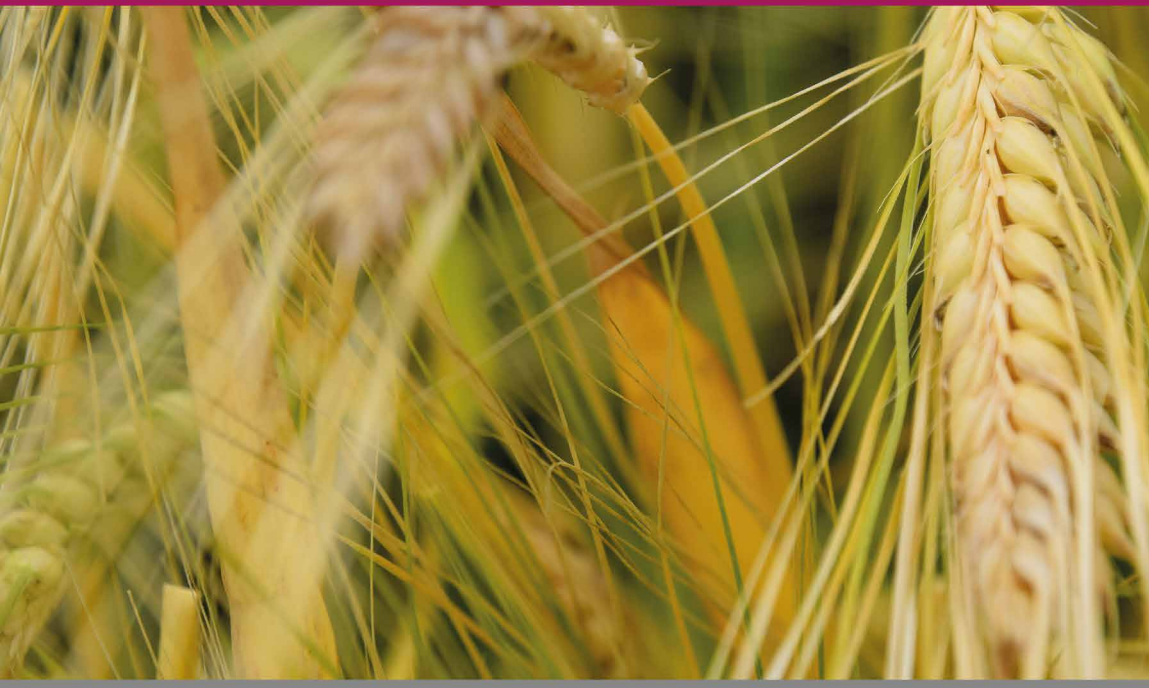

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Contents

Part 4 Quality

Introduction

This collection reviews advances in research on improving barley cultivation across the value chain. Part 1 reviews advances in understanding barley physiology in such areas as plant growth, grain development and plant response to abiotic stress. Chapters also review current developments in exploiting genetic diversity and mapping the barley genome. Building on this foundation, the second part of the book summarizes advances in breeding with chapters on breeding trial design as well as advances in molecular breeding techniques such as genome wide association studies (GWAS) and targeted induced lesions in genomes (TILLING). Part 3 looks further along the value chain at ways of optimizing cultivation practices. There are chapters on post-harvest storage as well as fungal diseases, weeds and integrated methods for their management. The final part of the book assesses current developments in optimising barley for particular end uses such as malting, brewing and animal feed as well as current research on the nutraceutical properties of barley.

Part 1 Plant physiology and genetics

Chapter 1 summarizes recent advances in understanding the genetics of barley development and architecture. In particular it discusses developments in understanding barley plant structure and morphology; molecular control of vegetative development; and molecular control of reproductive development. Finally, the chapter looks at the implications of these developments for breeding more resilient and productive varieties.

The next chapter addresses the importance of cold acclimation as a coordinated metabolic rearrangement leading to frost tolerance, before going on to consider new methodologies for understanding barley's resilience to drought. The chapter considers barley's adaptation to soil salinity, its resistance to low nitrogen, and the importance of environmental adaptation as a key target for future breeding improvement. The chapter concludes by looking ahead to future research trends in this area and gives detailed suggestions on further reading.

Chapter 3 highlights the progress in our understanding of barley grain, its functional architecture, and energy metabolism shaped by the constraints of internal hypoxia. The chapter provides a current view on the relevance of programmed cell death for grain development, and mechanisms regulating sugar intake. Finally, the chapter discusses the outcome of multiscale metabolic modelling studies and how they have advanced our understanding of grain

physiology. This provides an insider's view on the life of the developing barley grain and raises new questions, which remain to be answered by applying the most advanced approaches, including nuclear magnetic resonance imaging.

Following that, Chapter 4 reviews genetic diversity in barley and its role in improving varieties, including adaptation to abiotic stresses. Sulphur is an essential macronutrient required in plants for normal growth and development. Its deficiency in agricultural soils reduces grain yield and grain quality traits. Studies conducted with barley and wheat varieties demonstrate substantial variations among crops and varieties in their response to application of different levels of sulphur. The chapter looks at factors affecting sulphur nutrition in barley and the potential role of genetic differences in breeding more resilient varieties.

Finally, Chapter 5 discusses recent changes in the field of genetic mapping that allow for new possibilities of mapping barley genomes. The chapter evaluates ways in which these strategies can be used to efficiently breed for traits that will improve the resistance of barley to various stresses as well as to meet the requirements of its several uses. This section includes a case study of the shift from rym4 to rym11. The chapter concludes by looking ahead to future research trends in this area and suggests further reading on the topic.

