

The Biology of *Hordeum vulgare* L. (barley)



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This document provides an overview of baseline biological information relevant to risk assessment and risk management of genetically modified forms of the species that may be released into the Australian environment.

This document has been updated from Version 2 (April 2017).

For information on the Australian Government Office of the Gene Technology Regulator visit OGTR website.

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PREAMBLE

This document describes the biology of *Hordeum vulgare* L., with particular reference to the Australian environment, cultivation and use. Information included relates to the taxonomy and origins of cultivated *H. vulgare*, general descriptions of its morphology, reproductive biology, biochemistry, and biotic and abiotic interactions. This document also addresses the potential for gene transfer to occur to closely related species. The purpose of this document is to provide baseline information about the parent organism in risk analyses of genetically modified *H. vulgare* that may be released into the Australian environment.

H. vulgare is divided into two subspecies: *Hordeum vulgare* L. ssp. *spontaneum* and *H. vulgare* L. ssp. *vulgare* (C. Koch.) Thell. *H. vulgare* ssp. *spontaneum* is the wild progenitor of cultivated barley and will be referred to in this document as wild barley or by the subspecies name. *H. vulgare* ssp. *vulgare* is cultivated barley, including both six-row barley (var. *hexastichon*) and two-row barley (var. *distichon*). *H. vulgare* ssp. *vulgare* will be referred to as barley in this document.

Barley is one of the founder crops of Old World agriculture and was one of the first domesticated cereals. It is also a model experimental system because of its short life cycle and morphological, physiological, and genetic characteristics. Barley ranks fourth in world cereal crop production and is used for, in order of importance, animal feed, brewing malts and human food. Barley is a short season, early maturing grain found in widely varying environments globally. In Australia, barley is the second largest field crop (after wheat), and is grown in wheat production areas of all states.

SECTION 1 TAXONOMY

Barley belongs to the genus *Hordeum* in the tribe *Triticeae* of the grass family, *Poaceae* (also known as *Gramineae*). The *Triticeae* tribe contains about 350 species, including a number of economically important cereals and forages (von Bothmer et al., 1995; Blattner, 2018).

Hordeum species are annual or perennial, with the majority of species being perennial. Some species are primarily self-fertilising and other species are primarily outbreeders (von Bothmer et al., 1995).

There are 32 or 33 species within the *Hordeum* genus, all with a basic chromosome number of x=7. *Hordeum vulgare* L. is a diploid species with 2n=2x=14 chromosomes. Other *Hordeum* species are diploid, tetraploid (2n=4x=28) or hexaploid (2n=6x=42) (Komatsuda et al., 1999; Blattner, 2018).

The two species *H. vulgare* and *H. bulbosum* are considered to share a common basic genome, which is not shared by any other species in the genus. There are three other basic genomes in the *Hordeum* genus. Some polyploid *Hordeum* species contain two types of basic genome (Blattner, 2018).

SECTION 2 ORIGINS AND CULTIVATION

2.1 Centre of diversity and domestication

The genus *Hordeum* has centres of diversity in southwest Asia, central Asia, western North America and southern South America (von Bothmer et al., 1995). *Hordeum* species occur in a wide range of habitats. The majority of the wild perennial species grow in moist environments whereas the annual species are mostly restricted to open habitats and disturbed areas. Many species have adapted to extreme environments and tolerance to cold and saline conditions is common (von Bothmer, 1992).

Cultivated barley is grown in a range of diverse environments that vary from sub-Arctic to subtropical, with greater concentration in temperate areas. Other than the cool highlands, barley is rarely grown in the tropics as it is not suited to warm humid climates (Nevo, 1992). Barley was first domesticated about 10,000 years ago from its wild relative, *H. vulgare* ssp. *spontaneum*, in the area of the Middle East known as the Fertile Crescent (Civan et al., 2021). *H. vulgare* ssp. *spontaneum* still grows in the Middle East and adjacent regions of North Africa, in both natural and disturbed habitats, such as abandoned fields and roadsides (Nevo, 1992).

Until the late nineteenth century, all barleys existed as highly heterogeneous landraces adapted to different environments. Over the past 100 years, the landraces have mostly been displaced in agriculture by pureline varieties with reduced genetic diversity (Nevo, 1992). Extensive cultivation, intensive breeding and selection have resulted in thousands of commercial varieties of barley. For commercial purposes, barley varieties are classified into broad classes that are used as a basis for world trade. Major factors used to distinguish barley varieties include feed or malting barley, winter or spring growth habit, hulled or hull-less barley, and six- or two-row varieties (OECD, 2004). In two-row (*distichon*) varieties, only one spikelet at each node is fertile. In six-row (*hexastichon* or *vulgare*) varieties, all three are fertile (see Section 3.2).

The progenitor of cultivated barley, *H. vulgare* ssp. *spontaneum*, has a brittle two-row spike and a hulled grain. During the process of domestication, cultivated barley acquired mutations that confer a non-brittle spike, preventing natural seed dispersion at maturity and facilitating harvest (Pourkheirandish et al., 2015).

Domesticated six-row barley appeared about 8000 years ago (Komatsuda et al., 2007). This mutation was selected by some farmers to improve grain yield. The small, one seed arrow-like spikelets of two-row *H. vulgare* ssp *spontaneum* are adapted to reach the soil through stones and pebbles. However, the spontaneous six-row mutants, which produce larger three seed spikelets, do not have this evolutionary advantage and do not reach the soil as easily; therefore, they are naturally eliminated from wild barley populations. Thus, six-row barley occurs primarily as cultivars or volunteers in agricultural systems (Komatsuda et al., 2007).

Hulled barley has husks that adhere to the grain. In cultivated hull-less barley, which appeared about 8000 years ago, the husks are easily separated from the grain by threshing (Pourkheirandish and Komatsuda, 2007).

2.2 Commercial uses

Barley is the fourth most important cereal crop in the world after wheat, maize, and rice, in terms of both production quantity and area harvested. In 2019, about 159 million tonnes of barley was produced globally on about 51 million hectares. Countries producing the most barley are summarised in Table 1. The three leading exporters of barley in 2019 were France, the Russian Federation and Australia, while the three leading importers of barley were China, Saudi Arabia and Iran (FAOSTAT website, accessed 20 October 2021).

Country	Production ('000 tonnes)	Area harvested ('000 hectares)
Russian Federation	20,489	8,537
France	13,565	1,944
Germany	11,591	1,709
Canada	10,383	2,727
Ukraine	8,917	2,609
Australia	8,819	4,437

Table 1: Major barley producers in 2019	(FAOSTAT website.	accessed 20 October 2021)

Barley is used primarily for animal feed and to produce malt, with smaller amounts used for seed and direct human consumption. Barley is also used to produce starch, either for food or for the chemical industry (OECD, 2004). A small proportion of barley is used in biofuel production. Barley straw can be used as a fibre source for ruminants or as animal bedding (Tricase et al., 2018).

In 2018, the global use of barley grain was 62% feed, 24% processing, 7% seed, 6% food and 1% other non-food uses (<u>FAOSTAT website</u>, accessed 20 October 2021).

Animal Feed

Most barley grain produced is used for feeding animals, including cattle, swine and poultry. In most cases, the whole barley kernel is mechanically processed to break the hull, prior to being fed, to improve digestibility. Whole barley plants are also used as forage (OECD, 2004; Tricase et al., 2018).

Barley grain is considered to have a poorer nutritive value than wheat because its high fibre content means the energy is not easily utilised by animals. Although it has a higher protein content than maize, the diet of high-performing monogastric animals usually needs to be supplemented with other protein sources due to the low content and quality of protein in the barley grain (OECD, 2004).

<u>Malt</u>

The most economically valuable use of barley is for malt (Tricase et al., 2018). Malt is used mostly in beer, but also in whisky, baking and other food products. By-products from brewing, such as brewers' grain, are used as animal feed (OECD, 2004).

When barley is used for malt, it involves steeping in water, under controlled conditions, allowing the barley grain to germinate or sprout. It is then dried or roasted in a kiln, cleaned, and can be stored for extended periods. Malt itself is primarily an intermediate product and requires further processing, such as fermentation in beer and whisky production (OECD, 2004).

Malting barley requires uniform seed, plump kernels and relatively low protein content. Malting barley varieties that do not meet malting quality requirements at harvest can be sold as feed, at a lower premium (O'Donovan et al., 2011).

Human Food

Barley is an important food grain in several regions of the world, including Morocco, India, China and Ethiopia (OECD, 2004). For example, barley as flatbread or porridge is widely consumed in North Africa and parts of Asia. Food barley is generally found in regions where other cereals do not grow well due to altitude, low rainfall, or soil salinity (FAO, 2002).

In Western countries, barley has minor food uses such as barley flakes in breakfast cereal, barley flour in some bakery products and baby foods, and pearl barley in soups (Tricase et al., 2018). Barley starch is used in both the food industry as a sweetener and binder, and the brewing industry, in the production of beer and alcohol (OECD, 2004).

Barley must have its fibrous outer hull removed before it can be eaten, or alternatively, hull-less barley varieties can be used for human food. Pearl barley is dehulled barley that has been abraded (polished) to remove the bran and germ (Tricase et al., 2018).

2.3 Cultivation in Australia

2.3.1 Commercial propagation

In Australia, growers can either retain barley seed for sowing on-farm, or they can purchase seed from a commercial supplier. Growers cannot trade seed of a variety protected by Plant Breeders Rights for sowing unless they have an authorisation from the owner of the variety (GRDC, 2011b).

Seed purchased from a commercial distributor will be certified (GRDC, 2011b). Certification is in accordance with OECD Seed Schemes for export markets and the similar Australian Seed Certification Scheme for domestic markets. Certified barley seed must be at least 98% pure seed with at least 85% germination rate. Certified barley must be produced in a paddock that has not been sown to barley in the previous two years or any cereal species in the previous year, unless it was the same variety of barley. Certified barley production areas must be separated from other cereals by at least 2 metres or a physical barrier such as a fence. More stringent requirements apply to basic barley seed (Seed Services Australia, 2020).

2.3.2 Scale of cultivation

Barley is the main cereal other than wheat grown in Australia, with an estimated gross value of \$3 billion in 2019-20 (Australian Bureau of Statistics, 2021). It is grown in wheat production areas in New South Wales (NSW), Vic, Queensland (Qld), Western Australia (WA) and South Australia (SA) (Figure 1). A small amount of barley is also grown in Tasmania (Tas). The area sown to barley in Australia is forecast to be around 4.3 million hectares for 2020-21, with projected production of 10.1 million tonnes (ABARES, 2021).

While Australia produced less than 6% of the world's barley in 2019, it provided about 9% of world barley exports (FAOSTAT website, accessed 20 October 2021) and this is likely due to a relatively small domestic market. For 2019, about 52% of Australia's barley crop was exported, as malting barley (22%), feed barley (20%) and malt (10%) (ABARES, 2021). The remaining barley produced in Australia was used domestically. Domestic malting barley demand is about 1 million tonnes per year and feed barley demand is about 2 million tonnes per year (GRDC, 2016b).

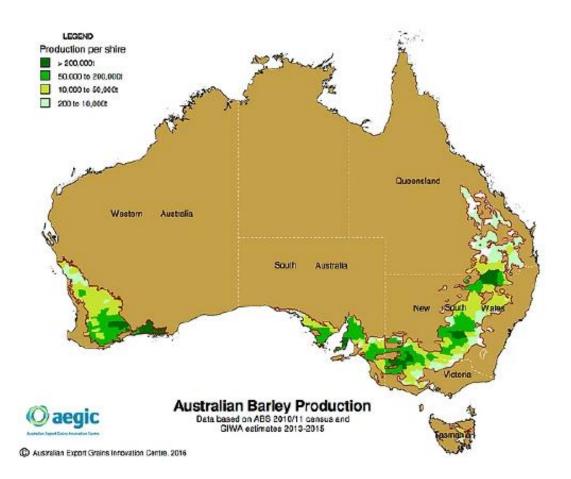


Figure 1: Barley growing shires of Australia (used with permission from Australian Export Grains Innovation Centre, South Perth, WA).

2.3.3 Cultivation practices

Australia produces almost exclusively two-row spring barley (<u>AEGIC website</u>, accessed 22 October 2021). Six-row barley varieties are rarely grown and are only suitable for feed (GRDC, 2016a). One winter barley variety is available in Australia (Birchip Cropping Group, 2018). Different barley varieties are suited to, and grown in, different areas of Australia, depending on soil type, climate, end use (malt or feed), and the incidence of pests and diseases. State-specific crop sowing guides with information on barley varieties are published annually by the relevant state department of agriculture or the Grains Research and Development Corporation (GRDC) and are available online. Additionally, the GRDC has published barley production guides for the Northern, Southern and Western regions of Australia (see <u>GRDC website</u>). Specific cultural practices presented in this section are for the Southern Region, encompassing central and southern NSW, Vic, Tas and south-east SA.

