

## REGISTRATION

## Mapping Population

# Oregon Wolfe barley genetic stocks – Research and teaching tools for next generation scientists

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## Abstract

The Oregon Wolfe Barley (OWB) mapping population (Reg. no. MP-4, NSL 554937 MAP) is a resource for genetics research and instruction. The OWBs are a set of doubled haploid barley (*Hordeum vulgare* L.) lines developed at Oregon State University from the F<sub>1</sub> of a cross between Dr. Robert Wolfe's dominant and recessive marker stocks. Exhibiting a high level of genetic and phenotypic diversity, the OWBs are used throughout the world as a research tool for barley genetics. To date, these endeavors have led to 56 peer-reviewed publications, as well as three reports in the Barley Genetics Newsletter. At the same time, the OWBs are widely used as an instructor resource at the K–12, undergraduate, graduate, and professional levels. They are currently used at universities and/or institutes in German, Italy, Norway, Spain, and

**Abbreviations:** AC, anther culture; AFLP, amplified fragment length polymorphism; DH, doubled haploid; HB, *Hordeum bulbosum*; ISS, Informative and Spectacular Subset; OSU, Oregon State University; OWB, Oregon Wolfe Barley; OWB-D, Oregon Wolfe Barley population parent with dominant marker stock; OWB-R, Oregon Wolfe Barley population parent with recessive marker stock; PCR, polymerase chain reaction; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STS, sequence-tagged site.

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the United States and are currently being developed further for educational use in other countries. Genotype and phenotype data, lesson plans, and seed availability information are available herein and online.

### Plain Language Summary

The Oregon Wolfe Barleys (OWBs) are a resource for genetics research and instruction. They are a population of barley plants that display a high degree of variation for traits such as height, leaf and seed color, and disease resistance as a result of differences in their genetic makeup. Because of these unique qualities, the OWBs have been used throughout the world as a research tool for barley genetics, and these endeavors have led to 56 peer-reviewed publications. At the same time, the OWBs are widely used as an instructor resource at the K–12, undergraduate, graduate, and professional levels. They are currently used at universities and/or institutes in German, Italy, Norway, Spain, and the United States and are currently being developed further for educational use in other countries. Genotype and phenotype data, lesson plans, and seed availability information are available herein and online.

## 1 | INTRODUCTION

The Oregon Wolfe Barleys (OWBs) are a mapping population (Reg. no. MP-4, NSL 554937 MAP) of barley (*Hordeum vulgare* L.) doubled haploids (DHs) developed at Oregon State University (OSU)—hence the “Oregon” in “OWB”—as an international resource for plant genetics research and instruction. Efforts to develop the OWBs began more than 50 years ago as Dr. R. I. (Bob) Wolfe—hence the “Wolfe” in “OWB”—worked to systematically integrate dominant and recessive alleles at several loci into contrasting marker stocks. As Dr. Wolfe conducted this work prior to the advent of molecular marker genotyping technologies, he relied on his observation of morphological traits to track the integration of their underlying dominant and recessive alleles. Once molecular marker technologies became available decades later, crossing the two OWB marker stocks created an optimal biparental mapping population which has since served as a foundational resource for identifying the genetic loci controlling a range of traits in barley related to floral morphology, domestication, growth habit, and disease resistance, among others.

Dr. Wolfe’s initial reliance on morphological traits to develop the OWB marker stocks produced an additional, highly beneficial outcome. The vast majority of traits in living organisms are quantitative, meaning they depend on the collective action and interaction of many genes each having a small influence. The effects of these genes are therefore difficult to disentangle, and the effects of each individual gene are not clearly or visibly apparent to the naked eye. In contrast, the qualitative single-locus morphological traits which Dr. Wolfe used were observable with sufficient ease that he could rely

on the phenotypes alone to track the underlying alleles in the absence of genetic markers. These highly visible traits are discernable to even the untrained eye, and therefore the OWBs represent an ideal instructional resource for demonstrating the concepts of genetics and heredity to students.

Over the years, molecular marker technologies and genomics tools have rapidly evolved, but the fundamental principles of heredity, which these tools are used to understand and explain, remain unchanged. By encapsulating and demonstrating these principles, the OWBs have traveled the world, serving as both research and teaching tools for the next generation. This work aims to describe the development of the OWBs, summarize their use in genetic studies, and highlight the educational activities designed by several institutions to-date that leverage the OWBs.

## 2 | METHODS

Dr. Wolfe systematically developed the parents of the OWBs by backcrossing recessive and dominant alleles at multiple morphological trait loci into two respective parents (summarized in Wolfe, 1972; Wolfe & Franckowiak, 1991). The two resulting parents are referred to as OWB-D (i.e., dominant marker stock) and OWB-R (i.e., recessive marker stock), respectively. The morphological trait loci at which the two parents differ are summarized in Table 1.

Following their development, the OWB-D and OWB-R stocks were subsequently crossed to generate an F<sub>1</sub> generation. From these F<sub>1</sub> plants, a population of DHs were created using the *H. bulbosum* (HB) method (Chen & Hayes, 1989).