Part 2 Advances in breeding

Part 2 begins with a review of key developments in experimental design in barley breeding. After a brief history to set the scene, Chapter 6 covers the background of experimental design for field trials, highlighting the key principles that are still fundamental for modern comparative experiments, including model-based design. The following section explores the quantification of genetic relationships through either pedigree or molecular marker information. Finally, the chapter presents the principles of multi-phase experiments for testing material both in the field and in the laboratory. Three case studies are included to highlight non-standard experimental designs that should be in the toolkit of every agricultural scientist and which are essential for modern plant breeding programs.

Chapter 7 begins by summarizing progress in barley breeding and how the advent of molecular markers has seen development in genome mapping with relation to malting quality and yield traits. This chapter shows how genome wide association studies (GWAS) have highlighted contrasts between different breeding germplasm groups, revealing where crossing between groups can produce greater advances than continuing to cross within. GWAS can also be used as training populations for genomic selection but will remain a key R&D technology as it provides a route to candidate gene identification and hence to suitable sources of genetic diversity to maintain breeding progress. Integration

of multi-environment GWAS with climatic variables is essential to breed for adaptation to climate change.

Chapter 8 introduces artificial mutagenesis and mutation discovery in TILLING, following a review of advances in TILLING resources in barley. The chapter evaluates the efficiency and TILLING versus other reverse genetics tools in barley. It also assesses the range of TILLING applications in barley. It gives special emphasis to developments in molecular screening approaches, and on opportunities for using TILLING in barley breeding.

Part 3 Cultivation techniques, pest and disease management

Part 3 opens with Chapter 9 which focuses on post-harvest storage and handling practices of barley grain and how these methods can be used to mitigate mycotoxin contamination. It also discusses management of insect pests in stored barley. The chapter goes on to review the various mycotoxins and fungi that are associated with barley, followed by the various post-storage treatments of feed and malting barley. It concludes by summarising how post-harvest storage is an important component in the sustainable production of barley and highlights potential areas for future research.

Chapter 10 reviews current research on the main fungal diseases affecting barley. It first reviews what we know about the mechanisms of barley genetic resistance to fungal pathogens. The chapter then focuses on the description of major fungal pathogens effecting barley production, new insights into their mechanisms of virulence and implications for achieving sustainable resistance to these important pathogens. The chapter reviews current knowledge about biotrophic foliar diseases: stem rust, leaf rust, stripe rust and powdery mildew. It then discusses necrotrophic diseases: spot blotch, net blotch, ramularia leaf spot, septoria speckled leaf blotch, scald and fusarium head blight. The chapter finally discusses barley stripe.

The following chapter, Chapter 11, looks at how integrated pest management (IPM) can be applied to barley production, considering the different disease threats, the tools available to counteract them and possible approaches to deploying them. The chapter evaluates varietal disease resistance, the range of crop protectants available and how agronomy can be used optimise protection. The chapter also discusses barriers to IPM use in practice. Finally, the chapter looks ahead to future research trends in this area. Chapter 12 examines the problem of weeds in barley and explains the application of integrated weed management (IWM) in barley cultivation. The chapter outlines weed control tactics and the practical implementation of IWM, focussing on specific examples of IWM in barley. Finally, the chapter provides detailed further reading on this issue.

Part 4 Quality

Chapter 13 introduces current challenges for improving malting barley. It goes on to review typical traits of malting quality, such as grain size, protein and germination. It also highlights the importance of malt extract obtained after the malting process, and reviews other important traits such as starch degrading enzymes, malt colour, grain hardness and other traits that are not routinely measured. A case study on modern varieties for twenty-first century brewing is also included. The chapter concludes by discussing requirements for successful programs in malting quality improvement and potential future trends in research.

Chapter 14 follows the application of barley throughout the brewing process. The chapter describes the different traits relevant during mashing, such as starch quality and heat stability of starch degrading enzymes; as well as traits during wort boiling, filtration, maturation as well as in the final product, putting special emphasis on barley-derived off-flavours. The chapter discusses breeding strategies to improve brewing quality. Finally, the chapter looks ahead to future research trends in this area.

Chapter 15 discusses the use of barley as feed for a range of livestock. The chapter reviews ways of optimising the use of barley for animal feed, from production and breeding through to the application of new technologies such as near infrared spectroscopy and molecular markers. The chapter then examines the specific grain quality and nutritional requirements of the major animal species routinely fed barley-based diets. The chapter concludes by assessing future research trends in optimising the use of feed barley.

The final chapter, Chapter 16, reviews the known and potential bioactive compounds in barley. Whole grain barley has been widely recognized as a valuable source of a number of biologically active compounds with unique health benefits. The great number of bioactive nutrients make barley an ideal raw material for the development of functional foods. This chapter discusses key issues and challenges currently faced by barley growers and manufacturers in producing high-quality products with health-promoting properties. It also reviews the known and potential bioactive compounds in barley, as well as research that has been carried out on barley and its health benefits. It concludes by discussing research that examines potential influences of barley bioactivity as well as future trends in research.