Australian barley producers generally grow spring barley varieties as a winter crop. Barley is grown mainly as a grain crop. It is occasionally grown as a fodder cereal, for the purpose of grazing and/or harvest as silage or hay (Mickan et al., 2009). Some barley varieties can be grown as dual-purpose crops for both early grazing and grain. In southern Australia, barley is generally planted between late April and June and harvested from October to December (GRDC, 2016b).

Barley is ideally sown at a depth of 3–6 cm into moist soil and a plant density of 80–120 plants/m². Sowing depth into dry soils would be 3–4 cm. Densities of less than 80 plants/m² can result in

reduced yield, and above 120 plants/m² can lead to a reduction in seed weight (GRDC, 2016b) In Australia, barley is often grown in rotation with other crops, including wheat, canola, pasture and legumes (Harries et al., 2015). Malting barley is best grown after a non-legume crop, to avoid the presence of too much nitrogen in the soil leading to high levels of grain protein (GRDC, 2017a). Similarly, nitrogen fertiliser should be applied to achieve optimum levels of grain protein for the end use (see Section 2.2).

There are a number of pests and diseases of barley, which may require management (e.g. application of herbicide or pesticide) during the growing season (see Section 7.2). Integrated weed management practices are used to control weeds in barley crops. Techniques employed include rotation cropping, increasing seeding rates, using a range of herbicides and harvest seed control (Weedsmart website, accessed 21 October 2021). Weed control by herbicides may include a knockdown treatment prior to sowing, pre-emergence herbicide treatment and post-emergent selective herbicide treatment (Llewellyn et al., 2016). A Western Australian survey found that barley crops received herbicide applications on average 3.1 times per year (Harries et al., 2020). A number of herbicides can cause a reduction in yield in some barley varieties (GRDC, 2016b).

Cultivation options for barley include no-till, direct-drill, reduced tillage, or conventional cultivation, with no-till becoming increasingly common over the last 30 years. In Australian grain growing regions in 2014, 80% of the crop was sown with no-till, 5% with direct-drill, and only 15% was sown with prior cultivation (Llewellyn and Ouzman, 2019). In combination with no- or reduced tillage, stubble can be retained (left standing or cut and spread for mulch), or removed, for example by grazing, burning or cultivation.

Barley dries down well and the use of desiccants is generally not required unless late weed growth needs to be controlled (GRDC, 2016b). Barley is usually harvested directly after the grain has ripened and dried to a moisture content of 12%. In some conditions, barley is harvested at higher moisture contents (up to 20%) and then aerated or dried (GRDC, 2016b). Alternatively, barley can be cut when the grain is physiologically mature (20 - 30% moisture) and allowed to dry in rows held together by straw (swathes). This method is more frequently used in areas with high likelihood of rain during harvest (Western Australian Department of Agriculture and Food, 2017).

2.4 Crop Improvement

2.4.1 Breeding

Barley has been intensively bred for improved performance and quality, resulting in reduced genetic diversity in the elite cultivars. Until the mid-1900s, breeders concentrated on conventional crossing to develop new cultivars (Pickering and Johnston, 2005). In these programs, hundreds of thousands of lines are often required to produce a new variety. However, since the 1950s, significant yield improvements have resulted from the application of more advanced plant gene technologies (Thomas, 2003; Pickering and Johnston, 2005). An extensive catalogue of genetic stocks, such as aneuploid lines, deletion stocks and translocation lines, is available for barley (Varshney et al., 2007). A number of high-density genome-wide profiling techniques are now available for barley breeding, including sequencing of the genome, high-density mapped single nucleotide polymorphism (SNP) arrays, and exome (the gene coding part of the genome) capture arrays (Dawson et al., 2015). High density molecular genetic maps are being used in marker assisted selection (MAS) for breeding as well as for map based cloning and comparative mapping studies. Marker assisted backcrossing used in combination with the production of doubled haploids can halve the time between the first cross and release of a variety compared to conventional breeding (Varshney et al., 2007).

Most of the proposed targets for marker assisted breeding in barley relate to disease resistance genes, with malting quality representing another important target. Several new Australian barley varieties have been developed using this technology (Varshney et al., 2007) and MAS continues to be applied to breeding barley to enhance disease resistance (Miedaner and Korzun, 2012). Whole genome breeding, in which large numbers of genes are targeted at once, is also being used in Australian breeding programs to develop new varieties (Varshney et al., 2007).

Since 1927, ionising radiation and chemical mutagens have been used to increase mutation rates in barley breeding programs (Horvath et al., 2001). Herbicide tolerant (Clearfield[®]) barley that is resistant to the imidizolinone class herbicides imazamox and imazapyr has been developed through a mutagenesis-based (non-GM) breeding program. The first Clearfield[®] barley variety was released in Australia in 2013 (GRDC, 2017a). In 2019, 50% of the barley growing area in Western Australia was planted to Clearfield[®] barley varieties (Shackley et al., 2020).

Molecular techniques such as embryo rescue have allowed the exploitation of wild relatives of barley as a source of genetic variation in crossing programs (Pickering and Johnston, 2005). Crossing between barley and its wild relatives is discussed in Section 9.

2.4.2 Genetic modification

Particle bombardment and *Agrobacterium*-mediated DNA delivery to immature embryos are the two main methods used for stable transformation of barley plants. Transformation efficiency of barley embryos is strongly genotype dependent. The cultivar 'Golden Promise' is one of the most efficiently transformable genotypes and has been widely used in genetic modification and genome editing of barley (Finnie et al., 2004; Travella et al., 2005; Hensel et al., 2008; Matres et al., 2021). Recently, a new method for *Agrobacterium*-mediated transformation of anther culture was reported to be genotype-independent and to efficiently transform four Australian commercial barley varieties (Han et al., 2021).

To date, no genetically modified (GM) barley varieties have been commercially released (ISAAA GM Approval Database, accessed 22 October 2021).

Field trials of GM barley have been previously approved in Australia (see <u>OGTR website</u> for details). These trials examined introduced traits such as increased tolerance to drought, salinity, cold, boron, and aluminium; yield improvement; altered grain starch composition; increased dietary fibre; and enhanced nitrogen utilisation.

SECTION 3 MORPHOLOGY

3.1 Plant morphology

Barley is an annual grass that stands 60–120 cm tall. Barley has two types of root systems, seminal and adventitious. The depth the roots reach depends on the condition, texture and structure of the soil, as well as on the temperature. The deepest roots are usually of seminal origin and the upper layers of the soil tend to be packed with the later developing adventitious roots. If the grain is deeply planted a 'rhizomatous stem' is formed, which produces leaves when it reaches the surface. The 'rhizome' may be one or several internodes in length, and may carry adventitious roots (Briggs, 1978).

The stems are erect and made up of hollow, cylindrical internodes, separated by the nodes, which bear the leaves (Gomez-Macpherson, 2017). A mature barley plant consists of a central stem and 2–5 branch stems, called tillers. The apex of the main stem and each fertile tiller carries a spike. At, or

near, the soil surface, the part of the stem carrying the leaf bases swells to form the crown. It is from the crown that the adventitious roots and tillers develop (Briggs, 1978).

Barley leaves are linear, 5–15 mm wide, and are produced on alternate sides of the stem (Briggs, 1978). The leaf structure consists of the sheath, blade, auricles and ligule. The sheath surrounds the stem completely. The ligule and auricles distinguish barley from other cereals as they are smooth, envelope the stem and can be pigmented with anthocyanins (Gomez-Macpherson, 2017).

3.2 Reproductive morphology

The inflorescence of barley is referred to as the ear, head or spike. The flowering units, the spikelets, are attached directly to the central axis, or rachis, which is the extension of the stem that supports the spike (Figure 2). There are three spikelets at each node, called triplets, alternating on opposite sides of the spike. Each spikelet is made up of two glumes, which are empty bracts, and one floret that includes the lemma, the palea, and the enclosed reproductive components. Depending on variety, each lemma is extended as an awn, or more rarely a hood. The sterile glumes in some varieties can also be awned. Awnless varieties are also known. In hulled or husked varieties, the palea and lemma adhere to the grain. In hull-less or naked varieties, the palea and lemma are not attached and separate from the grain on threshing (Briggs, 1978).

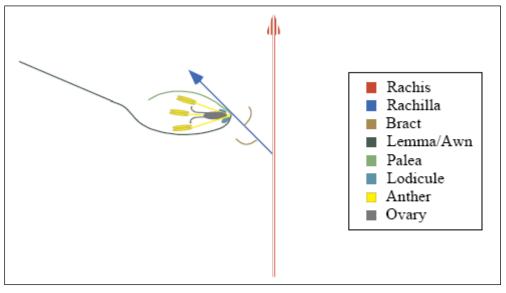


Figure 2: Depiction of the barley spikelet (Williams-Carrier et al., 1997). Reproduced with permission of the Company of Biologists.

In six-row barley, all of the spikelets in a triplet are fertile and able to develop into grains. The central seeds are round and fat, but the lateral seeds tend to be slightly asymmetric and, in some varieties (*intermedium* forms), they are also smaller than the central grain. In two-row barley, however, only the central spikelet is both male and female fertile. The two lateral spikelets are smaller with reduced stamens and a rudimentary ovary and stigma. Therefore, the lateral spikelets of two-row barley are sterile, and only a single seed is produced at each node of the spike, giving it a flat appearance (Komatsuda et al., 2007). Each spike may carry 25–60 kernels in six-rowed varieties or 15–30 kernels in two-rowed varieties (Briggs, 1978).

SECTION 4 DEVELOPMENT

4.1 Reproduction

4.1.1 Asexual reproduction

The production of rooted tillers has occasionally been described as a form of vegetative reproduction, as tillers separated from the plant can grow supported by the adventitious roots only (Briggs, 1978). Otherwise, barley is not capable of vegetative spread (Ellstrand, 2003).

4.1.2 Sexual reproduction

Winter barley varieties require a period of cold stimulus (vernalisation) to initiate floral development. Spring barleys do not require vernalisation (Ibrahim et al., 2016). Australian barley producers generally grow spring barley varieties as a winter crop. Flowering times depend on both degree days since planting and day length (Ibrahim et al., 2016). In Australian barley growing regions, flowering time ranges from August in the north to October in the south (GRDC, 2016b).

After a number of leaves have initiated the stem apex gives rise to spikelet initials which form the inflorescence or spike. The oldest spikelets are at the base of the spike, which terminates with the formation of one or more sterile florets. Initially, the spike is contained within the sheath of the flag leaf, which swells and is called the boot. In most varieties the spike eventually becomes clear of the boot, and flowering generally occurs in the newly emerged spike. Flowering usually begins in the florets around the middle of the ear and spreads upwards and downwards, taking 1–4 days to complete. Ears on different tillers may mature at varying times (Briggs, 1978).

The pollen and ovules in each floret mature together (Briggs, 1978). Barley pollen viability estimates range from a few hours to at least 26 hours (see Section 4.2), while stigma are receptive and able to be fertilised for a period of 6 to 8 days following the first flower opening (Riddle and Suneson, 1944). Barley can be either closed-flowering (cleistogamous) or open-flowering. In closed-flowering types the anthers remain inside each floret, thus self-pollination occurs. In open-flowering types, the lodicules become turgid and push the palea and lemma apart, so that the anthers may emerge (Briggs, 1978). In open-flowering barley, pollen shedding starts before the spikelet opens and continues after it opens, thus outcrossing is possible (Turuspekov et al., 2005). Nevertheless, most pollination occurs before the spikelet opens, so that self-fertilisation is usual (Briggs, 1978).

Floral traits such as high anther extrusion, large anthers and vigorous stigmas may increase the level of outcrossing in barley plants. Such traits are influenced by both genetic and environmental factors (Abdel-Ghani et al., 2005).

4.2 Pollination and pollen dispersal

Barley pollen grains are $35-45 \mu m$ in diameter and of spheroidal-ovoid shape. Within about five minutes of adhering to the stigma, pollen grains take up moisture and germinate. One to two hours after pollination the pollen tube starts to grow. The rate of pollen tube growth is temperature dependent, but generally the pollen tube takes about 45 minutes to grow. Fertilisation then occurs (Briggs, 1978).