TABLE 1 Morphological and phenological trait loci segregating in the Oregon Wolfe Barley populations.

Locus	Gene Name	Chromosome	Phenotype	GenBank Reference
<i>CER</i> ( <i>Cer-yy</i> )	<i>KCS</i>	1H	Wild type ( <i>Cer</i> )/glossy spike ( <i>cer</i> )	
<i>BLP</i>	<i>Amino acid (tyrosine) transporter</i>	1H	Black ( <i>Blp</i> )/yellow lemma and pericarp ( <i>blp</i> )	Pangenome (2020): [Horvu_13821_1H01G536500]
<i>Mla</i>	<i>Mla, RGH1</i>	1H	Powdery mildew resistant ( <i>Mla</i> )/susceptible ( <i>mla</i> )	GenBank: [OP561810.1]
<i>VRS1</i>	<i>HvHox1</i>	2H	Two-row ( <i>Vrs1</i> )/six-row inflorescence ( <i>vrs1</i> )	GenBank: [AB489122.1]
<i>ZEO1</i>	<i>HvAP2</i>	2H	Dwarf plant with compact head ( <i>Zeo1</i> )/normal height and head ( <i>zeo1</i> )	GenBank: [KC898653.1]
<i>WST1</i>		2H	Wild type ( <i>Wst</i> )/White variegation in young seedlings ( <i>wst</i> )	
<i>ALM1</i>	<i>HvGLK2</i>	3H	Green ( <i>Alm</i> )/albino lemma and nodes( <i>alm</i> )	GenBank: [LC570959.1]
<i>Pub1</i>		3H	Pubescent ( <i>Pub1</i> )/hairless leaf blade ( <i>pub1</i> )	
<i>HSH</i>		4H	Hairs ( <i>Hsh</i> )/lack of hairs on lower leaf sheaths ( <i>hsh</i> )	
<i>BKN3</i>	<i>Knox2</i>	4H	Hooded ( <i>BKn3, Kap</i> )/awned florets ( <i>bkn3, kap</i> )	GenBank: [AF022390.1]
<i>VRN-H2</i>	<i>ZCCT-H</i>	4H	Winter ( <i>VrnH2</i> )/spring growth habit ( <i>vrnH2</i> )	AY485977 and AY485978
<i>SRH</i>	<i>Enhancer region of SMR-like</i>	5H	Long ( <i>Srh</i> )/short hairs on rachilla ( <i>srh</i> )	GenBank: [XM_045094026] for SMR-like gene
<i>VRN-H1</i>	<i>HvBM5A</i>	5H	Spring ( <i>VrnH1</i> )/winter growth habit ( <i>vrnH1</i> )	AY785826
<i>ROB1</i>	<i>CAD?</i>	6H	Green ( <i>Rob</i> )/orange lemma and nodes ( <i>rob</i> )	
<i>WX</i>	<i>GBSS-1</i>	7H	Wild type ( <i>Wx</i> )/waxy endosperm starch ( <i>wx</i> )	GenBank: [AF486518.1]
<i>NUD</i>	<i>ERF family transcription factor</i>	7H	Hulled ( <i>Nud</i> )/hulless seed ( <i>nud</i> )	GenBank: [AP009567]
<i>LKS2</i>	<i>SH1-family transcription factor</i>	7H	Long ( <i>Lks2</i> )/short awns ( <i>lks2</i> )	GenBank: [AB678347.1]

A second set of DHs were subsequently developed using the anther culture (AC) method (Cistué et al., 2011).

### 3 | CHARACTERISTICS

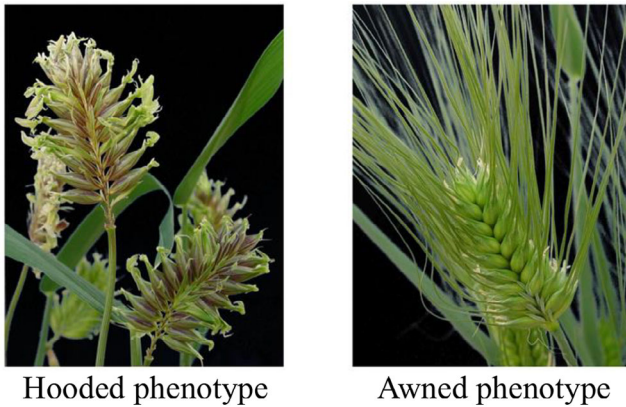
#### 3.1 | Morphological trait variation

Summarized in Table 1, the morphological loci at which the OWB-D and OWB-R differ control traits related to domestication, growth habit, and disease resistance, among others. For example, *VRS1* is a domestication locus, encoding the two-row/six-row inflorescence type. The two- to six-row *vrs1* mutation arose multiple times in different regions, resulting in improvements in yield that helped to establish barley as a founder crop for Near East Neolithic civilization (Komatsuda et al., 2007). The *Kap* gene, encoded by *BKN3*, contains a homoeotic mutation whereby the developing awn is supplanted by a duplicate spikelet (Müller et al., 1995; Figure 1), resulting in what is commonly referred to as a “hooded” floret. The *LKS2* locus controls awn length and is epistatic to

*Kap* (Yuo et al., 2012). Recessive *lks2/lks2* plants may possess the dominant *Kap* allele but will not present the hooded phenotype because it is masked by the *lks2* recessive allele (Figure 2). Lastly, due to different alleles at the *Mla* locus, the OWB marker stocks contrast in response to the powdery mildew pathogen *Blumeria hordei* (Halterman & Wise, 2004; Wise et al., 2024).