Part 1

Plant physiology and genetics

Chapter 1

Advances in understanding of barley plant physiology: plant development and architecture

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1 Introduction

Crop plant improvement for food security in the face of population growth and climate change remains a key challenge for breeders. The intensive crop production achieved in recent years, which relies heavily on fertilizers, insecticides and fungicides, is not sustainable and is not a viable strategy for the future. Future crops need to be more resource-efficient and also able to adapt to their environment in a better way. Plant adaptation to environmental conditions can be enhanced by manipulating the architecture of agronomic traits with a consequent increase in grain yield.

The barley genome is large and complex but, at the same time, it offers a large reservoir of genes that can be exploited by breeders to develop new varieties with increased grain yield. The availability of barley mutants and the advances in genomics have led to an increased knowledge of the genetic factors controlling plant architecture that can be exploited by breeders. Epigenetics can also play an important role in generating further information that can be related to phenotypic response in adaptation to climate change. Ideotype breeding, proposed by Donald in 1968, is an alternative breeding strategy that aims at designing crops with optimal adaptation to target environments by combining a set of predefined traits. The recent advances in genetics and genomics could facilitate the obtainment of such ideotypes.

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The on-going revolution in genomics also needs to be complemented by high-throughput phenotypic approaches to increase the rate of identification of trait-gene associations. Combining genetic knowledge of plant architecture with information about developmental processes is crucial for breeding varieties with increased adaptation and resilience to climate change, which are able to deliver competitive yields with lower inputs.

This chapter summarizes recent advances in understanding the genetics of barley development and architecture. In particular it discusses developments in understanding: (i) barley plant structure and morphology, (ii) molecular control of vegetative development and (iii) molecular control of reproductive development. Finally, the chapter looks at the implications of these developments for breeding more resilient varieties.

2 Barley plant structure/morphology and growth habit

The barley embryo is located dorsally near the basal mark of the seed. It is composed of four parts: scutellum, radicle, epicotyl and a nodal region between the epicotyl and radicle (MacLeod and Palmer 1966; Rossini et al. 2018). Vegetative development starts a few days after germination with the formation of the radicle from the apical-basal axis. The roots are then originated from the radicle. The epicotyl also starts to grow from the axis and initiates the shoot (Shaaf et al. 2019). The barley embryo contains shoot and root apical meristems (SAM and RAM, respectively). The SAM and the leaf primordia enclosed by the coleoptile are part of the epicotyl, while the RAM together with the radicle is part of the coleorhiza.

Both SAM and RAM are responsible for the architecture of the aerial and basal parts of the barley plant. SAM controls the development of the above-ground structures including the nodes, internodes, leaves, axillary meristems and the inflorescence (Sussex 1989; Babb and Muehlbauer 2003). The SAM structure has been described by Doring et al. (1999) as a system composed of two main and clonally distinct layers: L1 (*tunica*) and L2 (*corpus*). Its activity starts with the development of three or four-leaf primordia during embryogenesis. As reviewed by Rossini et al. (2014), lateral buds are located in the axil of the coleoptile and in the axil of the first leaf. Shoot architecture consists of units or modules called phytomers. The basic module of plant architecture, the phytomer, is made of an internode, a leaf and axillary bud (Sharman 1942). The basal region of the plant (also known as the crown) is composed of the first set of phytomers developed from the SAM. Their internodes do not elongate. In fact, internode elongation in barley occurs only after the transition from the vegetative to reproductive phase. The number of leaves produced, and consequently the number of basal internodes at the barley culm, depends on genetic and environmental factors. A part of those internodes will elongate after

the transition from the vegetative to reproductive phase (Kirby and Appleyard 1987).

Tiller development in barley occurs as a result of the proliferation of axillary meristems (AXMs) located between the stem and the leaf/coleoptile. As reported by Hussien et al. (2014), the AXM produce an axillary bud that may produce a lateral shoot with a structure similar to the main culm. The AXM produces a stem cell accumulation in the leaf axil. Afterward, cells differentiate into primordial leaves that finally result in axillary buds. Tillers will finally originate only from subsets of the axillary buds while the rest will remain at the bud stage. Tillers are usually classified as primary tillers if they are originated from the main stem and as secondary tillers when they are derived from the primary tillers (Hussien et al. 2014). Tillers production is reiterative and further tillers (tertiary tillers) can originate from secondary tillers. Tiller outgrowth is controlled by a tangled network of hormonal and regulatory signals that leads to a high morphological diversity between genotypes and within the same genotypes (Kebrom et al. 2012; Shaaf et al. 2019). In barley and wheat the number of stems produced depends on genetic and environmental factors and, in general, winter genotypes produce more tillers (Kirby and Appleyard 1987). The transition phase between the vegetative stage and the reproductive stage is also under the control of genetic, environmental and hormonal factors.

Stem elongation begins when aerial internodes start to grow at the beginning of the reproductive phase. Elongation starts from basal internodes located at the bottom of the plant and continues toward the top with the elongation of internodes located in a more apical position (Briggs 1978). The process is complete when the last internode (called a peduncle) completes its elongation. Usually the internodes of a mature barley plant are longer than those immediately below. The only exception in some cases is the peduncle (Briggs 1978). A meristematic zone located in the base of the internode ensures the restoration of a vertical stem orientation in the case of lodging. The meristem is capable of asymmetric growth and delayed lignification (Briggs 1978). The inner layer of the internode is composed of a cavity pith surrounded by the parenchyma that contains additional vascular bundles and by a ring of sclerenchyma. The outer part is composed of alternate vertical files and sclerenchyma fibers supporting vascular bundles surrounded by a silicified epidermis (Briggs 1978).