Pollen production in barley per spike is about 10% of that of rye (Eastham and Sweet, 2002). Early studies of pollen viability suggest that barley pollen is extremely sensitive to drying and remains viable for only a few hours after dehiscence (Pope, 1944; Bennett et al., 1973). In addition, pollen viability was found to fall to 54% at distances of 1.5–3 m from the parent plants (Giles, 1989). A more recent study found that barley pollen viability in anthers remained above 80% 4 hours after

anthesis at about 21°C and 73% humidity (Gupta et al., 2000). Similarly, pollen viability in nonextruded anthers remained above 80% after 8 hours at 20°C and about 48% humidity, and remained above 60% after 8 hours at 30°C and about 28% humidity (Parzies et al., 2005). After a 26 hour treatment of high/low/high temperatures, pollen viability in anthers remained above 40%. This study found considerable variation in pollen viability between the three barley cultivars tested (Parzies et al., 2005).

Barley reproduces almost entirely by self-fertilisation (~99%) (Wagner and Allard, 1991; von Bothmer, 1992; Ellstrand, 2003). Barley is not pollinated by insects and does not attract bees (USDA, 2018). Any outcrossing occurs by wind pollination. Gene flow rapidly decreases at distances beyond a few metres (Gatford et al., 2006), and most outcrosses that have been detected in cultivated barley result from pollen migrations between closely adjacent plants (Wagner and Allard, 1991).

The extent of outcrossing also varies with genotype and weather conditions (Ritala et al., 2002). In general, cool and moist conditions promote outcrossing in barley (Chaudhary et al., 1980; Parzies et al., 2000; Abdel-Ghani et al., 2004; Gatford et al., 2006), possibly because pollen viability may be extended under these conditions.

Prevailing wind direction has also been suggested to influence pollen migration, but differences observed are often small (for example Wagner and Allard, 1991). Interestingly, prevailing winds were mostly opposite to the direction of gene flow in an Australian study of wheat and barley (Gatford et al., 2006).

The extent of outcrossing in *H. vulgare* ssp. *spontaneum* was estimated as varying from 0–9.6%, with a low overall average of 1.6% (Brown et al., 1978). Outcrossing rates for cultivated barley are very similar, with frequencies of 0–10% being reported, as detailed below.

Average outcrossing rates in barley landraces in Jordan and Syria have been estimated at 0.2% (plants collected about 1 m apart and within 2 to 3 km of cultivated barley landraces) and 1.7% (plants collected at least 2 m apart), respectively (Parzies et al., 2000; Abdel-Ghani et al., 2004). Outcrossing rates in barley populations in Canada ranged from 0–0.8%, with a mean of 0.35% (Chaudhary et al., 1980).

Doll (1987) reported outcrossing rates in autumn and spring sown-barley of 5% and less than 0.5%, respectively. Interestingly, particular lines sown in both autumn and spring showed different levels of outcrossing and the author suggested this was due to the fact that the sex organs of the autumn-sown barley were more exposed during flowering than spring-sown barley, which tend to flower even before spikes emerge from the sheath. Gorastev and Popova (1977, as cited in Doll, 1987) also noted stamen extrusion and relatively high rates of outcrossing in their study of winter barley varieties. In Doll's study (1987), for about one third of the outcrosses, the pollen may have come from neighbouring plots, but for another one third the nearest pollen donor was at least 10 m away. One of the autumn sown lines had about 10% outcrosses, possibly due to the early flowering of this variety, its genetic background, or a combination of the two.

In a study of pollen flow from GM barley, when the non-GM recipient was an open-flowering barley variety with normal fertility, cross-pollination frequency was about 3% at a distance of 1 m. When the non-GM recipient was a male-sterile open-flowering barley variety (providing extremely favourable conditions for pollen flow), the recipient plants produced about 0.4 GM seeds per head at a distance of 1 m, and this declined with distance to about 0.1 GM seeds per head at 6 m, 0.02 GM seeds per head at 12 m and 0.0003 GM seeds per head at 50 m. No pollen flow was detected at 100 m (Ritala et al., 2002).

A study of pollen flow from GM barley under Australian field conditions used a closed-flowering barley variety with normal fertility as the non-GM recipient. The recipient population was at a distance of 1-12 m from the GM donor plots, and cross-pollination occurred at a frequency of 0.005% (Gatford et al., 2006).

An Icelandic study of pollen flow between two-row and six-row barley varieties, where both varieties were closed-flowering types and the two varieties were planted in alternating rows 65 cm apart, found that only 0.0003% of seeds were hybrids (Hermannsson et al., 2010).

In experiments designed to measure outcrossing rates of plants in physical contact with each other, the average rate of outcrossing was about 0.8% (Allard unpublished, discussed in Wagner and Allard, 1991). The rate of outcrossing fell to 0.2% when physical contact was virtually eliminated by spacing plants 30 cm apart, and when plants were 60 cm or 90 cm apart, the pollen migration rate fell to approximately 0.1%. Pollen migrants were only detected sporadically when pollen donor and recipient plants were separated by 3 m, and no outcrossing was detected when plants were separated by 10 m.

In observations of pollen migration between commercial barley fields, outcrossing rates were 0.05% and 0.01% for distances of 1 m and 10 m, respectively. No pollen migrants were observed in these studies at distances of 20 m or 50 m (Allard unpublished, discussed in Wagner and Allard, 1991). However, cross fertilisation with very low frequencies has been observed at distances of up to 60 m (Wagner and Allard, 1991).

To certify both basic and certified barley seed in Australia, the crop must be separated from other cereals by at least a two metre strip or a physical barrier such as a fence to prevent any mixture of seed during harvest (Seed Services Australia, 2020). In Canada, certified, registered and foundation barley seed must be grown at least 3 m from other barley varieties to ensure varietal purity and 2 m from non-barley cereals to ensure mechanical purity (Canadian Seed Growers' Association, 2021).

Hybrid barley seed production uses a male-sterile female parent, so all seeds are cross pollinated. For hybrid barley to be classified as certified seed, a minimum isolation distance of 50 m is needed in the United Kingdom. A minimum isolation distance of 100 m is needed for seed to be classified as pre-basic and basic of female component (Animal and Plant Health Agency, 2017). The OECD Seed Schemes require an isolation distance of 25 m for production of hybrid barley certified seed which does not use cytoplasmic male sterility (CMS), an isolation distance of 50 m for production of hybrid barley certified seed that uses CMS, and an isolation distance of 100 m for production of hybrid barley basic seed that uses CMS (OECD, 2021).

4.3 Fruit/seed development and seed dispersal

Double fertilisation occurs in barley and results in a diploid embryo with equal nuclear contributions from the male and female gametes, and the triploid endosperm, which is derived from a second fusion between one male gamete from the pollen and two polar nuclei from the embryo sac (Briggs, 1978). The total number of cells in the endosperm is higher than in wheat or rice, which is why barley grains contain more cell wall material such as β -glucans than these cereals (Gomez-Macpherson, 2017).

In addition to varieties being awned or hooded and husked or husk-less, grain shapes and sizes can vary widely. Grain development progresses through a number of stages; watery ripe, milk, soft dough, hard dough, grain hard and harvest ripe (NSW Department of Industry and Investment, 2010).

During domestication a strong selection for tough rachis was made for easier reaping, threshing and sowing, with the result that cultivated barley is not prone to shattering. Instead, the single seed is broken off at the base at maturity (von Bothmer, 1992). Some *Hordeum* species, including *H. lechleri* and *H. jubatum*, have small, light seeds and spikelets that serve as an elegant flying aparatus for wind dispersal. Other species, including *H. vulgare, H. bulbosum* and *H. murinum*, have large, heavy seeds and special bristles on the spikelets which make them adhere well to the furs of larger animals, the feathers of birds and the clothing of people, and the seeds may get dispersed in this way (von Bothmer, 1992; von Bothmer et al., 1995). Viable seed may also be transported on the muddy feet/legs of birds (Sorensen, 1986; Cummings et al., 2008).

Approximately 15% of the faecal dry matter from cattle fed whole barley seeds was composed of whole and undamaged barley seeds (Beauchemin et al., 1994). Although the viability of the intact seeds was not determined, this study suggests there is the potential for some livestock to disperse viable barley seed after consumption. However, when sheep and goats were fed *H. vulgare* ssp. *spontaneum* seeds, no intact seeds were recovered from faeces (Oveisi et al., 2021).

In feeding studies with corellas, galahs, house sparrows, mallard ducks, pheasants, red-winged black birds and rock pigeons fed whole barley seeds, no birds excreted any intact barley seeds (Cummings et al., 2008; Woodgate et al., 2011). However, viable seed from other cereal crops has been reported to survive passage through the digestive tract of some birds. Viable oat seeds, a grass from the same subfamily as barley (Pooideae), were detected in emu droppings (Calvino-Cancela et al., 2006). It has also been reported that wheat seeds will germinate after passage through an emu's digestive system, although no experimental evidence was provided (Davies, 1978). Corellas have been shown to excrete some (about 3%) viable wheat seeds after passage through the digestive tract (Woodgate et al., 2011).

4.4 Seed dormancy and germination

Dormancy is defined as the inability of viable seed to germinate under favourable conditions. Dormancy of the barley grain is typically imposed by the seed covering structures (lemma, palea, pericarp and seed coat). Primary dormancy is intrinsic, whereas secondary dormancy arises as a result of external factors. Water sensitivity is a form of secondary dormancy in which germination is reduced under excessive moisture conditions (Briggs, 1978).

Long dormancy is not desirable in malting barley as the malting process requires grain to germinate rapidly and uniformly. Malting barley standards require a minimum of 95% of seeds to germinate within 72 hours when exposed to moisture (Grain Trade Australia, 2021a). Therefore, during barley domestication, farmers selected for non-dormancy of seeds. In a study of 4365 cultivated barley accessions, immediately after harvest about 20% of cultivars had germination rates over 90% within 4 days when exposed to moisture; after 5 weeks storage about 50% of cultivars had germination rates over 90%. In comparison, germination rates of all *Hordeum vulgare* spp. *spontaneum* accessions were negligible immediately after harvest and about half of the seeds germinated after 10 weeks storage (Takeda and Hori, 2006).

Australian barley varieties generally have low to very low dormancy levels (NSW Department of Industry and Investment, 2010).

While low dormancy is desirable in malting barleys, too little dormancy can lead to pre-germination or pre-harvest sprouting, where germination of the grain begins on the mother plant in rainy conditions before harvest. Both pre-germination and pre-harvest sprouting trigger the hydrolysis of the endosperm and can have adverse effects on the yield, malting quality and storage life of the

grain. Pre-harvest sprouting susceptibility is determined mainly by genotype; some varieties are resistant due to deep dormancy, others are highly susceptible, and a third group are intermediate (Rodriguez et al., 2001). Traditionally, Australian malting barley varieties have relatively good tolerance to pre-harvest sprouting. However, Harrington barley, which has been widely used in Australian breeding programs, is highly susceptible to pre-harvest sprouting (Li et al., 2003).

In addition to the influence of variety, dormancy varies with the conditions during grain ripening, harvest and storage. Cool, moist conditions during ripening encourage the expression of dormancy, while low dormancy is generally associated with high temperatures, short days, low moisture and high nitrogen levels (Rodriguez et al., 2001).

In wild barley (ssp. *spontaneum*) growing in the Fertile Crescent, the release of seed dormancy is promoted by after-ripening during the dry summer (Chen et al., 2004). Similarly, in cultivated barley, dormancy is commonly relieved by after-ripening, which is achieved by post-harvest storage in warm temperatures and low humidity (Leymarie et al., 2007). Coat imposed dormancy in barley may last 0.5–9 months in dry storage. In contrast, storing grain in cold and moist conditions can maintain dormancy, and barley seeds have remained dormant for 3 years at 2°C under high humidity (Pickett, 1989).

Shed grain may exhibit more prolonged dormancy than grain in dry storage, possibly because wet periods following harvest encourage retention of dormancy, so that self-sown grain often germinates just before the following crop (Pickett, 1989).

In a study in Germany, seeds and spikes of freshly-ripened barley kernels were incorporated into the ground at depths of 1-15 cm and 15-30 cm directly after harvest in the summer of 1981 and again after harvest in 1982. Chopped straw was added to half of the plots. Depth of incorporation, application of straw and incorporation as seed or as spikes had no consistent effect on seed viability. A small proportion of seed, 1% for the 1981 trial and 0.02% for the 1982 trial, remained viable after 15 months. The different seed viability rates for the two trials was attributed to lower temperatures while the seed was developing and maturing in 1981 compared to 1982. The cooler temperatures lead to greater dormancy as demonstrated by the germination rates immediately after harvest which were only 10% in 1981 compared to 59% in 1982 (Rauber, 1988).

In a Scottish study, winter barley was buried in the autumn to depths of 5, 10, 15 and 20 cm and emergence was measured in the following three years. In the first season following burial, emergence occurred from seed buried from all depths, and was highest from seed buried at 5 cm and lowest from seed buried at 20 cm. No plants emerged in the second or third year after burial. No data as to the initial viability of the buried barley seeds in this trial were provided (Davies and Wilson, 1993).