#### 3.2 | Molecular marker genotyping, linkage maps, QTL analysis, and genome assembly

Costa et al. (2001) provided the first published report on the OWBs, based on 94 of the HB-derived DH lines. In total, 734 markers were scored on this set of 94 progeny and the two parents. Markers included the original twelve morphological markers targeted by Dr. Wolfe (Table 1) as well as an array of molecular marker types available at that time: 87 restriction fragment length polymorphisms (RFLPs), five random amplified polymorphic DNAs (RAPDs), one sequence-tagged site (STS), one intron fragment length polymorphism, 33 simple



**FIGURE 1** The hooded and awned phenotypes of barley. The hooded phenotype consists of an additional spikelet (i.e., flower) in the place of an awn as the barley spikes develop. This homoeotic mutation is caused by a 305-bp duplication within an intron in the hooded allele (*Kap*), a transcription factor that regulates shoot and awn development that is not present in the non-hooded allele (*kap*) (Müller et al., 1995). Heterozygous plants display the hooded phenotype, meaning that the *Kap* allele is dominant to the *kap* allele. Images and text modified from Wise et al. (2024).

sequence repeats (SSRs), and 586 amplified fragment length polymorphisms (AFLPs).

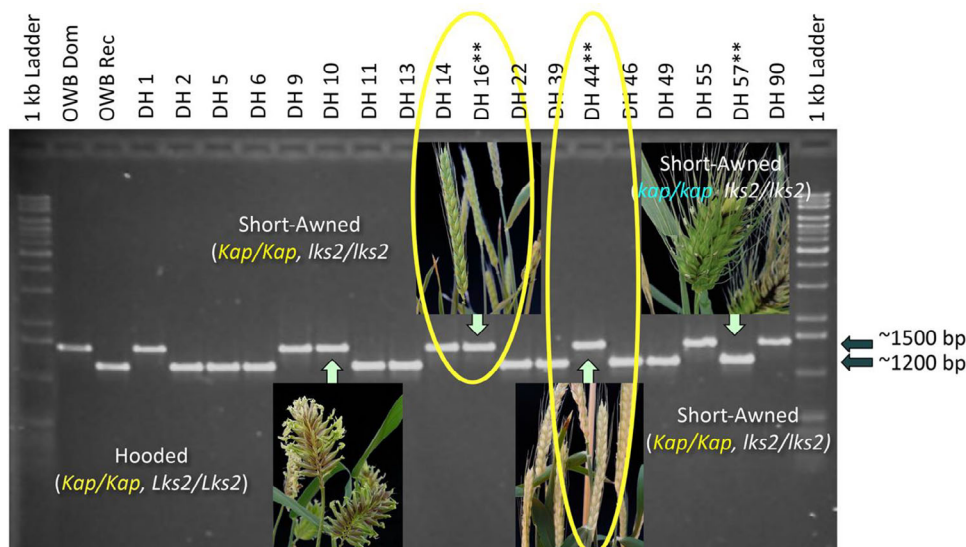
The integration of the morphological trait markers with molecular markers represented an important first step in aligning classical and contemporary linkage maps. The integrated linkage map revealed technical and biological phenomena of interest. Technically, the RAPD and AFLP markers—while considered cost-effective and high-throughput at the time—were often clustered, and missing data were problematic. Furthermore, as dominant markers, these were less informative than co-dominant alternatives. Biologically, the integrated linkage maps revealed localized areas of segregation distortion, which the authors of Costa et al. (2001) hypothesized could be related to gametophytic selection during the DH process. The *ZEO1* locus, for example, encodes alleles determining plant height (Houston et al., 2013). The dominant dwarf allele (*Zeo1*) appeared at a significantly lower frequency than the expected 1:1 ratio for  $F_1$ -derived DHs, suggesting this allele may have a pleiotropic effect on fitness during the tissue culture phase of the DH process.