Leaves originate from a ring of founder cells recruited on the SAM flank (Bossinger et al. 1992; Shaaf et al. 2019). The leaf primordium is located in the insertion disk at the node (Sharman 1942). A barley leaf has a strap-like appearance and is divided into a distal blade and proximal sheath that wraps around the culm and supports the blade (Rossini et al. 2018). The blade is the major photosynthetic organ of the plant. The ligular region, composed of a ligule and two auricles, separates the blade from the sheath (Rossini et al.

2018). Cell division ensures the differentiation and the growth of the leaf. The expansion pattern occurs in a basipetal wave, from the tip to the base, with the sheath cells still dividing when the blade cells are fully differentiated (Sylvester et al. 1990; Kołodziejek et al. 2006). Cell division undergoes both longitudinal and transverse divisions to support leaf growth in width and length (Sylvester and Smith 2009). The final leaf size (an important factor for photosynthesis efficiency) and leaf shape depends on the spatial and temporal coordination of these processes (Shaaf et al. 2019). Leaf orientation and angle also plays an important role in photosynthesis efficiency and is determined by the lamina joint that connects the blade to the sheath (Shaaf et al. 2019).

Inflorescence development starts with the transition of the SAM to an inflorescence meristem through the so-called double ridge phase. During this phase the apex is elongated about 1 mm. Each inflorescence primordium is composed of a leaf primordium and a lateral meristem (Kirby and Appleyard 1987). The lateral meristem then becomes the main growing point from where three spikelet triplet meristem (one central and two laterals) will originate, while the leaf primordium will not develop further (Bossinger et al. 1992; Rossini et al. 2014). The three-spikelet meristem stage is also called the triple-mound stage where each spikelet meristem will evolve into a floral meristem. Afterward, two outer glumes primordia originate from the floral meristems to give rise to the floral organ primordia. The final step of floral development is the sequential differentiation of glume, lemma and stamen primordium in the mature spikelet (Rossini et al. 2014). A spikelet located in the central region of inflorescence axis will develop earlier than basal and apical spikelets. The final number of spikelets is defined by the apex that continues to initiate new spikelet meristems until the awn primordium stage. At this stage the spike layout and spikelet structure are completed (Kirby and Appleyard 1987). The fully developed barley spike is composed of floral units (spikelets) located on the floral stem. Each spikelet consists of a floret and two subtending bracts called the outer glume. The spikelets are usually organized in triplets at each rachis node.

The spikelet axis also bears the so-called lemma, an abaxial floral bract that encloses single florets and carries the awn. Several authors have reported that this complex can be considered to be a reduced vegetative leaf where the lemma corresponds to the leaf blade while the awn is considered as the sheath (Dahlgren et al. 1985; Clifford 1988; Pozzi et al. 2000; Rossini et al. 2014). Spikelet fertility is different between two- and six-rowed barley varieties. In the first, each triplet contains only one fertile spikelet while, in the second, all three are fertile. Lateral spikelets develop slower than the central spikelet and this may result in the development of very rudimentary and sterile structures that represent the typical two-rowed barley structure. The six-rowed barley spike structure is the result of a recessive mutation at the Vrs1 gene locus. Vrs1 locus promote the development of the lateral spikelets that become morphologically

indistinguishable from the central spikelet, resulting in the typical six-rowed barley spike (Kirby and Appleyard 1987; Komatsuda et al. 2007).

3 Molecular control of vegetative development

The pre-anthesis developmental phase in barley consists of the vegetative and early and late reproductive stages. All of these three phases impact grain yield. During the vegetative and early reproductive phases in particular, the number of tillers, plant height, biomass accumulation and leaf angle and area can have a direct impact on grain yield.

Tillering is one of the most important and critical traits for improving grain yield in temperate cereals (Sreenivasulu and Schnurbusch 2012; Jia et al. 2011). Cereals such as barley are able to increase grain yield through an increased number of tillers (Evers and Vos 2013). On the other hand, as reviewed by Shaaf et al. (2019), tillering potential needs to be carefully balanced to avoid a number of problems (Peng et al. 1994; Kennedy et al. 2017; Tripathi et al. 2003; Kuczynska et al. 2013; Mew 1991):

1 an excessive number of tillers will result in unfertile spikes;
2 unfertile tillers divert resources from developing spikes;
3 tillers can have negative effects on other traits related to biomass accumulation; and
4 a crowded canopy can foster the spread of disease.

Numerous mutants have been identified and characterized recently. Many of them have been used to develop near-isogenic (NI) lines in the cv. Bowman collection (Druka et al. 2011). These have been classified into:

1 mutants which fail to develop auxiliary buds (single culms mutants);
2 mutants with weak auxiliary bud development;
3 mutants with low tillering reduction; and
4 mutants producing high tiller number.

A comprehensive and more detailed review of available barley tillering mutants can be found in a recent publication from Shaaf et al. (2019). Tillering is considered as a complex trait that is strongly influenced by environmental and growing conditions such as light intensity and water availability and in which phytohormones also play a crucial role (Kebrom et al. 2013; Alqudah et al. 2016). As reviewed by several authors, phytohormones regulate bud growth through a very complex pathway in which different phytohormones interact (Evers and Vos 2013; Kebrom et al. 2013). Recent evidence suggests that

sugars might also be involved in the branching process through the regulation of phytohormonal gene expression (Barbier et al. 2015).