In a Scottish survey, volunteer winter barley was reported to persist for up to five years in some rotations (Davies and Wilson, 1993). In most cases the authors could not distinguish whether this was due to genuine seed persistence or production of seed by volunteers from year to year. It is also possible that cereal seed for planting may have been contaminated with barley seed. However, there was evidence suggesting that barley volunteers can emerge from seeds shed two seasons previously, in cases where ploughing has caused deep burial of seeds and subsequent ploughing has brought seeds back nearer to the soil surface (Davies and Wilson, 1993).

There is a difference in germination rates between buried grain and grain lying on the surface. Cereal seeds remaining on the surface can generally easily germinate and become established (Ogg and Parker, 2000). Exposure to periods of rain interspersed with dry conditions may encourage

germination in grains on the soil surface. On the other hand, deep cultivation soon after harvest encourages dormancy by placing the grain in a cool, moist environment (Pickett, 1989).

Germination can occur at temperatures between 5°C and 38°C, with 29°C being optimal. Successful germination also requires both water and oxygen. Germination begins with the grain absorbing moisture and swelling. The rate of grain imbibition increases rapidly with increasing temperature (Briggs, 1978).

Soil type and condition, including pH level, can also affect germination of barley seeds. Deep cultivation in certain soil types can prevent emergence by encouraging prolonged dormancy in seeds as a result of low oxygen availability (Pickett, 1989; Ogg and Parker, 2000). In addition, any seeds germinating at depth would be unlikely to successfully emerge (Pickett, 1989).

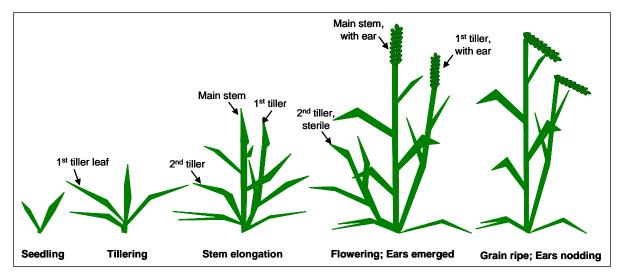
4.5 Vegetative growth

Cultivated barley is an annual grass. Barley growth can be divided into a number of stages; germination, seedling development, tillering, stem elongation, heading (ear emergence), flowering and ripening (Figure 3).

The duration of the different developmental stages varies widely. Growth rate depends on the weather, water supply, soil fertility, the degree of competition with other plants, the presence of pests and diseases, and the time of planting. Initially growth is slow while the seedlings establish and the tillers form. Total time to maturity depends on variety, location and planting date. In south-east Qld, barley plants take between 105 and 157 days to reach maturity (Thomas et al., 1995). In Victoria, twelve barley varieties planted at three different sowing times took between 155 and 193 days from sowing to maturity (Birchip Cropping Group, 2018).

Roots

The seminal rootlets of barley emerge when the seed germinates and form a fibrous branched mass of roots, some of which extend deeply downwards. Later, at the tillering stage, the adventitious root system arises from the crown, and this tends to be thicker and less branched. Under some conditions such as drought, the adventitious roots may not develop at all. In other cases, the seminal roots cease functioning during the life of the plant. Different barley varieties can vary significantly in rooting system, and this can impact on their competitive ability (Briggs, 1978).





<u>Leaves</u>

After germination, the coleoptile (a leaf sheath that encloses the embryonic plant) reaches the surface and the first leaf emerges at its tip. The leaves grow rolled up from the tube formed by the bases of earlier leaves, unrolling once emerged (Briggs, 1978). Leaves emerge continuously on the main stem and tillers until the final (flag) leaf emerges. Emergence of the flag leaf is an important growth stage for timing the application of certain growth regulators. The mature leaves progressively senesce and gradually the whole plant dries out until full maturity, when the grain is ripe (Briggs, 1978).

Stems and Tillers

Stem elongation usually starts when the plant is about 5 cm in height and coincides with leaf emergence, tillering and spike formation (Briggs, 1978). During stem elongation the developing spike is carried upwards.

Tillers start to develop at about the 3-leaf stage (Figure 3). The number of tillers and duration of tillering vary with variety and growth conditions (Briggs, 1978). Some older genotypes produce many tillers but develop few spikes, while most modern genotypes have a higher percentage of tillers that develop spikes (Gomez-Macpherson, 2017). In addition, winter varieties usually produce more tillers than spring varieties during the vegetative growth period over winter. In general, field grown barley plants typically produce 2–5 tillers (Briggs, 1978; Gomez-Macpherson, 2017). Most tillers initiate adventitious roots, although later appearing tillers often remain unrooted and die prematurely (Anderson-Taylor and Marshall, 1983).

SECTION 5 BIOCHEMISTRY

5.1 Toxins

Barley is generally not considered toxic. However, a number of allergens and anti-nutritional factors occur in barley and in extreme cases may have a toxic effect. These are described in Sections 5.2 and 5.3.

5.2 Allergens

Barley is a well-known allergenic food in the human diet and is associated with several adverse reactions. A food allergy to barley is more common in children than in adults, and most people who are sensitive to barley are also allergic to wheat. Symptoms following barley consumption include atopic dermatitis, gastrointestinal complaints, respiratory symptoms and anaphylaxis (Armentia et al., 2002; Lee et al., 2020). Contact dermatitis and anaphylaxis can also be induced by barley proteins present in beer. Two barley proteins were identified as beer allergens, with lipid transfer protein 1 causing strong positive skin prick responses in all beer-allergic patients (Garcia-Casado et al., 2001). Lipid transfer proteins are common food allergens in other plant species, including wheat (Gonzalez-Klein et al., 2021).

Inhalation of barley flour can cause baker's asthma, an occupational allergy. Barley alpha-amylase inhibitor proteins have been identified as major allergens associated with baker's asthma (Sanchez-Monge et al., 1992).

Barley pollen contains proteins that are homologues to common grass pollen allergens (Astwood et al., 1995). Most of a group of people with allergic rhinitis due to grass pollen allergies were also found to be sensitive to barley pollen. However, barley produces far less pollen than most wild grasses, and barley pollen grains are much larger than most wild grass pollen so are less easily dispersed by wind. Therefore, barley pollen is considered a negligible aeroallergen for the general population, although it could potentially cause occupational allergy in barley farm workers (Damialis and Konstantinou, 2011).

Coeliac disease

Coeliac disease is an immune-mediated condition that develops in genetically susceptible individuals when exposed to gluten proteins, which are found in wheat, barley and rye grains (Abadie et al., 2011; Walker et al., 2017).

Coeliac disease is a multigenic disorder involving particular alleles of the human leukocyte antigen (HLA) gene and a range of other genes. Individuals with an HLA allele associated with susceptibility and many other genetic risk factors usually develop coeliac disease early in life. Individuals with an HLA allele associated with susceptibility and a few other genetic risk factors may develop coeliac disease later in life, triggered by viral infection or other environmental factors, or may never develop the disease (Abadie et al., 2011).

Coeliac disease is more prevalent in females than in males and is particularly common in Caucasians compared to other ethnic groups (Abadie et al., 2011). In Australia, the prevalence of coeliac disease is estimated at 1.2% in adult men and 1.9% in adult women, which is similar to rates in Europe (Walker et al., 2017).

Coeliac disease damages the small intestine, resulting in malabsorption of nutrients and a range of gastrointestinal and systemic symptoms (Abadie et al., 2011; Walker et al., 2017). Symptoms of coeliac disease vary and patients with clinical coeliac disease may have few or many symptoms. Common symptoms include diarrhoea, abdominal pain, fatigue, weight loss or growth failure, iron deficiency anemia and osteoporosis due to calcium malabsorption. The treatment for coeliac disease is a gluten free diet, which alleviates symptoms and prevents further damage to the small intestine (Walker et al., 2017).

Development of the first ultra-low gluten barley variety has been reported. The variety contains less than 5 ppm hordeins, the type of glutens found in barley, which is well below the World Health

Organization's upper limit of 20 ppm for classification as gluten-free (Tanner et al., 2016). The variety was commercially released under the trade name Kebari[™] and the first commercial product made with this barley was a gluten-free beer in Germany (<u>CSIRO website</u>, accessed 15 Oct 2021).

5.3 Other undesirable phytochemicals

Enzyme Inhibitors

Both protease and alpha-amylase inhibitors are present in the barley grain. Protease inhibitors, especially trypsin inhibitors, may decrease the digestibility of dietary proteins while amylase inhibitors may affect the digestibility of dietary starch. However, these inhibitors do not appear to pose a serious risk to human health as they tend to be heat labile (OECD, 2004), although members of the trypsin/alpha-amylase inhibitor protein family are major allergens associated with baker's asthma (see Section 5.2).

The most common barley protease inhibitors are inhibitors of trypsin, chymotrypsin and microbial proteases (Casaretto et al., 2004). However, due to the low levels of protease inhibitors in the barley grain, it is unlikely that they have a significant negative influence on protein digestibility (Newman and Newman, 1992).

Lectins

Lectins are glycoproteins that bind to specific carbohydrate groups on cell surfaces, causing lesions to form. In the intestinal tract, these lesions can seriously impair the absorption of nutrients (OECD, 2003). Although more commonly associated with legumes, cereal grains including barley are also known to contain lectins, although their possible physiological significance is unknown (OECD, 2004). As lectins are usually inactivated by heat treatment, they are really only of interest when raw or inadequately cooked food or feed is consumed (OECD, 2003). Therefore, in the case of barley, they are more likely to be an animal feed concern.

Phytic acid

Phytic acid may reduce the bioavailability of trace elements in animal diets through chelation of minerals such as iron, zinc, phosphate, calcium, potassium and magnesium. This anti-nutrient is of particular importance to monogastric animals, whereas ruminants possess digestive enzymes which degrade phytate and release the chelated minerals (OECD, 2004). Mature seeds of most traditional crops contain about 75% of total phosphorus as phytic acid (Raboy, 2000). The excretion of feed phytic acid phosphorus by livestock (e.g. poultry, swine, and fish) contributes to water pollution and is a major environmental issue (Raboy, 2000).

Low phytic acid mutants, with reduced phytic acid accumulation and increased inorganic phosphorous, have been identified in barley (Larson et al., 1998; Raboy, 2000), but typically have an associated yield penalty (Raboy, 2000; Raboy et al., 2014). Recent studies indicate that the low phytic acid 1-1 trait in barley is a seed-specific or filial determinant of barley endosperm total phosphorus, suggesting low phosphorus varieties with good agronomic performance could be developed (Raboy et al., 2014). A hulless, low phytate barley with improved feed conversion and nutrient digestibility compared to a regular-hulled barley has been released in Canada (Woyengo et al., 2012).

Phenolic compounds

The phenolic compounds proanthocyanidins and catechins are found in barley seed coats, and these can form insoluble complexes with proteins inhibiting nutrient utilisation (Newman and Newman, 1992). Proanthocyanidins also cause haze formation in beer, an undesirable characteristic for most breweries. Proanthocyanidin-free barley mutants have been released commercially (von Wettstein, 2007).

5.4 Beneficial phytochemicals

Barley is an excellent source of dietary fibre, protein, and complex carbohydrates, and is a good source of certain vitamins and minerals. Barley composition varies markedly in different environments and between varieties (OECD, 2004). The concentration of starch is inversely related to the content of total dietary fibre and protein. In malting barley, lower protein content (8–10.5% dry matter) and corresponding high starch content is preferred. In feed barley, grains with low fibre, higher protein (10–15%) and higher starch content are preferred (OECD, 2004).

Carbohydrates, including starches, sugars and non-starch polysaccharides, comprise about 80% of the barley grain (Newman and Newman, 1992). Most of the carbohydrates is starch, which makes up 60% of the grain and provides energy for germination (OECD, 2004). Starch is the major source of readily available energy for food and feed. In most barleys, the predominant starch is amylopectin and the remainder is amylose (Newman and Newman, 1992).

The non-starch polysaccharides are collectively called total dietary fibre and include ß-glucans and arabinoxylans. The fibre content of barley is relatively high, and the benefits of dietary fibre on human health are well known. The soluble fibre ß-glucan, for example, can lower both post-prandial blood glucose levels and blood cholesterol (McIntosh et al., 1991; OECD, 2004). In contrast, arabinoxylans and ß-glucans can have a deleterious effect on digestion in monogastrics (OECD, 2004). In addition, ß-glucans are known to negatively impact poultry, especially young birds, by reducing the intestinal viscosity (Newman and Newman, 1992).

Protein is the second major component of the barley grain. Protein content of barley grains is strongly affected by the growth conditions and nitrogen fertilisation regime, but is usually about 10–12%. Barley proteins can be classified by solubility as albumins, globulins, prolamins and glutelins (Newman and Newman, 1992). Prolamins (or hordeins) are the major storage protein and account for up to half of the total nitrogen in the grain. The other groups consist mainly of structural and metabolic proteins (OECD, 2004).