Following the initial genotyping and mapping efforts of Costa et al. (2001), subsequent research projects added additional types of markers to the OWB dataset as they were developed. GrainGenes provides an online open-access platform that researchers have utilized to store OWB marker datasets (<https://wheat.pw.usda.gov/ggpages/maps/OWB/>). Sponsored by the USDA, GrainGenes is an international database and web resource for barley, wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), oat (*Avena sativa* L.), and other small grains that has been providing

genetic and genomic data to the plant research communities for more than three decades (Yao et al., 2022). Over the years, genetic maps created using the OWBs were curated into GrainGenes database records upon publication, and interactive genetic maps were made available for use in CMap, a comparative mapping tool (Youens-Clark et al., 2009). CMap enables researchers to visualize and align maps from different populations to compare the order and positions of molecular markers, mapped genes, and quantitative trait loci (QTL). Curated map sets include *Barley, OWB* (Wolfe et al., 1996); *Barley, OWB, 2004* and *Barley, OWB, 2005* (P. M. Hayes, pers. comm.); *Barley, OWB, SNP* (Rostoks et al., 2005); and *Barley, OWB, OPA2008* (Szűcs et al., 2009). By incorporating the 159 RFLP and STS markers from the original 1996 maps with 2,383 single nucleotide polymorphism (SNP) and Diversity Arrays Technology (Wenzl et al., 2004) markers generated in 2008, the *Barley, OWB, OPA2008* map highlights the progress in marker development made over the course of twelve years (Szűcs et al., 2009). The *Barley, OWB, OPA2008* map also contains known genes and 154 QTL related to malting quality.

Over time, markers that were dominant, prone to missing data, and/or equivocal allele calls were dropped, leading to progressively more robust mapping datasets. The most recent linkage map was reported by Cistué et al. (2011) and is based on 1,328 SNPs and a new subset of OWB DHs derived by AC. These authors compared linkage maps developed using DHs derived by the HB method, a technique which samples gametes formed in megagametogenesis, with those developed via AC, which samples gametes formed in microsporogenesis. Only minor differences in linkage map length were found, and, importantly, there were no differences in locus ordering. Using both sets of OWB DHs (HB and AC), segregation distortion associated with the *ZEO1* locus was again detected, as in Costa et al. (2001). Furthermore, the use of the high-quality SNP marker data unequivocally identified genetically identical DHs within the HB and AC sets, even as earlier marker datasets showed these lines to be highly similar but not identical. The authors hypothesized that the differences observed using earlier marker datasets were due to errors in allele scoring, and that the lines with indistinguishable SNP genotypes are indeed genetically identical. The most likely explanation for the presence of these genetically identical sets of lines is that multiple haploids were inadvertently advanced from tissues regenerated from a single embryo. After removing genetically identical lines, the resulting OWB populations consisted of 82 and 93 individuals derived by HB and AC, respectively, for a total population size of 175. A detailed explanation of the genetically identical lines and the resulting re-numbering is available online (<https://barleyworld.org/data>).

Cistué et al. (2011) used the full set of 175 DHs and 1,328 SNPs for linkage map construction and for demonstrating the



**FIGURE 2** Amplification of the *Kap* gene alleles, encoded by *BKN3*, to dissect the epistatic interaction between *Kap* and *Lks2* alleles. The *Kap* and *LKS2* loci each encode proteins that influence the production of barley spikes. The *LKS2* locus determines whether the spikes will possess long awns (*Lks2*/\_) or short awns (*lks2*/*lks2*). The *Kap* allele will form an additional spikelet in place of an awn (i.e., “hooded”) if there is a dominant *Lks2* allele present. Recessive *lks2*/*lks2* plants may have the dominant *Kap* allele but will not express the *Kap* phenotype (i.e., hooded florets) because the *lks2* alleles mask the effects of *Kap*. This is a classic example of epistasis, whereby the gene products from two or more genetic loci influence a single trait. Amplification of *Kap* in the OWB-ISS reveals the 305-bp insertion in the *Kap* allele visibly segregating from the *kap* allele. DH 16 and DH 44 (circled in yellow) represent examples of epistasis, whereby the *Kap* amplicon displays the characteristic 305-bp insertion. However, the dominant hooded *Kap* allele is masked by the recessive *lks2* allele, resulting in the short-awned phenotype. Modified from Wise et al. (2024).

utility of biparental QTL mapping of reproductive fitness phenotypes (spike length, grain number, floret number, hundred grain weight, plant height, spike number, and heading date). The OWBs have been leveraged for a range of other QTL mapping projects over the years, such as to map flowering time (Börner et al., 2002) and, most recently, non-host resistance to *Puccinia* isolates (Haghdoust et al., 2021). The OWBs have also been useful for assigning linkage map coordinates to genes of known function, including *Ds* transposon insertion sites (Cooper et al., 2004) and QTLs reported in other populations (Szűcs et al., 2009). Most importantly, the OWB linkage map was used to establish genetic map positions during the construction of the physical map of the barley genome (IBGSC, 2012).

The barley community now has a next-generation reference sequence of the barley cultivar ‘Morex’ (Barley cv. Morex V3; Masher et al., 2021) and a forthcoming barley pangenome consisting of 76 genotypes (Jayakodi et al., 2024; Leibniz IPK, 2025). Barley genome browsers regularly provide genome sequence information along with bioinformatically-derived genome annotations, including the location and structure of predicted genes. The JBrowse tool (Skinner et al., 2009), adopted by GrainGenes, also enables the alignment of curated data such as functional genes and QTL. QTL for malting quality, previously aligned on the *Barley*, *OWB*, *OPA2008* maps, are now aligned on the genome browser for Morex V3.