Several QTL and GWAS studies in the past decade have suggested a correlation between tillering and other important regulatory traits such as vernalization and photoperiod sensitivity, flowering time and plant height (Abeledo et al. 2004; Borràs et al. 2009: Alqudah and Schnurbusch 2013; Alqudah et al. 2016). Several authors reported that Vrn-H1, Vrn-H2 and Ppd-H1 have a significant effect on tiller production. In particular, it was reported that tiller number increased in genotypes with a strong vernalization requirement and reduced photoperiod sensitivity (Karsai et al. 1999; von Korff et al. 2006; Wang et al. 2010). On the basis of these studies, it was suggested that those three genes may regulate tillering indirectly by controlling flowering time (Corbesier et al. 2007; Tamaki et al. 2007).

The Vrs1 and Int-C genes are responsible for spike morphology and are also related to tillering. Vrs1 is a major gene controlling the row type of barley spike. Vrs1 encodes for the two-row spike while mutations in the gene result in the six-row spike. Alqudah and Schnurbusch (2014) found significant differences in tiller number associated with row number under different growing conditions. Furthermore pleiotropic effects on tiller number are associated with allelic status at the Vrs1 locus. Int-C is the barley homolog of maize TB1 (*TEOSINTE BRANCHED 1*). It inhibits bud outgrowth by regulating the GT1 gene (*GRASSY TILLERS* 1; Alqudah et al. 2016). Int-C has been identified as a major gene-controlling lateral spikelet development that also represses the number of tillers in barley early in development (Ramsay et al. 2011).

A more recent study revealed that there are five major row-type loci (Vrs1, Vrs2, Vrs3, Vrs4 and Vrs5) that can convert spikes from the two-rowed to six-rowed type (Zwirek et al. 2019). The recessive alleles at vrs4 and vrs5 also affect tillering. Genotypes carrying Vrs1 and paired vrs3, vrs4 or vrs5 recessive alleles showed increased spikelet fertlity and variation in tillers numbers. In the case of vrs3 in vrs4 background, Zwirek et al. (2019) reported loss of spikelet identity and determinacy, improved grain homogeneity and increased tillering, while vrs5 in the same background was reported to be associated to decreased tiller number and increased grain weight.

A further study by Alqudah et al. (2016) investigated QTL underlying natural variation in number of tillers per plant at different pre-anthesis stages, and differences in plant height at harvest based on differences in row type and photoperiod response in a spring barley collection. This work clearly demonstrated a link between tillering, heading date and plant height. The study from Alqudah et al. (2016) revealed that the major loci controlling tillering were Vrs1 and Ppd-H1. Accessions carrying the Ppd-H1 allele showed several QTL associated with an increased number of productive tillers. Two-rowed accessions showed a more complex genetic make-up of tillering than six-rowed

accessions. The Vrs1 gene was seen to have a pleiotropic effect in increasing the number of tillers, probably as compensation for the reduced number of seed produced. Experimental evidence suggests that the Vrs1 gene also controls plant height. Accessions carrying this allele were taller than accession carrying the Vrs1 allele. The QTL detected for plant height seems to colocate with genes involved with flowering time regulators and sugar-related genes. Interestingly, QTL for tillering and plant height are co-localized in genomic regions harboring plant stature-related phytohormones and sugar-related genes.

Brassinosteroids, gibberellins and stringolactones are the three phytohormones that play a key role in determining plant height. Dwarf and semi-dwarf phenotypes can be the results of lower production or insensitivity to those phytohormones, which usually arise from disorders in their biosynthesis or signaling pathways (Marzec and Alqudah 2018). Semi-dwarf varieties were one of the key factors in the success of the Green Revolution. Shorter plants usually have increased stem sturdiness, lodging resistance, improved response to fertilizers and enhanced grain yield. These semi-dwarf varieties were achieved using mutations in gibberellin pathway genes and metabolism (Hedden 2003). Semi-dwarf wheat and rice varieties introduced during the Green Revolution have dramatically increased yield due to the repartitioning of assimilate from stems to grain production (Khush 2001).

Since the beginning of Green Revolution, reduced plant height has always been a priority for breeders, especially to increase lodging resistance. For this reason numerous loci involved in plant height have been identified through QTL mapping and GWAS (Salvi et al. 2013; Rossini et al. 2018). A huge number of mutants have been identified and classified into different categories on the basis of their phenotypic changes, pleiotropic characteristics that often accompany short culm phenotypic changes, parental background such as brachytic (brh), breviaristatum (ari), dense spike (dsp), erectoides (ert), semibrachytic (uzu), semi-dwarf (sdw) or slender dwarf (sld) (Franckowiak and Lundqvist 2013; Dockter et al. 2014). More information about barley mutants can be found in recent reviews (Rossini et al. 2014; Dockter and Hansson 2015). These mentioned pleiotropic effects have limited the use of semi-dwarfing genes in breeding programs due to the negative effects on phenotypes such as low vigor and reduced yield (Rossini et al. 2018).

Uzu is a semi-dwarf gene that seems to be the ortholog of Arabidopsis and rice BRASSINOSTEROID-INSENSITIVE1 (BRI1), encoding a brassinosteroid receptor (Li and Chory 1997; Chono et al. 2003). It is one of the few genes shaping plant height that have been successfully used in breeding. Accessions carrying the uzu1.a allele show sturdy culm, lodging resistance and tolerance to dense planting due to leaf erectness. Uzu1.a expression is sensitive to temperature, exhibiting a mild phenotype at 14°C and a stronger phenotype at 26°C as reported by Dockter et al. (2014). This sensitivity to heat is a

limitation to the diffusion of this allele that is mainly restricted to East Asia. However recently new uzu alleles, less sensible to high temperature, have been identified and they could represent an alternative to uzu1.a (Dockter and Hansson 2015).