Barley contains 2–3% minerals, although the mineral content can vary markedly with variety, growing conditions and fertiliser application. The major minerals are magnesium, phosphorus, potassium, calcium and sodium. Although much of the phosphorus is unavailable to monogastric animals, barley contains more phosphorus and has higher phosphorus bioavailability than other grains (OECD, 2004).

Barley also contains 2–3% lipids, including several with health promoting activities such as carotenoids and tocopherols (Newman and Newman, 1992). Vitamin E, a mixture of tocopherols, is present in barley oil. Barley grains also contain B vitamins (OECD, 2004).

Barley contains a number of other compounds, some of which may have a role in protection against diseases when ingested at high levels. These include simple phenolic acids, flavenoids and lignans, all of which have good antioxidant properties (OECD, 2004).

SECTION 6 ABIOTIC INTERACTIONS

6.1 Abiotic stresses limiting growth

6.1.1 Nutrient stress

Common nutrient deficiencies of Australian soils for production of barley crops are nitrogen, phosphorus and zinc. Potassium deficiency is also common in Western Australia. In some soil types and growing areas, sulphur, copper, manganese or molybdenum may also be lacking (GRDC, 2016a, b, 2017a).

Nitrogen deficiency leads to small, pale green plants with reduced grain yield and protein levels. The amount of nitrogen fertiliser required for barley crops varies with rainfall, soil type and rotational history of the field. Nitrogen fertilisers are generally applied between sowing and early to mid-vegetation. In malting barley, nitrogen fertilizer application must be managed to limit protein content, as markets for malting barley demand moderate protein levels (GRDC, 2016b).

Phosphorus is essential for two distinct phases of growth: during early development (emergence to end of tillering) and for seed formation and grain filling. Potassium deficiency leads to stunted plants with dark green leaves and reduced grain number. Phosphate fertiliser is generally applied with the seed during sowing to meet the requirements during the early development phase (GRDC, 2016b).

Zinc is a micronutrient, essential for protein shape and thus important for enzyme function in the plant. Deficiencies manifest as stunted plants with short, thin stems and pale green leaves. Severe deficiencies result in reduced tillering, delayed maturity and little or no grain production. Zinc can be applied at sowing or as a foliar application (Norton, 2014; GRDC, 2016b).

Potassium deficiencies can lead to poor root growth and leaf development, and to fewer and smaller grains. As potassium has high soil persistence, potassium fertilisers can be applied at planting, during fallow prior to planting, or applied to a preceding crop (GRDC, 2017a).

6.1.2 Temperature stress

Barley is a temperate climate plant that is grown as a winter crop in Australia. It can suffer frost damage at low temperatures or heat stress at high temperatures.

Barley seeds will not germinate at soil temperatures under 4°C. Barley crops have high susceptibility to frost damage from late booting to milk development stages, and are most susceptible at flowering. Irreversible freezing damage typically occurs at canopy temperatures under -2°C, although florets can also be aborted due to chilling damage at temperatures above freezing. Frost damage during flowering reduces grain number, but remaining grain is of normal quality. Frost damage during grain fill produces light, shrivelled grains with compromised ability to germinate and establish (GRDC, 2016b).

Soil temperatures over 33°C during barley emergence cause significant seedling mortality, reducing the number of plants that establish (GRDC, 2016b). High temperatures during reproductive growth affect pollen development, with temperatures of 30°C/25°C (day/night) for five days reported to cause male sterility (Sakata et al., 2000). In addition, heat stress during spike growth and grain setting affects grain yield. In a study in Argentina, a mean temperature of 15.4°C during this period (the lowest temperature measured) gave highest barley grain yield, and yield was reduced by about 10% per degree of mean temperature increase (Garcia et al., 2015).

6.1.3 Water stress

Barley has high water use efficiency compared to other crops (GRDC, 2016b). In Western Australia, barley yield is reported to be strongly dependent on water supply at growing season rainfall levels of up to 285 mm, and very weakly dependent on water supply at higher rainfall levels. Therefore, barley can be successfully grown in low, medium and high rainfall zones (van Gool and Vernon, 2006). In WA grain growing regions, the climate is Mediterranean with a concentration of rainfall during the winter months, which matches the growing season for barley. The winter-dominant rainfall of WA differs from the generally higher and more evenly distributed rainfall of Vic and southern NSW, and the summer-dominant rainfall of the northern grain growing areas (Cramb et al., 2000).

Drought stress occurs when soil moisture does not meet the needs of a crop at a particular growth stage. Symptoms of drought stress in barley include slower plant growth, deeper root systems and leaf wilting, as well as reduced yield (Saade et al., 2018).

Waterlogging is an important constraint to barley production. Barley is more susceptible to waterlogging than wheat or oats. Barley is able to tolerate short periods of waterlogging but should not be grown on soils where waterlogging may occur for periods of more than two weeks. Waterlogging damages roots, resulting in decreased plant growth or plant death, and reducing crop yield (van Gool and Vernon, 2006; GRDC, 2016a).

6.1.4 Other stresses

Soil acidity is a major constraint to crop growth. Acidic soils solubilise toxic aluminium ions, and barley is among the most aluminium-sensitive of the cereal crops (van Gool and Vernon, 2006; Saade et al., 2018). Barley yield is affected by soil pH <5.0 and soils with pH <4.5 are unsuitable for barley cultivation (van Gool and Vernon, 2006; GRDC, 2017a).

Barley is also sensitive to boron toxicity, which can occur due to high boron levels in some Australian soils (GRDC, 2017a; Saade et al., 2018).

6.2 Abiotic tolerances

Barley is the most salt-tolerant cereal crop (Munns and Tester, 2008), and barley and cotton are the two most salt-tolerant broad-acre crops grown in Australia. Barley yields begin to decline in very saline soils (soil salinity class 3: 8-16 dS/m) and extremely saline soils (soil salinity class 4: >16 dS/m) are unsuitable for barley cultivation (NSW Department of Environment and Climate Change, 2008).

Barley is also more tolerant of alkalinity than other cereals (van Gool and Vernon, 2006).

SECTION 7 BIOTIC INTERACTIONS

7.1 Weeds

Barley is considered a competitive crop. Among the nine major winter crops grown in Australia (ABARES, 2021), the relative competitive ability is ranked as oats > barley > wheat > canola > field pea > faba bean > lupin = chickpea = lentil (GRDC, 2011a). In Australian grain growing regions in 2014, the estimated revenue loss from competition with in-crop weeds was \$8 per hectare for barley, which was lower than the revenue losses due to weed competition in oats, wheat, canola, pulses and sorghum (Llewellyn et al., 2016). However, weeds in barley crops still need to be controlled by weed management practices (see Section 2.3.3).

The major in-crop weeds in Australian winter cereals, in order of economic importance, are ryegrass (*Lolium rigidum*), wild radish (*Raphanus raphanistrum*), wild oats (*Avena fatua*), brome grass (*Bromus* spp.) and wild turnip (*Brassica tournefortii*). Ryegrass is particularly difficult to manage due to its high levels of herbicide resistance (Llewellyn et al., 2016). A Western Australian survey found that the mean weed density in barley crops at anthesis was 13 grass weeds/m² and 4 broadleaf weeds/m² (Harries et al., 2020).

7.2 Pests and pathogens

Birds often damage cereal crops, including barley, in Australia (Bomford and Sinclair, 2002). The main bird pests of winter cereals are cockatoos including the galah (*Eolophus roseicapilla*), little corella (*Cacatua sanguinea*), long-billed corella (*Cacatua tenuirostris*), cockatiel (*Nymphicus hollandicus*) and sulphur-crested cockatoo (*Cacatua galerita*) (Bomford and Sinclair, 2002; Tracey et al., 2007; DELWP, 2018). Cockatoos cause most damage to germinating cereal crops in the autumn, but may also feed on ripening crops or grain residue in stubble (DELWP, 2018). Invasive bird pests such as the house sparrow (*Passer domesticus*), common starling (*Sturnus vulgaris*) and common myna (*Acridotheres tristis*) also feed on cereal crops (Tracey et al., 2007). Native Australian crows and ravens sometimes feed on grain (Tracey et al., 2007), and the Australian raven (*Corvus coronoides*) and Torresian crow (*Corvus orru*) are known to engage in food caching behaviour (de Kort and Clayton, 2006). Emus (*Dromaius novaehollandiae*) can cause significant damage by feeding on grain crops, especially when bird numbers are high, and also damage crops by trampling (Department of Environment and Conservation, 2009a).

Kangaroos are reported to damage grain crops by feeding on seedlings or trampling mature plants. Eastern grey kangaroos (*Macropus giganteus*) may feed on young green cereal crops when native grasses are dry and producing no new growth (Hill et al., 1988). Western grey kangaroos (*Macropus fuliginosus*) may feed on cereal crops near remnant bush and damage is most likely to occur in late autumn and early winter when little green feed is available elsewhere (Department of Environment and Conservation, 2009b). Rabbit (*Oryctolagus cuniculus*) grazing can reduce crop yield in Australian agricultural areas (Brown et al., 2020). Like kangaroos, rabbits prefer soft, green, lush grass (Myers and Poole, 1963) and select the most succulent and nutritious plants first (Croft et al., 2002).

The main rodent pest in Australian agricultural crops is the house mouse (*Mus domesticus*). In most years, mice cause <5% yield loss in winter cereals, but they can cause >25% yield loss in years when there is a mouse plague (Brown et al., 2007). Mice dig out newly sown seed, gnaw on vegetative growth and can climb to flowers and developing grain, as well as feeding on grain residue after harvest (GRDC, 2017b). Mice may eat seeds at the seed source or they may hoard seed (AGRI-FACTS, 2002). In agricultural regions, the median territory size of mice is reported as 0.014 ha in the breeding season (spring/summer) and as 0.2 ha in the non-breeding season (autumn/winter) (Krebs et al., 1995). Mice may travel around 100 m to forage for food (GRDC, 2017b). Reduced plant cover is reported to deter movement of mice (AGRI-FACTS, 2002). Mice prefer wheat or barley grains over lentils. The only in-crop rodenticide bait approved in Australia is zinc phosphide coated wheat, but mice in barley fields may not consume wheat bait if there are abundant supplies of equally preferred barley grain (Henry et al., 2021).

Damage from field insects and other arthropod pests is not generally a major factor for barley crops, although significant damage can occur if conditions favouring the build-up of pest populations occur. Potential insect and arthropod pests of barley in Australia include: blue oat mite (*Penthaleus* spp.), redlegged earth mite (*Halotydeus destructor*), Bryobia mites (*Bryobia* spp.), Balaustium mite (*Balaustium medicagoense*), cutworms (*Agrotis* spp.), aphids (*Rhopalosiphum padi, R. maidis* and *Metopolophium dirhodum*), earwigs (*Nala lividipes*), common armyworm (*Leucania convecta*), *Helicoverpa* spp., pasture webworm (*Hednota* spp.), pasture cockchafers (*Acrossidius tasmaniae*),

grass anthelids (*Pterolocera* sp.), lucerne flea (*Sminthurus viridis*), leaf hoppers (family: *Cicadellidae*), millipedes (*Ommatoiulus moreleti*), slaters (*Porcellio scaber*, *Armadillidium vulgare* and *Australiodillo bifrons*) and locusts (*Chortoicetes terminifera*, *Austroicetes cruciata* and *Phaulacridium vittatum*). Management of insects in barley crops is similar to that of wheat and involves cultivation, good weed control and the use of insecticide sprays. Slugs (*Deroceras reticulatum*, *D. invadens*, and *D. laeve*) and snails (*Cernuella virgate*, *Cochlicella acuta*, *Theba pisana* and *Prietocella barbara*) can also cause damage to barley crops, especially in the early seedling stage (GRDC, 2016b).

Nematodes are microscopic, worm-like animals that can cause yield loss to crops. Two important nematodes affecting barley and other cereals are the root-lesion nematodes (*Pratylenchus* spp.) and the cereal cyst nematode (*Hererododera avenae*). The cereal cyst nematode can cause yield losses of up to 80%. Nematode management includes cultivation of tolerant or resistant cereal varieties and rotation with resistant crops to reduce nematode populations (GRDC, 2016b).