### 3.3 | Instructional resources

With uncanny prescience, Costa et al. (2001) identified the potential for the OWBs as a tool for genetics research and instruction, stating “The full power of this population as an interactive, collaborative teaching and research tool will come as participants generate additional genotype and phenotype data. Consider, for example, a university lab generating abundant DNA-level polymorphism while a high school science class measures plant height and heading date. If each group operates in isolation, the marker data generates just another map, and the plant growth data are just another quantitative data set. However, through this collaborative network, the two can be integrated, and through QTL analysis, the determinants of the maturity and plant height can be assigned to chromosome positions. Chromosome location information, in turn, provides tools for physiology, developmental genetics, and finer structure genetic analysis. We hope that this networking will also lead to long-term partnerships.”

#### 3.3.1 | From living plants to digital image galleries

Growing the OWBs is an ideal activity to engage students in hands-on research, and, to that end, detailed growing

instructions are provided on the OWB website (accessible at <https://barleyworld.org/owb>). For users who are interested in working with living plant material but may not be equipped to grow a complete population, the OWB Informative and Spectacular Subset (OWB-ISS) was formed as a smaller population of manageable size and exhibits high levels of phenotypic and genotypic diversity. The OWB-ISS is comprised of the two OWB marker stock parents and 18 DH lines developed with the HB method.

While it is ideal for students to engage in growing plants for phenotyping and de novo molecular analyses, this is not always possible given staffing and infrastructure limitations. Therefore, digital image galleries of inflorescences are provided for the HB and AC populations, and a digital image gallery of seeds is available online for the HB population (accessible at <https://barleyworld.org/main/images>). Another alternative to growing live plants is the use of dried specimens which can be stored and maintained for successive generations of students without the need to cultivate new lines annually. Dried specimens allow students to conduct phenotyping studies throughout the entire duration of a course, whereas the use of live plants requires advanced preparation such that the appropriate phenological stages align with class sessions. This can be particularly challenging to achieve when considering academic calendars and necessitates significant allocation of staff and infrastructure resources. Although phenotypic studies of dried specimens are (as with photographs) limited to the preserved plant parts, this type of observation is more akin to the act of phenotyping live plants.

### 3.3.2 | OWB in the classroom

The full OWB population, the HB or AC subsets, and the OWB-ISS have been used internationally as resources for teaching the principles of Mendelian analysis, linkage mapping, biparental QTL analysis, and more. These exercises may involve living plant material, the digital image galleries, and/or preserved specimens. Complete lesson plans are available online (accessible at <https://barleyworld.org/education>). Brief summaries, ordered alphabetically by country, are presented to highlight the educational efforts which have utilized OWB resources to-date:

#### *Germany – University of Düsseldorf*

The OWB population has been integrated into the University of Düsseldorf's second-year bachelor's curriculum of the "Quantitative Biology" interdisciplinary program (<https://www.qbio.hhu.de/en/>) within the Cluster of Excellence on Plant Sciences, in which up to 40 students enroll annually. The lecture series covers critical population and quantitative genetics topics, laying the theoretical groundwork for prac-

tical sessions using the OWB material. Students engage in hands-on phenotypic data collection by observing live OWB plants in the greenhouse. Divided into groups, students measure spike length and plant height as quantitative traits. Each group is also assigned two qualitative traits, such as two-row/six-row inflorescence (*VRS1*), long/short awns (*LKS2*), hooded/awned florets (*BKN3*, i.e., *Kap*), hulled/hulless seed (*NUD*), black/yellow lemma and pericarp (*BLP*), and leaf variegation (*WST1*). Students can refer to the online image galleries (accessible at <https://barleyworld.org/main/images>) when uncertain about specific traits such as caryopsis color or hull adherence. Using the R statistical programming software (R Core Team, 2021), the "R/qtl" package for R (Broman et al., 2003), and the molecular marker and linkage data available online (accessible at <https://barleyworld.org/data>), students are introduced to the fundamentals of linkage mapping and QTL analysis. They begin by constructing their own barley linkage maps using a selection of markers. Using the entire marker and linkage datasets, the students then investigate linkage between their assigned qualitative traits and molecular markers. Measurements for plant height and spike length are also used to perform QTL analyses in R. Finally, the students compile their results into a publication format, simulating the identification of genes for the investigated traits and reinforcing their understanding of the concepts.