Two mutations that are more extensively used by breeders in Europe and North America and Australia include sdw1 and denso (Hellewell et al. 2000), both located on chromosome 3H and thought to be allelic (Hellewell et al. 2000; Jia et al. 2009). Beside mutants, many QTL and GWAS studies have identified loci and candidate genes controlling plant height, like the brassinosteroid biosynthesis gene DWARF4 (HvD4), that has not been described in other species, and HvCPD that encodes a protein involved in brassinosteroid biosynthesis (Alqudah et al. 2018; Dockter et al. 2014; Marzec and Alqudah 2018).

Leaf angle is regulated by phytohormones, among which brassinosteroids play a major role (Sakamoto et al. 2006a; Hartwig et al. 2011). Brassinosteroids have been reported to control many physiological processes like cell expansion, stomata development, photo-morphogenesis, plant height, grain size and stress response (as reviewed by Shaaf et al. 2019). The leaf angle is controlled at the lamina joint level by brassinosteroids. The phytohormone seems to promote cell proliferation on the adaxial side while it suppresses cell division on the abaxial side (Sun et al. 2015). Enlarged leaf angle is associated with increased brassinosteroid content or increased brassinosteroid signaling, while brassinosteroid-deficient mutants have erect leaves (Shaaf et al. 2019).

As mentioned before, the uzu1.a allele is associated with erect leaves, making genotypes carrying this allele suitable for high-density planting. Uzu1.a was the first gene cloned among the brassinosteroid mutants in barley. Gruszka et al. (2016) identified two semi-dwarf barley mutants for HvDWARF, the barley ortholog of rice OsDWARF. A mutation on the rice gene causes reduced plant height and erect leaves (Hong et al. 2002). Resequencing of the barley mutants showed that missense mutation in the coding sequence can potentially affect the conserved sequence of the protein. The mutants also showed a reduced transcription of HvBAk1, another component of the brassinosteroid signal pathway. In rice and Arabidopsis this is associated with changes in plant height, leaf erectness, grain morphological features and disease resistance (Li et al. 2009). HvDWARF4 has been identified as one of the genes from the brassinosteroid pathway controlling plant height in barley that could also play a role in controlling leaf angle as its rice ortholog OsDWARF4. Further work on its functional characterization may confirm the role of HvDWARF4 in determining leaf angle (Sakamoto et al. 2006b; Dockter et al. 2014).

Leaf area is also an important trait for breeding. The size and the shape of leaves and their position are closely related to photosynthesis, photosynthetic efficiency and with the amount of assimilates produced by plants (Jiang et al. 2015; Driever et al. 2014). The amount of assimilates produced by plants

generally means more fertile tillers, more spikes and increased spikelets survival with direct consequences on grain yield (Marzec and Alqudah 2018).

A recent GWAS analysis identified nine genes involved in the genetic control of leaf area in barley, all involved in different phytohormone pathways (gibberellins, brassinosteroids and stringolactones), suggesting that these phytohormones might regulate the leaf area independently from other traits such as branching (Marzec and Alqudah 2018; Alqudah et al. 2016). Results showed the effect of the Ppd-H1 gene, while the less photoperiod-sensitive allele (ppd-H1) was also associated with increased leaf areas when associated with the Ppd-H1 allele. Other associations with leaf area were found for other genes related to heading time and sugar-related genes (Alqudah et al. 2018). Molecular regulation of leaf area development needs to be further investigated in order to identify genes and use these genes in breeding programs.

4 Molecular control of reproductive development

Because of its relationship with grain yield and grain number, understanding the genetic control of barley inflorescence development is of primary importance in plant breeding. Dozens of barley mutants with altered spike and spikelet morphology have been described and mapped, providing an ideal starting point for the genetic analysis of inflorescence development (Druka et al. 2011; Franckowiak and Lundqvist 2010; Sreenivasulu and Schnurbusch 2012; Rossini et al. 2014). Most of the differences in spike morphology in grasses are reflected by the extent of inflorescence branching (Koppulu and Schnurbusch 2019). Several studies suggest that branched inflorescence is the basis of the evolution of most evolved/derived inflorescences (Vegetti and Anton 1995; Endress 2010; Kellogg et al. 2013).

Mutants with non-canonical spike branching have been identified in barley. Recessive mutations at the branched1 (brc1) and compositum (com1 and com2) loci cause the development of branches from rachis nodes in the basal portion of the spike (Franckowiak and Lundqvist 2010; Druka et al. 2011). Wild-type COM1 and COM2 genes determine spikelet meristem characteristics and branching (Franckowiak and Lundqvist 2013; Koppulu and Schnurbusch 2019). Paired or supernumerary spikelet formation in barley seems to be under the control of the flo locus. The underlying genetic mechanism is not known but was recently elucidated in wheat (Boden et al. 2015; Dixon et al. 2018). The loss of function of the photoperiod-insensitive Ppd-D1 allele attenuates FT1 expression, thus promoting paired spikelet formation (Boden et al. 2015). Interestingly, TB1 (that also regulate the expression of FT1) promotes paired spikelet formation by interacting directly with FT1. TB1 competitively binds FT1, making it less available and delaying meristem maturation (Dixon et al. 2018).