Pathogens, particularly fungi and viruses, can reduce grain yield and quality in barley. Disease management strategies include using resistant varieties and rotation with non-host crops. The main diseases that affect barley in Australia are: barley yellow dwarf virus, cereal yellow dwarf virus, Fusarium head blight (*Fusarium graminearum*), covered smut (*Ustilago segetum var. hordei*), loose smut (*U. nuda*), leaf rust (*Puccinia hordei*), barley grass stripe rust (*Puccinia striiformis*), net blotch net form (*Pyrenophora teres f. teres*), net blotch spot form (*P. teres f. maculate*), powdery mildew (*Blumeria graminis f.s.p. hordei*), scald (*Rhynchosporium secalis*), stem rust (*Puccinia graminis*), crown rot (*Fusarium pseudograminearum*), common root rot (*Bipolaris sorokiniana*), take-all (*Gaeumannomyces graminis var. tritici*), rhizoctonia root rot (*Rhizoctonia solani*), pythium root rot (*Pythium sp.*), wirrega blotch (*Drechslera wirreganensis*), and ringspot (*Drechslera campanulata*) (GRDC, 2016b, a; DAFWA, 2017).

7.3 Other biotic interactions

Endophytic actinobacteria, belonging to the genera *Streptomyces, Microbispora, Micromonospora* and *Nocardioidies*, have been isolated from surface sterilised healthy wheat and barley plants (Coombs et al. 2004). Actinobacteria are recognised as prolific producers of bioactive compounds and may have a role in disease resistance and maintaining the health of the plants (Conn and Franco, 2004; Coombs et al., 2004).

Arbuscular mycorrhizal fungi and endophytic fungi have also been associated with barley (Hause et al., 2002; Waller et al., 2005). Such symbioses can improve the plants nutrient uptake and can protect the plant from disease resistance and abiotic stress.

SECTION 8 WEEDINESS

Barley shares some characteristics with known weeds, such as self-compatibility, wind-pollination (although it is predominantly self-pollinating, see Section 4.2) and the ability to germinate or to produce some seed in a range of environmental conditions (Section 2.1). However, it lacks most characteristics that are common to many weeds, such as long lived seed (Section 4.4), rapid growth to flowering (Section 4.5), continuous seed production as long as growing conditions permit (Section 4.1.2), very high seed output (Section 3.2), high seed dispersal and long-distance seed dispersal (Section 4.3) (Baker, 1965; Keeler, 1989).

During domestication of the modern barley plant, characteristics that benefited farmers were selected. Mutations conferring non-shattering seed heads were selected to enable effective harvesting (Pourkheirandish et al., 2015). Losing the primary seed dispersal system of wild barley

reduces the fitness of cultivated barley outside agricultural environments. Many barley cultivars have also been bred to have low seed dormancy (see Section 4.4).

All cereals, especially barley, have been reported to be allelopathic. A hundred years of breeding has resulted in a decrease in allelopathic activity in barley, although there is considerable variation between cultivars (Bertholdsson, 2004; Belz, 2007). In eight current Australian commercial cultivars and elite breeding lines, the weed suppressive ability of different barley cultivars, as measured by reduced weed seed yield, varied more than twofold (Mahajan et al., 2020).

8.1 Weediness status on a global scale

Barley is naturalised in many countries, which are located in every continent except Antarctica. In some of these countries barley has been reported as an agricultural weed, and much less frequently barley has been reported as an environmental weed (Randall, 2017).

An important element in predicting weediness is documented weediness of plants from the same taxon (Panetta, 1993). Among the 32 species of the *Hordeum* genus, the three species *H. murinum*, *H. marinum* and *H. jubatum* have become established as weeds in many parts of the world (von Bothmer et al., 1995; Randall, 2017). *H. murinum* and *H. marinum* are annual, originally Mediterranean species, and *H. jubatum* is a perennial North American species (von Bothmer et al., 1995).

8.2 Weediness status in Australia

Barley is a cultivated cereal that often escapes into the wild. It is naturalised in all six states of Australia, but not the Northern Territory (<u>Atlas of Living Australia</u>, accessed 5 Oct 2021; <u>VicFlora</u>, accessed 5 Oct 2021). Barley can be a minor weed in natural ecosystems, but is primarily an agricultural or ruderal weed (Groves et al., 2003). Barley is frequently found on roadsides in Australia (<u>NSW FloraOnline</u>, accessed 5 Oct 2021) and Europe (Eastham and Sweet, 2002), likely due to grain spills during transport.

Other Hordeum species present in Australia include *H. marinum* (including subspecies *gussoneanum* also known as *H. hystrix*), *H. murinum* (including subspecies *glaucum* and *leporinum* also known as *H. glaucum* and *H. leporinum*) and *H. secalinum* (Atlas of Living Australia, accessed 5 Oct 2021; VicFlora, accessed 5 Oct 2021). *H. murinum*, or barley grass, is a widespread weed (NSW FloraOnline, accessed 5 Oct 2021) which is a major agricultural weed in Australian grain growing regions (Llewellyn et al., 2016) and *H. marinum* is a weed of disturbed sites and saline areas (NSW FloraOnline, accessed 5 Oct 2021). However, none of these species can cross with cultivated barley under natural conditions (see Section 9.2).

8.3 Weediness in agricultural ecosystems

Volunteer barley is a weed of canola, other cereals, pastures and vegetables (Randall, 2017). For example, in a Canadian study where canola crops were grown the year after barley crops, in 50% of the canola crops volunteer barley was the dominant weed species and in 25% of the canola crops volunteer barley was the second most common weed species (Harker et al., 2015). Unmanaged volunteer barley significantly impacts canola production; even a low volunteer barley density of 10 plants/m² emerging at the same time as a canola crop is reported to reduce yield by about 12% (O'Donovan, 1992).

Volunteer barley can also reduce the yield of wheat crops, and volunteer barley seeds mixed with wheat seeds at harvest can decrease the grain grade and price (O'Donovan et al., 2007; Jhala et al.,

2021). In spring wheat grown at moderate density (150-200 wheat plants/m²), unmanaged volunteer barley at 10 plants/m² is reported to reduce yield by 13-17%. Seeding wheat at a higher rate alleviates losses due to volunteer barley (O'Donovan et al., 2007).

8.4 Weediness in natural ecosystems

In Victoria, the environmental weed risk rating of barley is assessed as medium, which is the second lowest rating in a scale of five. Barley is considered moderately invasive and able to colonise an extensive range of susceptible habitat types, but rarely has significant impact on natural systems (White et al., 2018).

In the southwest region of Western Australia, the environmental weed risk rating of barley is medium, which is the middle ranking in a scale of five. Plants with medium weed risk rating can be used, but it is recommended that offsite spread should be monitored and managed. In other regions of Western Australia, the environmental weed risk rating of barley is low or negligible (Environmental weed risk assessments, accessed 6 Oct 2021).

No environmental weed risk ratings were found for barley in other Australian states and territories. It is likely that barley would pose a medium environmental weed risk in temperate regions of Australia (as assessed in Victoria and southwest Western Australia) and a lower weed risk in subtropical or tropical regions of Australia (as assessed in northern regions of Western Australia).

8.5 Control measures

If harvest efficiency is low, volunteer barley can grow densely in a subsequent crop. Shallow tillage after harvest, followed by irrigation, will germinate much of the seed lying on the surface. After germination, shallow tillage or the application of herbicide will kill volunteer plants (Ogg and Parker, 2000).

During the growing season, volunteer barley in broadleaf crops can be controlled using selective herbicides, such as acetyl-CoA carboxylase inhibitors (Jhala et al., 2021). Barley volunteers in wheat crops are harder to control. Selective cultivation early in the season can control volunteer barley in interrow spaces, but volunteers in the rows will persist (Ogg and Parker, 2000). In the US, the herbicide sulfosulfuron (MON 37500) provides reasonable control of volunteer barley in wheat, but can also damage the wheat crop. In imidazolinone-resistant wheat cultivars, herbicides from the imidazolinone class can be used to manage volunteer barley (O'Donovan et al., 2007), unless the barley is also an imidazolinone-resistant cultivar.

SECTION 9 POTENTIAL FOR VERTICAL GENE TRANSFER

Barley has three gene pools in the genus *Hordeum*, based on relationships to barley and the ease of interspecific hybridisations. The primary gene pool comprises *H. vulgare* ssp. *vulgare* and *H. vulgare* ssp. *spontaneum*, the secondary gene pool consists of *H. bulbosum* L., and the tertiary gene pool includes the remaining *Hordeum* species (von Bothmer et al., 1995).

9.1 Intraspecific crossing (primary gene pool)

H. vulgare ssp. *spontaneum* is the only wild *Hordeum* taxon that is cross-compatible and fully interfertile with cultivated barley. *H. vulgare* ssp. *vulgare* and *H. vulgare* ssp. *spontaneum* are morphologically similar and hybrids of the two subspecies show normal chromosome pairing and segregation in meiosis and are fully fertile. When *H. vulgare* ssp. *vulgare* and *H. vulgare* ssp. *spontaneum* grow together, spontaneous hybridisation occurs sporadically (Nevo, 1992). In Israel, where cultivated barley and wild barley grow in proximity, there is ongoing gene flow from barley

cultivars to *H. vulgare* ssp. *spontaneum* populations (Hubner et al., 2012). Barley breeding programs make extensive use of *H. vulgare* ssp. *spontaneum* accessions (Rehman et al., 2021). *H. vulgare* ssp. *spontaneum* is not known to be present in Australia.

9.2 Interspecific and intergeneric crossing

9.2.1 Interspecific

With a few exceptions the isolation barriers (usually hybrid sterility) are very strict between the *Hordeum* species and there is little or no genetic exchange in nature due to a lack of chromosome pairing, even where two taxa have sympatric distribution (von Bothmer et al., 1995).

9.2.2 Intergeneric

Intergeneric crosses invariably require the application of growth regulators during crossing, followed by embryo rescue (Fedak, 1992).

9.3 Crossing under experimental conditions

There are a number of problems associated with interspecific crossing of barley including hybrid instability and chromosome pairing, endosperm degeneration and hybrid infertility (Pickering and Johnston, 2005). Nonetheless, a number of interspecific crosses between *H. vulgare* and wild *Hordeum* species have been performed using tissue culture techniques (see below and Table 2). Both ploidy level and taxonomic group are important for crossability. Seedling lethality is common in the resulting hybrids and the majority are seed and pollen sterile (Von Bothmer and Jacobsen, 1986; Ellstrand, 2003).

Intergeneric crosses have also been performed with *in vitro* techniques between barley and various species from the genera *Triticum*, *Aegilops*, *Elymus* and *Secale*, plus *Psathyrostachys fragilis* and *Thinopyrum intermedium* (see Table 2). Intergeneric hybrids are usually sterile, very few respond to colchicine doubling and backcrossing can be difficult. To transfer genetic material, recombination must be induced to overcome the strong meiotic pairing control mechanisms (Fedak, 1992).

9.3.1 H. bulbosum (secondary gene pool)

Hordeum bulbosum is a perennial, highly self-incompatible species that occurs as both a diploid and an autotetraploid (von Bothmer et al., 1995). The genomes of *H. vulgare* and *H. bulbosum* are genetically very closely related and the two can be readily crossed under artificial conditions. However, seed setting on crosses between some *H. vulgare* varieties and *H. bulbosum* can be very low due to incompatibility, which manifests as pollen tube bursting within stylar tissue (Pickering and Johnston, 2005).

In crosses between *H. vulgare* and diploid *H. bulbosum*, the *H. bulbosum* genome is quickly eliminated in over 95% of hybrids, resulting in haploid barley embryos. Homozygous diploid barley lines can be produced from the haploid plants through application of colchicine (Devaux, 2003). Chromosome elimination is strongly influenced by the parental genotypes and temperature during embryo formation, and true hybrids can still occur. However, due to endosperm degeneration, embryos must be rescued to regenerate plants. In addition, diploid true hybrids are infertile and must be treated with colchicine to double the chromosome number and restore fertility (Pickering and Johnston, 2005).

Crosses between *H. vulgare* and tetraploid *H. bulbosum* can produce triploid hybrids. These are generally infertile, although partially fertile triploid hybrids exist (Pickering and Johnston, 2005).

A considerable number of fertile diploid introgression lines have been derived from tetraploid or triploid *H. vulgare* x *H. bulbosum* hybrids for use in barley breeding programs (Wendler et al., 2015). *H. bulbosum* is the only species other than *H. vulgare* that has been successfully used in barley breeding to date (Rehman et al., 2021).

H. bulbosum is not reported to be present in the environment in Australia (<u>Atlas of Living Australia</u>, accessed 19 October 2021).

9.3.2 Other interspecific hybrids (tertiary gene pool)

H. vulgare has been crossed to numerous other *Hordeum* species using tissue culture and embryo rescue techniques (see Table 2). The hybrids are almost totally sterile, and the genome of cultivated barley does not readily recombine with the genome of the other species, therefore genetic material is generally not exchanged (Jorgensen et al., 1986). In several interspecific combinations involving various wild *Hordeum* species and either *H. vulgare* or *H. bulbosum*, chromosome elimination occurs, resulting in haploids of one of the parents (Von Bothmer and Jacobsen, 1986). Plant regeneration from calli of hybrids has been used in some studies to induce karyotypic variations and subsequent transfer of genetic material (Jorgensen et al., 1986; Jorgensen and Andersen, 1989).