#### *Italy – University of Bologna*

At the University of Bologna, the OWBs are used as part of the second cycle degree (i.e., 2-year master's degree) in Plant and Agricultural Biotechnology as well as in the "Biotechnologies Applied to Breeding Annual Crops" course. The 93 OWB lines developed by the AC method are used for the practical activities of the course. The objectives of these activities are to expose the students to Mendelian and quantitative genetic variation and provide hands-on experience in phenotyping, biometrical analysis, and genetic mapping of both Mendelian loci and QTL. Each OWB line is grown in pots in four replicates in the greenhouse. Students observe the hooded/awned florets (*BKN3*, i.e., *Kap*), long/short awns (*LKS2*), and two-row/six-row inflorescence (*VRS1*) as qualitative traits. The quantitative traits measured are awn length, spike length, number of florets, and plant height. These data are used for running standard biometric analyses. As an example, the collected trait data are used to show a statistically significant correlation of plant height with spike length but not with the number of florets. The students also take advantage of the publicly accessible molecular marker data. Students perform QTL mapping using MapQTL6 (Ooijen, 2009). Single-marker and interval mapping QTL analyses for both plant height and spike length show a strong logarithm of odds peak on chromosome 2H at approximately 150–160 cM, which corresponds to the *ZEO1* locus and illustrates

the concept of pleiotropy. Other results are used to illustrate QTL genetic effects, the proportion of phenotypic variation explained, and the indefinite separation between QTL and Mendelian loci. The assessment of variation for root architectural traits (e.g., number of seminal roots, root growth angle, total root length) at the seedling stage using the OWB AC population following a semi-hydroponic protocol is a planned addition to the activity, with the corresponding QTL analysis to follow.

#### Norway – Norwegian University of Life Sciences

The bachelor-level plant breeding course at the Norwegian University of Life Sciences offers an introduction to applied methods in plant breeding. The mandatory activities currently include five practicals with submission of lab reports. The OWB HB population has been used for these activities for approximately 25 years (Professor Emeritus Åsmund Bjonstad, personal communication, 2024) to demonstrate the concepts of qualitative and quantitative traits as well as QTL analysis. For qualitative traits, the students phenotype the population in a greenhouse for two-row/six-row inflorescence (*VRS1*), hulled/hulless seed (*NUD*), dwarf plant with compact head/normal height and head length (*ZEO1*), long/short awns (*LKS2*), and hooded/awned florets (*BKN3*, i.e., *Kap*). The concept of epistatic interaction is demonstrated because the long/short awned phenotype can only be observed for awned, not hooded, florets. Variation in plant height is used as an example of a quantitative trait. However, plant height is also affected by the *ZEO1* locus for dwarfing, thereby demonstrating the impact of major-effect loci on quantitative trait expression. Comparison of plant height in the  $F_1$  generation to the height of each parent is used to demonstrate heterosis. The students then perform QTL mapping using the “R/qtl” package (Broman et al., 2003) for R (R Core Team, 2021), the qualitative and quantitative trait data they recorded, and the publicly-available OWB marker data (accessible at <https://barleyworld.org/data>). Since computer skills vary among the undergraduate students, they are also provided with example phenotype data (accessible at <https://barleyworld.org/data>) that is pre-formatted for importing into R. The students generally appreciate that they can work with the same population for several exercises, and it makes the otherwise theoretical or abstract activity of QTL mapping more understandable, as they have observed and collected the phenotypic differences themselves.

#### Spain – Universidad Politécnica de Madrid

The Plant Breeding Group (<https://blogs.upm.es/geneticaymejora/en/>) of the Biotechnology and Plant Biology Department at the Universidad Politécnica de Madrid uses the OWB HB population in various courses related to plant breeding at both the undergraduate and

master's degree levels, with adjustments made according to academic level. During each academic year, three replicates of the OWB HB population is grown in a greenhouse, where students collect measurements of plant height and spike length. During laboratory sessions, students genotype the population using two polymerase chain reaction (PCR)-based molecular markers: the *Knox*-dup dominant marker and the *Bmac 0130* co-dominant SSR marker. Using these phenotypic and molecular data along with a molecular marker matrix (Chutimanitsakun et al., 2011; Cistué et al., 2011) provided by the faculty, students conduct QTL mapping for the quantitative traits using the free software MapDisto (accessible at <http://mapdisto.free.fr/>; Lorieux, 2012). This practice enables students to understand the importance of accurate phenotyping and genotyping, the differences between dominant and co-dominant molecular markers, and how to perform analyses to link molecular markers with quantitative trait variation. As an additional resource, the OWB-D and OWB-R parents were used to develop an  $F_2$  population, which demonstrates Mendelian segregation ratios (Giménez et al., 2021).

#### Spain – Ciheam-Zaragoza and the University of Lleida

The OWB population has been used for over a decade as part of an international program run by the International Center for Advanced Mediterranean Agronomic Studies (CIHEAM)-Zaragoza and the University of Lleida (<https://www.iamz.ciheam.org/agendas/xxiii-international-master-in-plant-genetics-genomics-and-breeding/>). In this 2-year biennial program, 20–25 students from 10–12 countries—primarily in West Asia, North Africa, and Europe—work with a reduced set of 82 OWB HB lines and 252 molecular markers. They are responsible for the phenotypic characterization of field-grown OWB plants for the following qualitative and quantitative traits: two-row/six-row inflorescence (*VRS1*), dwarf plant with compact head/normal height and head length (*ZEO1*), hooded/awned florets (*BKN3*, i.e., *Kap*), plant height, length of the last internode, spike length, awn length, and phenological stages. Using these data, the students perform quantitative genetic analyses using the open-access software QTL IciMapping 4.2 (accessible at <https://isbreedingen.caas.cn/software/qtlcimapping/294607.htm>; Wang et al., 2015) for both genetic map construction and QTL mapping. The master's degree program has more than 25 practical units, several of which include the OWB activities, and students are surveyed with regards to their interest in each unit. The OWB-related units received the highest score compared to all other practicals when ranked by the 2010, 2014, and 2016 student cohorts and the second-highest score when ranked by the 2012 and 2018 cohorts, thereby demonstrating the students' high level of interest in working with the OWB

population. Notably, the OWB-related units received a lower score from the 2020 cohort, which completed the practical online during the COVID-19 pandemic and therefore did not have the opportunity to perform hands-on phenotyping.