A characteristic feature of the barley spike is the triplet spikelet meristem. In wild and two-rowed barley the lateral spikelets are sterile. Each spike node develops one central fertile grain-bearing spikelet and two lateral sterile spikelets. The characteristic two-row shape comes from the position of the two fertile central spikelets that are located at the rachis. Six-rowed barley shows three fertile spikelets that form six rows of grains (Zohary and Hopf 2000). Lundqvist and Lundqvist (1988) identified 11 loci that can modify lateral spikelet fertility and that can independently convert two-rowed barley into a six-rowed barley. The genes are vrs1, vrs2, vrs3, vrs4 and Int-C. In natural six-rowed barley the predominant genotype is vrs1.a that promotes lateral spikelet fertility. Vrs1 also affects leaf primordium size and leaf area (Thirulogachandar et al. 2017). Different combinations of vrs1 and Int-c alleles are found in barley germplasm, leading to various levels of lateral spikelet development and fertility (Ramsay et al. 2011). For example, Int-c.a is the less functional allele of HvTB1, the barley orthologous of the maize teosinte branched gene. It facilitates the growth of lateral spikelets together with the vrs1.a allele and contributes to increased grain size (Ramsay et al. 2011). On the other hand, the recessive int-c.b allele is commonly found in two-rowed (Vrs1) cultivars where it inhibits anther development in lateral florets, while in six-rowed (vrs1) cultivars it results in reduced lateral spikelet development (Rossini et al. 2014). It has been found that in several six-rowed barley accessions the mutation in Vrs1 is absent and this accession also does not show alterations in Vrs1 expression (Komatsuda et al. 2007; Youssef et al. 2017b), suggesting that the suppression of lateral spikelet fertility might also be controlled by other genes.

It has also been reported that intermediate spike morphology is under the control of Vrs3. Genotypes carrying the Vrs3 mutant allele show a two-rowed condition in the lower third of their spike. The weaker six-rowed genotype may indicate a specific developmental regulation for Vrs3 across the spike (Koppulu and Schnurbusch 2019).

Rachis length and the number of spikelets per spike units (spike density) are generally correlated traits. In fact, longer rachis correlates positively with longer internodes and also negatively with spike density. The allelic variation that controls rachis development results in a range of phenotypes, often associated with alterations in other traits (Rossini et al. 2014). As reviewed by Terzi et al. (2017) several loci are involved in the modulation of spike density, such as dense spike, zeocriton, lax spike and laxatum.

The laxatum mutant phenotype is characterized by long rachis internode, a large base of lemma awns and five anthers instead of the regular three (Civáň and Brown 2017; Jost et al. 2016). The spike density seems to be under the control of several major genes, for example, dense spike genes (dsp) which control rachis internode length, resulting in dense or compact spikes. The Dsp.ar and Dsp1 gene seems to be located on the same position on chromosome

7H close to the lks2 gene that produces short awns (Shahinnia et al. 2012; Taketa et al. 2011). As reviewed by Terzi et al. (2017), the transcription factor HvAP2 (ortholog of APETALA2), which is driven by microRNA 172, regulates the length of the critical developmental window required for the elongation of the inflorescence internodes which has a critical effect on spike density.

The awn, the extension of the lemma, is a photosynthetic organ that plays an important role in grain size and yield and also shows a wide natural variation in length and shape (Terzi et al. 2017). Mutants can show this variation. A study conducted by Yuo et al. (2012) on the short awn 2 (lks2) gene demonstrated that this mutation affects the awn's cell proliferation, resulting awns 50% shorter than the wild type. Natural recessive variants of the lks2 gene are widespread in Eastern Asia, possibly offering adaptation to high-precipitation conditions (Rossini et al. 2014). The so-called hooded phenotypes are another example of awns morphology variation, where a mutation causes the appearance of an extra flower of inverse polarity on the lemma. The mutant phenotype is caused by a 305-base pair duplication in intron 4 at the single dominant genetic locus Knox3 (Müller et al. 1995). Hooded barley cultivars represent an interesting option for feed use as the presence of awns is unsafe for animals (Blake et al. 2011).

In covered barleys, the typical barley is used for both animal feed and malting. A lipid layer is present and favors the adhesion of the hull to the caryopsis surface. Hulless or naked barley results from a mutation on the nud gene that regulates the deposition of lipids on the epidermis of the pericarp. The nud gene is a transcription factor of the ethylene response factor (ERF) family regulating the lipid biosynthesis pathway (Terzi et al. 2017; Taketa et al. 2008). Naked barley is becoming popular in several countries as a health food due to its nutritional properties (i.e. b-glucans). Naked barley does not need to be pearled, thus reducing production costs while also preserving its nutraceutical content.

Barley is an autogamous plant and its stigmas become receptive before anther extrusion. When the anthers become ready for pollination, the stigmas are able to capture sufficient pollen. Another feature of barley florets is cleistogamy that ensures self-pollination through floret morphology. Florets show smaller lodicules in comparison with the non-cleistogamous types. In typical barley florets, palea and lemma remain tightly closed throughout the period of pollen release. Lodicule development is under the control of the Cly1 gene, an APETALA2 transcription factor (Nair et al. 2010). A mutation on the recessive allele of Cly1 gene leads to an increased expression that produces lodicule reduction. In genotypes carrying the Cly1 dominant allele, the downregulation of the Cly1 gene expression promotes the growth and the swelling of the lodicules, making the plant chasmogamous (Rossini et al. 2014).