<u>Triticum</u>

Intergeneric hybrids can be obtained between barley cultivars and diploid, tetraploid and hexaploid wheats (see Table 2) (Fedak, 1992), but only with extensive intervention such as hormone applications, chemical treatment and embryo rescue (Koba et al., 1991; Molnar-Lang and Sutka, 1994; Molnar-Lang et al., 2000). There is no evidence that cultivated wheat x barley hybrids exist naturally (Eastham and Sweet, 2002). Barley and wheat chromosomes normally do not pair in the hybrids, which are sometimes referred to as *Tritordeum* (Shepherd and Islam, 1992). Crosses between diploid barley and hexaploid wheat (*Triticum aestivum* L.) are most common, and this combination forms the focus of the following discussion.

Wheat x barley hybrids are usually wheat-like in morphology and completely self-sterile, but female fertile (Fedak, 1992). Many wheat x barley hybrids have been produced with barley as the female parent. The chromosome numbers of most of the resulting hybrids are 2n=28 and the chromosomes are somatically stable. Chromosome number at meiosis is more variable, and meiotic chromosome pairing is generally low. In addition, backcross lines are difficult to produce because of pistilloidy (the conversion of other floral parts into pistils) and/or male and female sterility in the backcross plants (Fedak, 1992).

Hybrids with wheat as the maternal parent are more difficult to obtain due to low crossability and result in more variable chromosome numbers and low yield in the hybrids (Fedak, 1992). This combination, however, can avoid the problem of pistilloidy in backcross progeny (Taketa et al., 1998). Backcross plants can still be difficult to obtain, but tissue culture can be used to multiply wheat x barley hybrids and produce enough plants for pollination (Molnar-Lang et al., 2000; Molnar-Lang et al., 2005).

The majority of reports of wheat x barley crosses have used varieties with high crossability in intergeneric crosses, which is controlled by parental genotype, but poor agronomic traits. In a study by Molnar-Lang et al. (2000), winter wheat x winter barley hybrids between agronomically useful varieties were produced. In this study, a six-row barley was the male parent and embryo rescue was

used to produce hybrids. The hybrids showed a high degree of male and female sterility and reduced seed set. Thirteen barley cultivars tested as pollinators could not be crossed with wheat.

Aegilops

In a cross with *Aegilops crassa* (syn. *Triticum crassum*), vigorous but sterile hybrids were produced in tissue culture (Fedak and Nakamura, 1981). In contrast, only subviable hybrids were obtained in the cross between barley and *Ae. squarrosa* (syn. *Triticum tauschii; Ae. triuncialis*) (Fedak, 1992).

<u>Elymus</u>

Some species of *Hordeum* and some of *Elymus* can intercross naturally. However, hybrids between *H. vulgare* and *Elymus* species have only been produced with embryo rescue. The majority of the hybrids involved barley with tetraploid *Elymus* species (see Table 2). Viable hybrids are generally vigorous, self-sterile and difficult to backcross. There is little homology between the genomes and intergenomic pairing does not usually occur (Mujeeb-Kazi, 1985; Fedak, 1992).

Hybrids involving hexaploid *Elymus* species have also been produced. For example, Torabinejad & Mueller (1993) obtained sterile hybrids of the Australian hexaploid species *E. scabrus* and *E. rectisetus* with *H. vulgare* using embryo culture.

<u>Secale</u>

Crosses between *H. vulgare* and species of *Secale* are characterised by high seed set and a relatively high yield of embryos, but also a very high seedling necrosis from some cultivar combinations. Various progeny can be obtained using embryo rescue including haploids, hybrids with incomplete genomic numbers and hybrids. Surviving hybrids, sometimes referred to as *Hordecale*, are only reasonably vigorous and are self-sterile and often completely sterile. Most of the hybrids lack pairing between chromosomes, precluding any intergenomic gene transfer (Fedak, 1992).

In crosses between cultivated rye (*Secale cereale*) and barley, pre-fertilisation barriers mean that rye pollen growth is retarded in the style after initiation (Heslop-Harrison, 1982). Post-fertilisation barriers also exist, and *H. vulgare* and *S. cereale* crosses are incompatible because of an early abortion of the endosperm and embryo (Bajaj et al., 1980). Wojciechowska & Pudelska (1992) overcame incompatibility barriers using embryo rescue, tissue culture and colchicine treatment to produce barley x rye hybrids. The numbers of embryos obtained was low and lethality of seedlings was strong: from 62 crosses of different varieties, only 69 seedlings and 9 plants were obtained. The plants were completely sterile and chiasma frequency was very low (Wojciechowska and Pudelska, 1992).

Trigeneric hybrids

Trigeneric hybrids involving *Hordeum, Triticum* and *Secale* are commonly produced. For example, a trigeneric hybrid can be obtained by crossing barley and *Triticale*, which is a commercially grown artificial hybrid of rye and wheat. The cross requires embryo rescue and the hybrids are generally sterile (Balyan and Fedak, 1989; Fedak, 1992).

Trigeneric hybrids can also be produced by crossing *Secale* onto *Hordeum-Triticum (Tritordeum)* hybrids, or by intercrossing *Triticale* and *Tritordeum* (for example, see Fedak and Armstrong, 1980). In some combinations, the resulting hybrids can produce viable seed without embryo rescue (Fedak, 1992).

Species	Common name	Ploidy level	Hybrids under natural conditions?	References
Hordeum vulgare	Wild barley	2x	Yes	see Section 9.1
ssp. spontaneum				
H. bulbosum	Bulbous barley	2x, 4x	No	see Section 9.3.1
H. arizonicum	Arizona barley	6x	No	Linde-Laursen & von Bothmer
				(1988)
H. bogdani	-	2x	No	Von Bothmer & Jacobsen (1986)
H. brachyantherum	Meadow barley	4x, 6x	No	Jorgensen et al. (1986)
H. brevisubulatum	_	2x, 4x, 6x	No	Jorgensen et al. (1986)
H. capense	Cape wild barley	4x	No	Jorgensen et al. (1986)
H. chilense	-	2x	No	Thomas & Pickering (1985)
H. comosum	-	2x	No	Von Bothmer & Jacobsen (1986)
H. cordobense	-	2x	No	Von Bothmer & Jacobsen (1986)
H. depressum	Dwarf barley	2x, 4x	No	Von Bothmer & Jacobsen (1986)
H. erectifolium	-	2x	No	Von Bothmer & Jacobsen (1986)
H. euclaston	Argentine barley	2x	No	Von Bothmer & Jacobsen (1986)
H. flexuosum	-	2x	No	Von Bothmer & Jacobsen (1986)
H. fuegianum	-	4x	No	Von Bothmer & Jacobsen (1986)
H. intercedens	Bobtail barley	2x	No	Von Bothmer & Jacobsen (1986)
H. jubatum	Foxtail barley	4x	No	Orton (1979)
)	· · · · · · · · · · · · · · · · · · ·		-	Jorgensen et al. (1986)
H. lechleri	_	6x	No	Von Bothmer et al. (1999)
		•		Jorgensen et al. (1986)
H. marinum	Sea barley	2x, 4x	No	Finch (1983)
H. murinum	Mouse barley	2x, 4x, 6x	No	Von Bothmer & Jacobsen (1986)
H. muticum	-	2x	No	Von Bothmer & Jacobsen (1986)
H. parodii	_	6x	No	Jorgensen et al. (1986)
H. patagonicum		2x	No	Von Bothmer & Jacobsen (1986)
H. procerum	_	6x	No	Jorgensen et al. (1986)
H. pubiflorum	Antarctic barley	2x	No	Von Bothmer & Jacobsen (1986)
H. pusillum	Little barley	2x	No	Von Bothmer & Jacobsen (1986)
H. roshevitzii		2x, 4x	No	Jorgensen et al. (1986)
H. secalinum	_	4x	No	Von Bothmer & Jacobsen (1986)
H. stenostachys	Centenillo	2x	No	Von Bothmer & Jacobsen (1986)
H. tetraploidum	-	4x	No	Jorgensen et al. (1986)
Triticum aestivum	Bread wheat	6x	No	Fedak (1992)
	bread wheat	0	110	Molnar-Lang et al. (2000)
T. dicoccum	Cultivated emmer	4x	No	Fedak (1992)
+	wheat			5 1 1 (1000)
T. monococcum	Einkorn wheat	2x	No	Fedak (1992)
T. persicum	Persian black wheat	4x	No	Fedak (1992)
T. timopheevi	Sanduri wheat	4x	No	Fedak (1992)
T. turgidum	Rivet wheat, Poulard wheat	4x	No	Fedak (1992)
Aegilops crassa	Persian goatgrass	6x	No	Fedak & Nakamura (1981)
Ae. sqarrosa	Barbed goatgrass	2x	No	Fedak (1992)
Elymus arenarius	Blue lime grass	6x	No	Fedak (1992)
E. canadensis	Canada wild rye	4x	No	Dahleen (1999)
E. caninus	Bearded	4x	No	Fedak (1992)
	wheatgrass			

Table 2: Species that can be crossed with *Hordeum vulgare* under experimental conditions.

Species	Common name	Ploidy level	Hybrids under natural conditions?	References
E. elongatus	Rush wheatgrass	4x	No	Dahleen (1999) Mujeeb-Kazi (1996)
E. humidus	-	6x	No	Muramatsu et al. (1993)
E. lanceolatus	Thick spike wheatgrass	4x	No	Fedak (1992)
E. mollis	American dunegrass	4x	No	Fedak (1992)
E. patagonicus	-	6x	No	Mujeeb-Kazi (1985)
E. rectisetus	-	6x	No	Torabinejad & Mueller (1993)
E. scabrus	Common wheatgrass	6x	No	Torabinejad & Mueller (1993)
E. trachycaulus	Slender wheatgrass	4x	No	Aung (1991)
Secale cereale	Rye	2x	No	Fedak (1992)
S. africanum	Wild rye	2x	No	Fedak (1992)
S. kuprijanovii	-	2x	No	Fedak (1992)
S. montanum	Mountain rye	2x	No	Fedak (1992)
S. vavilovii	-	2x	No	Fedak (1992)
Psathyrostachys fragilis (syn. Elymus fragilis)	-	2x	No	Von Bothmer et al. (1984)
Thinopyrum intermedium (syn. Elymus hispidus)	Intermediate wheatgrass	6х	No	Fedak (1992)

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Appendix A Weed Risk Assessment of Barley

Species: *Hordeum vulgare* (barley)

Relevant land uses:

- 1. Intensive¹ uses (ALUM² classification 5)
- 2. Production from dryland agriculture (ALUM classification 3.3)
- 3. Production from irrigated agriculture (ALUM classification 4.3)

Background: The Weed Risk Assessment (WRA) methodology is adapted from the Australian/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol. The questions and ratings used in this assessment are based on the South Australian Weed Risk Management Guide (Virtue, 2004). Questions 1 - 5 relate to invasiveness and questions 6 - 11 relate to impact of barley on the relevant land use areas. The terminology is modified to encompass all plants, including crop plants.

Weeds are usually characterised by one or more of a number of traits, these including rapid growth to flowering, high seed output, and tolerance of a range environmental conditions. Further, they cause one or more harms to human health, safety and/or the environment. Although barley has some traits associated with weeds, it is not generally considered an invasive weed in Australia. Groves et al. (2003) categorises barley as a minor weed in natural ecosystems, but primarily an agricultural or ruderal weed. Other than agricultural areas where it is cultivated, barley can occur along the sides of roads and railway lines that have acted as routes for its transportation.

Unless cited, information in this weed assessment is taken from this document *The Biology of* Hordeum vulgare L. (*Barley*) v3 (OGTR 2021). This WRA is for **non-GM barley volunteers** and includes non-GM herbicide tolerant varieties of this crop. Reference made to barley as a cultivated crop is only to inform its assessment as a volunteer.

¹ Intensive use includes areas of intensive horticulture or animal production, areas of manufacture or industry, residential areas, service areas (e.g. shops, sportsgrounds), utilities (e.g. facilities that generate electricity, electrical substations, along powerlines) areas of transportation and communication (e.g. along roads, railways, ports, radar stations), mine sites and areas used for waste treatment and disposal.

² ALUM refers to the Australian Land Use and Management classification system version 7 published May 2010.