#### *United States – University of California-Davis*

Undergraduate plant breeding students at the University of California-Davis use qualitative phenotype information from the OWB to enter binary trait values (e.g., 0 for two-row inflorescence, 1 for six-row inflorescence) into an Excel worksheet (accessible at <https://barleyworld.org/education>). The Excel worksheet contains scores for 2846 molecular markers, and a Manhattan plot embedded in the worksheet adjusts in real-time to show the significance of marker-trait associations. An additional worksheet within the Excel file contains formulas to calculate *t*-tests and associated *p*-values. Using a total of 25 lines including all of the OWB-ISS lines, the students are able to map the two-row/six-row and compact/normal inflorescence traits to the *VRS1* and *ZEO1* loci, respectively.

#### *United States – University of Illinois Urbana-Champaign*

At the University of Illinois Urbana-Champaign, first-year undergraduate students grow the HB OWB population and collect data on spike morphology traits, including two-row/six-row inflorescence (*VRS1*), hooded/awned florets (*BKN3*, i.e., *Kap*), and awn length, among others. Using both linked and unlinked molecular markers, students create pairwise genotypic classes from marker-trait combinations and analyze them using chi-square tests. The data substantiate the concepts of genes, alleles, and Mendel's Law of Segregation, and the activity illustrates the concepts of parental and recombinant genotypic classes and the effect of linkage on recombination. The students are able to grasp how scientists implement genetic analyses to identify the location of genes within the genome. For many first-year undergraduates, this empirical and interactive activity is their first exposure to the concept of improving crop plants through the use of genetics. The lab instructions and associated lectures are accessible at <https://barleyworld.org/education>.

#### *United States – Huntington University*

The OWB-ISS is used in an upper-division undergraduate plant breeding course at Huntington University in Indiana to train students to recognize variation in plant phenotypes. Students work with live plants to record phenotypic differences between the two OWB marker stock parental lines at early growth stages. As the plants develop, the students observe visible or measurable differences between the 18 non-parental lines and the parents. In addition to measuring quantitative traits such as plant height and number of tillers, the students devise a relative (1–5) rating scale for traits which

are more difficult to measure accurately (e.g., tiller erectness). They also categorize the following qualitative traits: green/orange lemma and nodes (*ROB1*), pubescent/hairless leaf blades (*Pub1*), black/yellow lemma and pericarp (*BLP*), hooded/awned florets (*BKN3*, i.e., *Kap*), and two-row/six-row inflorescence (*VRS1*). In their final report, the students each return a spreadsheet containing the mean, maximum, and minimum for each quantitative trait and the number of lines of each qualitative trait phenotype, based on the data they recorded. The phenotyping and lab report instructions are accessible at <https://barleyworld.org/education>.

#### *United States – Purdue University*

The OWB-ISS was grown at Purdue University's controlled environment phenotyping facility in Indiana which employs an automated system using pots on conveyer belts to collect plant measurements with imaging technology (<https://ag.purdue.edu/aapf/virtual-tour.html>). Three times each week, the facility's Red Green Blue camera system was used to acquire color images of each plant (one top and 12 side views), automatically segment the images, and extract predefined parameters to quantify growth and color-related traits of each line. In addition to these image-based traits, ground-reference observations of plant height and tiller number were collected twice weekly over the course of a 42-day evaluation period. This gave rise to a dataset containing 324 total observations of image-based and ground-reference metrics which may be used for a variety of instructional applications. These may include teaching descriptive statistics and exploratory plotting of image-based and manual measurements, and teaching simple modeling of ground reference measurements using various combinations of image-based variables. These exercises may be most appropriate for high school or university-level students. All data along with experimental details are publicly available within the Zenodo repository (accessible at <https://doi.org/10.5281/zenodo.10999418>).