5 Implications for breeding

Barley is generally known as an adaptable crop with the ability to cope with both abiotic and biotic stresses. However, even though it could be considered as a more resilient 'climate change' crop, it is still susceptible to major abiotic and biotic stresses. To ensure food security, breeders need to continue to develop new varieties that are capable of adapting to climate change and lower inputs. Current knowledge of plant architectural traits can be exploited to achieve improved genotypes.

In the case of rice, grain yield increase through the manipulation of tiller numbers was achieved by the International Rice Research Institute (IRRI; www.irri.org) by reducing the number of unproductive tillers (Peng et al. 2008). The Chinese 'super rice' initiative also achieved a significant grain yield increase by exploiting natural variation available in rice germplasm, combining optimal plant architecture and number of tillers (Qian et al. 2016; Wenfu et al. 2007). In barley the genetic factors controlling tillering plasticity are still only partially understood. However, it has been shown that vernalization and flowering time can be manipulated to increase grain yield by optimizing the number of fertile tillers (Shaaf et al. 2019). New insights on genes controlling tillering at the pre-anthesis developmental phase can be found in a GWAS study by Alqudah et al. (2016). This identified a QTL on chromosome 2H shown to be associated with the production of productive tillers in six-row genotypes. The QTL overlaps with the HvDRM1 gene in wheat. The expression of the DRM1-like gene is associated with tiller bud dormancy in a tiller inhibition mutant (Kebrom et al. 2012). Alqudah et al. (2016) identified three further QTL on chromosomes 1H, 2H and 5H associated with productive tiller production. The QTL on 2H is located near the HvBRD locus, an important regulator of plant height in barley. Once validated, these QTLs might be useful for breeders to genetically manipulate the number of tillers in barley to increase grain yield. The same study also identified novel QTL for plant height and suggested the role of Vrs1 in controlling plant height in addition to lateral spikelet development and tillering.

Alqudah et al. (2016) also reported a QTL for plant height located in a putatively sugar-related chromosomal region on chromosome 3H, suggesting that sugars may also be involved in plant height regulation. A few genes controlling plant height have been used in breeding such as uzu1.a, sdw and denso. New uzu alleles which are less sensitive to temperature have been identified which might help to overcome the limitations of uzua.1 (Dockter and Hansson 2015). Recent GWAS studies have demonstrated the effect of loci-involved brassinosteroid biosynthesis (i.e. HvD4 and HvCPD) in regulating plant height. The link between tillering and plant height needs to be further investigated for a better understanding of the genetic relationship between the two traits that may then lead to the identification of useful genes for breeders.

Table 1 Table of genes involved in barley plant development and architecture

Gene abbreviation	Chromosome	Gene product annotation	References
Vrs-3	1H	Putative jumonji C-type (JMJC) H3K9me2/3 histone demethylase	Bull et al. (2017), van Esse et al. (2017)
Ppd-H1	2H	PPR pseudo response regulator	Turner et al. (2005)
Vrs-1 (HvHox1)	2H	Homeodomain-leucine zipper class I protein (HD-ZIP I)	Komatsuda et al. (2007)
HvAP2	2H	APETALA2 transcription factor	Houston et al. (2013)
Cly1	2H	Cleistogamy 1 gene	Wang et al. (2015)
HvDRM1	2H	DORMANCY-ASSOCIATED1	Alqudah et al. (2016)
HvBRD	2h	BRASSINOSTEROID-DEFICIENT DWARF2	Dockter et al. (2014), Alqudah et al. (2016)
Vrs-4 (HvRA2)	3H	RAMOSA2	Koppolu et al. (2013)
uzu (HvBR1)	3H	Brassinosteroid insensitive	Chono et al. (2003), Dockter et al. (2014)
uzu1.a (HvBR1)	3H	Brassinosteroid insensitive	Chono et al. (2003), Dockter et al. (2014)
sdw1/denso	3H	Gibberellin 20-oxidase gene (HvGA20ox2)	Kuczynska et al. (2013), Xu et al. (2017)
Vrn-H2 (HvZCCT-Hb)	4H	ZCCT gene family member	von Ziztewiz et al. (2005)
Vrs-5 or Int-C (HvTB1)	4H	TEOSINTE BRANCHED1	Ramsay et al. (2011)
HvD4	4H	Brassinosteroid biosynthesis gene DWARF4	Dockter et al. (2014), Alqudah et al. (2018)
Vrn-H1 (HvBM5A)	5H	HvBM5A; MADS-box	von Ziztewiz et al. (2005)
Vrs-2	5H	Homolog of the Arabidopsis SHORT INTERNODES gene	Youssef et al. (2017a)
HvCPD	5H	BRASSINOSTEROID C-23 HYDROXYLASE	Dockter et al. (2014)
FT1	7H	FLOWERING LOCUS T	Faure et al. (2007)
Iks2	7H	*NA*	Shahinnia et al. (2012), Taketa et al. (2011)
Dsp1	7H	*NA*	Shahinnia et al. (2012), Taketa et al. (2011)
Dsp.ar	7H	*NA*	Shahinnia et al. (2012), Taketa et al. (2011)
Nud	7H	Ethylene response factor (ERF) family	Taketa et al. (2008)

Index