Invasiveness questions	Barley
1. What is barley's ability to establish amongst existing plants?	Rating: Low to medium in all relevant land uses
	Barley is a domesticated crop that grows best under agricultural conditions. Barley volunteers would mainly be derived from seed that is shed before or during harvest and can establish in <i>dryland and irrigated cropping areas</i> , especially along the margins of fields or in subsequent crops sown on the same land where barley was grown and harvested. Seed losses can also occur in <i>intensive use areas</i> involved in the transport, storage and processing of barley; volunteers often grow along roadsides or near grain silos.
	Barley is considered a competitive crop, and barley volunteers can establish in other crops. Barley does not compete well with more vigorous plants such as established native species, pasture species or weeds.
2. What is barley's tolerance to average	Rating: Low in all relevant land uses
weed management practices in the land use?	Weed management practices (preventive, cultural and chemical) are used extensively in <i>dryland and irrigated cropping areas</i> to prevent crop yield loss. Weed management is used to some extent in <i>intensive use areas</i> , for example on roadsides to maintain good sight lines.
	Volunteer barley can be controlled by cultivation. A range of herbicides, from at least four different mode-of-action groups, are registered by the APVMA for control of volunteer barley. The registered herbicides include commonly used glyphosate and paraquat knockdown herbicides, as well as acetyl-CoA carboxylase inhibitors that selectively kill grass weeds. The imidazolinone chemical class of herbicides are effective on most barley volunteers but not on imidazolinone-resistant barley cultivars.
3. Reproductive ability of barley in the land use:	
3a. What is the time to seeding in the land	Rating: < 1 year in all relevant land uses
uses?	Barley is an annual crop that generally takes four to seven months to complete its lifecycle under agricultural conditions in Australia. Volunteer barley behaves in a similar way.
3b. What is the annual seed production in the land use per square metre?	Rating: Low to high in cropping areas; low in intensive use areas
	Barley volunteers can grow in subsequent crops in <i>dryland and irrigated cropping areas</i> . In a UK study, when wheat was grown the year after barley, and weed management practices were a pre-sowing cultivation and knock-down herbicide application, the percentage of volunteer barley grains in the

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Invasiveness questions	Barley
	harvested wheat ranged from 1.2 – 9.2% (Christian and Carreck, 1997). Considering the yields in this study, and assuming an average seed weight of 3.4 g/100 seeds for wheat and 4.2 g/100 seeds for barley (<u>Agriculture Victoria Website</u> , accessed 7 Oct 2021), volunteer barley seed production ranged from 200 – 1300 seeds/m ² . Similarly, in a US study, when wheat was grown the year after barley with an intervening summer field pea crop, and weed management practices were cultivation before each sowing, the wheat harvest contained 5.0% barley kernels by weight before cleaning (Shinn et al., 1999). Considering the yield in this study, and assuming an average barley seed weight of 4.2 g/100 seeds, the volunteer barley seed production was about 1000 seeds/m ² .
	Volunteer barley growing outside a cropping environment, for example in <i>intensive use areas</i> , would likely have lower seed production due to the lack of farmer interventions to improve crop yield.
3c. Can barley reproduce vegetatively?	The production of rooted tillers has occasionally been described as a form of vegetative reproduction, as barley tillers separated from the main plant can grow supported by the adventitious roots only. Otherwise, barley is not capable of vegetative spread.
4. Long distance seed dispersal (more than 10	10 m) by natural means in land uses:
4a. Are viable plant parts dispersed by	Rating: Unlikely to occasional in all relevant land uses
flying animals (birds and bats)?	There is no evidence that flying animals play a major factor in the dispersal of barley seeds. Corellas, galahs, house sparrows, mallard ducks, pheasants, red-winged black birds and rock pigeons fed barley seeds did not excrete any intact barley seeds.
	Barley has special bristles on the spikelets, which allows them to adhere well to the feathers of birds, and the seeds may be dispersed in this way. Viable seed may be transported on the muddy feet/legs of birds.
4b. Are viable plant parts dispersed by land	Rating: Occasional in all relevant land uses
based animals?	Barley has special bristles on the spikelet, which allows them to adhere well to the fur of larger animals, and the seeds may be dispersed in this way. Whole and undamaged barley seeds have been reported in the dung of cattle, suggesting there is the potential for large mammals to disperse viable barley seed after consumption.
	Dispersal in the hooves of animals is also probable, but not well reported, thus the frequency is not known. Rodents which hoard seeds could disperse barley seed.

Invasiveness questions	Barley	
4c. Are viable plant parts dispersed by water?	Rating: Unlikely in all relevant land uses	
	Barley seed has a high specific gravity and will typically sink in water (Derr, 1910). Thus barley seed is not well adapted for dispersal by water. Barley seed is also intolerant of waterlogging.	
4d. Are viable parts dispersed by wind?	Rating: Unlikely in all relevant land uses	
	Barley seeds are heavy and do not possess appendages that are designed to facilitate wind dispersal (e.g. they are not "winged"). Although short-range dispersal could occur in high winds, long-distance dispersal is unlikely.	
5. Long distance seed dispersal (more than	5. Long distance seed dispersal (more than 100 m) by human means in land uses:	
5a. How likely is deliberate spread via	Rating: Common in/from all relevant land uses	
people	Barley is a crop species that is deliberately transported over long distances for cultivation. During cultivation, there is typically 5-10% loss of barley grain (<u>Agriculture Victoria Website</u> , accessed 7 Oct 2021). These seeds are dispersed in the cropping area and surrounds, and may establish as volunteers.	
	Whole unprocessed barley seeds are also deliberately transported and used as supplemental feed for sheep (<u>Queensland Department of Agriculture and Fisheries</u> , accessed 7 Oct 2021), and could be dispersed in the feedlot and paddocks.	
	Barley volunteers are treated as weeds and their seeds would not be spread deliberately.	
5b. How likely is accidental spread via	Rating: Occasional to common in/from all relevant land uses	
people, machinery and vehicles?	Where barley is planted as a crop, it is common for seed to be accidentally dispersed by people, machinery and vehicles. Large quantities of harvested seed are transported to silos, and further afield for processing or replanting, and can be spilt along roadsides and railway lines, as well as near storage facilities. Seed can remain on machinery after harvesting and be dispersed when the machinery is transported or next used.	
	Where barley grows as a volunteer, accidental spread of seed by people, machinery and vehicles could occur occasionally. Cultivation of fields or mowing of weeds along roadsides could lead to spread of seeds by machinery. Barley also has special bristles on the spikelets, which allows them to adhere well to the clothes of people, and the seeds may be dispersed in this way.	

Invasiveness questions	Barley
5c. How likely is spread via contaminated produce?	Rating: Occasional in/from all land use areas.
	Barley farming in <i>dryland and irrigated cropping areas</i> is characterised by rotation with other crops, such as wheat, canola, legumes or pasture. Barley volunteers may grow in the subsequent rotation crop. In some rotation crops, the harvested seed could be contaminated with barley seed. The contaminated seed may be transported for use as animal feed and dispersed in the feedlot and paddocks, or farmers may retain a portion of the contaminated seed for replanting on other parts of their properties. Long distance dispersal via contaminated hay and forage may occur from cropping areas or from <i>intensive use areas</i> (such as along roadsides) if harvested for hay or forage.
5d. How likely is spread via domestic/farm animals?	Rating: Occasional in all relevant land uses
	If livestock are grazed in barley fields after harvest, or in areas that contain barley volunteers, then it is possible that viable barley seeds may be spread either in their hooves, fur, wool or excrement. Barley has special bristles on the spikelet, which allows them to adhere well to the fur of larger animals, and the seeds may be dispersed in this way. Some barley seeds remain intact after digestion by cattle. Survival of whole seed through other animals is not known.

Impact Questions	Barley
6. Does barley reduce the establishment of desired plants?	Rating: Reduces establishment by < 10% in all relevant land uses
	In <i>dryland and irrigated cropping areas</i> , if a field is weedy, weed management practices are usually applied prior to planting a crop. This would remove large barley volunteers capable of significantly reducing the establishment of desired crop plants.
	<i>Intensive use areas</i> may have no particular desired plants, or land managers may prefer native flora or introduced trees, bushes and shrubs. Barley is a domesticated crop plant and is not expected to be competitive amongst vigorous vegetation.

Impact Questions	Barley
7. Does barley reduce the yield or amount of desired plants?	Rating: Reduces yield/amount by < 10% or between 10-25% in all relevant land uses
	In <i>dryland and irrigated cropping areas</i> , barley is commonly used in rotation with other crops, and may emerge as a volunteer while follow-on crops are being grown. In less competitive crops, such as canola, unmanaged barley volunteers may reduce yield by over 10%. Although weed control for volunteer barley is available, the volunteers may not be managed if herbicide application is not economically justified.
	In <i>intensive use areas</i> , barley, as a domesticated crop plant, is not expected to be competitive amongst vigorous vegetation.
8. Does barley reduce the quality of	Rating: Low in all relevant land uses
products or services obtained from the land use?	In some rotation crops, if barley volunteers are present and not effectively managed before harvest, the harvested seed could be contaminated with barley seeds. Although this does not make the crop unsalable, significant levels of barley contamination may reduce the quality grade and therefore the price. For example, in wheat, having over 50 barley seeds per half litre of grain can reduce the quality of the crop (Grain Trade Australia, 2021b). Lentil markets are particularly sensitive to cereal contamination, and having more than 2 barley seeds per 200 g of lentils can lower the quality of the crop (Pulse Australia, 2021).
9. What is the potential of barley to restrict	Rating: Low in all relevant land uses
the physical movement of people, animals, vehicles, machinery and/or water?	Even as a densely planted mature crop, barley is never impenetrable. Sparser barley volunteers have low potential to restrict the physical movement of people, animals or water.
10. What is the potential of barley to	Rating: Low in all relevant land uses
negatively affect the health of animals and/or people?	Barley has been part of the human diet for thousands of years and there is no evidence that barley is toxic to humans. A small number of people do suffer from barley induced allergies via inhalation of barley flour, chiefly caused by proteins in the cereal trypsin/ α -amylase inhibitor family. Coeliac disease (gluten intolerance), characterised by damage to the intestinal wall and a failure to absorb the nutrients found in food, is an autoimmune disorder induced by an intolerance to cereal storage proteins. However, volunteer barley is very unlikely to enter the human food chain.
	When barley is used as animal feed, elevated levels of phytic acid in barley can be a problem, chelating minerals and preventing their dietary use after digestion by animals. Similarly, the lectins found in

Impact Questions	Barley
	barley grain can cause lesions in the intestinal tract and thus impair absorption of nutrients by animals. In awned barley, the awns can irritate the mouths of livestock. The proportion of volunteer barley in animal feed (e.g. hay) is unlikely to be great enough to negatively affect the health of animals. Thus the potential for barley to negatively affect the health of animals is considered low.
11. Major positive and negative effects of bar	ley on environmental health in each relevant land use:
11a. Does barley provide food and/or	Rating: Minor or no effect in all land uses
shelter for pathogens, pests and/or diseases in the land use?	Barley is associated with a number of insect pests that infect multiple crops. It is susceptible to a range of pathogens, such as viruses, nematodes and fungi, that affect primarily cereal crops. Infected barley volunteers can act as a reservoir of these pathogens, allowing them to infect crops in the subsequent growing season. It is therefore recommended to control barley volunteers in cropping areas over the summer and autumn.
	However, most of these pathogens can also use weedy grasses as a green bridge over the summer, so barley volunteers have a limited effect on pathogen persistence.
11b. Does barley change the fire regime in	Rating: Minor or no effect in all relevant land uses
the land use?	Barley has similar characteristics to other widespread grasses. The density of barley volunteers is expected to be low for all relevant land uses, and would not be expected to affect fire regimes.
11c. Does barley change the nutrient levels in the land use?	Rating: Minor or no effect in all relevant land uses
	Barley has similar characteristics to other widespread grasses. The density of barley volunteers is expected to be low for all relevant land uses, and would not be expected to affect nutrient levels.
11d. Does the species affect the degree of	Rating: Minor or no effect in all relevant land uses
soil salinity in the land use?	Barley has similar characteristics to other widespread grasses. The density of barley volunteers is expected to be low for all relevant land uses, and would not be expected to affect soil salinity.
11e. Does the species affect the soil stability	Rating: Minor or no effect in all relevant land uses
in the land use?	Barley has similar characteristics to other widespread grasses. The density of barley volunteers is expected to be low for all relevant land uses, and would not be expected to affect soil stability.

Impact Questions	Barley
11f. Does the species affect the soil water	Rating: Minor or no effect in all relevant land uses
table in the land use	Barley has similar characteristics to other widespread grasses. The density of barley volunteers is expected to be low for all relevant land uses, and would not be expected to affect the soil water table.
11g. Does the species alter the structure of	Rating: Minor or no effect in all relevant land uses
nature conservation by adding a new strata level?	Barley has similar characteristics to other widespread grasses. The density of barley volunteers is expected to be low for all relevant land uses, and would not be expected to add a new strata level.