#### *United States – USDA-ARS and Iowa State University*

The USDA-ARS and Iowa State University have leveraged the OWB-ISS since 2010 within the comprehensive Inheritance of Traits and Genes (iTAG) curriculum to train high school and community college instructors in 6-week summer Research Experiences for Teachers and genotype-to-phenotype short courses (Wise et al., 2024). iTAG uses the OWB-ISS to help students associate observable phenotypic traits with variations in the genotype, or DNA sequence. Students observe segregating differences in the following qualitative traits: hooded/awned florets (*BKN3*, i.e., *Kap*), two-row/six-row inflorescence (*VRS1*), and long/short awns (*LKS2*). Students are trained in molecular protocols such as DNA extraction, PCR, restriction digestion, and gel electrophoresis in order to compare and contrast the different

types of DNA polymorphisms (e.g., indels, SNPs) among plants with distinctive phenotypes. Instructors then discuss with the students about how researchers are able to connect genotypes to phenotypes. Thus, the students gain meaningful exposure to concepts in genetic segregation, developmental mutations, and gene interactions (i.e., epistasis), as well as principles essential to breeding crops. The ultimate objective is to train students to initially understand and monitor a few genes as a foundation for tracking and leveraging hundreds to thousands of genes as they engage with modern genomics applications later in their careers. From 2010 to 2024, 50 instructors implemented the iTAG curriculum in more than 200 grade level 7–12 biology classes, affecting over 5000 students from both urban and rural communities. The laboratory exercises are accessible at <https://doi.org/10.1094/PHI-E-2023-09-0009>.

#### *United States – Cornell University*

At Cornell University in New York, the OWB HB population was used for over 20 years as part of an introductory course entitled “Plants, Genes and Global Food Production”. The 93 OWB HB progeny and the two parental marker stocks were planted in a greenhouse in Ithaca, NY, in mid-August at the beginning of the fall semester, and the plants were fully mature and ready for use in a hands-on learning exercise by the end of October or the first week of November. Students worked in groups of three to collect phenotypic data on leaf- and spike-morphology traits for all plants in the population, guided by photographs of the mutations observed in the parental lines. Each group was assigned a different set of three qualitative phenotypic traits, with overlapping trait observations across groups. Collectively, the class evaluated six qualitative phenotypes: two-row/six-row inflorescence (*VRS1*), long/short awns (*LKS2*), hooded/awned florets (*BKN3*, i.e., *Kap*), dwarf plant with compact head/normal height and head length (*ZEO1*), pubescent/hairless leaf blades (*Pub1*), and hairs/lack of hairs on lower leaf sheaths (*HSH*). As part of an in-class exercise, the trait records were cross-checked among groups and compared to the online digital image gallery (<https://barleyworld.org/main/images>) to resolve any scoring differences. Datasets were then merged to generate a consensus dataset for the population, which served as the basis for a linkage mapping exercise. Students used these data in conjunction with the OWB genotypic information available online (accessible at <https://barleyworld.org/data>) to perform chi-square tests to evaluate linkage. Use of the OWB population captured student interest; deepened their understanding of Mendelian laws of inheritance and segregation; helped to clarify the meaning of “genes”, “genetic loci”, and “alleles”; heightened awareness of the relationship between plant genetics and breeding; and provided an opportunity to discuss how linkage between genes and molecular markers is utilized to predict phenotypes in the context of both human genet-

ics and crop improvement. The lab manual and homework assignment associated with these exercises are accessible at <https://barleyworld.org/education>.

#### *United States – Oregon State University*

The OWB population is used at OSU in undergraduate- and graduate-level courses in the Plant Breeding and Genetics program (<https://catalog.oregonstate.edu/courses/pbg/>). In PBG430/530, an upper-level undergraduate/introductory graduate course on plant genetics, the OWBs are used to demonstrate the principles of Mendelian analysis and its continued relevance in an era of abundant and cost-effective whole genome sequencing. In PBG620/621, a graduate-level course on genetic markers and mapping, the OWBs are leveraged to demonstrate molecular marker development and application. Students perform hands-on phenotyping to measure spike length and categorize hooded/awned floral morphology (*BKN3*, i.e., *Kap*). An online option is available for students attending remotely. The phenotype data are then used in genetic mapping and genotype-to-phenotype association exercises. Lectures, videos, and an exercise for the “R/qt1” package for R (Broman et al., 2003) are accessible at <https://barleyworld.org/education>.

#### *United States – Oregon K–12 in English and Spanish*

The OWB populations serve as the basis of lesson plans developed for K–12 education in conjunction with the Oregon Agriculture in the Classroom Foundation (OAITC). Available in both English and Spanish, these lesson plans were designed based on the Next Generation Science Standards (NGSS), which are nationally-developed science standards that have been formally adopted in 20 states and adapted for use in 26 more (<https://www.nsta.org/science-standards>). The lesson plans were crafted to meet the NGSS standards for grade levels 1, 3, 6–8, and 9–12 in the core discipline area of “Heredity: Inheritance and Variation.” These standards were written to encourage students to develop an understanding of the concept of inheritance by making observations and drawing upon them as evidence to support conclusions. The OWBs are well-suited to the NGSS and K–12 instruction because of their visible diversity, thereby making the practice of phenotyping more accessible to a young audience.

The lesson plans were translated into Spanish by M. Martínez-Subirà. With the number of Spanish speakers in the United States expected to surpass that of all other countries by 2050 (Pentón-Herrera, 2018), offering the lesson plans in Spanish in addition to English supports bilingual education encourages a more inclusive and effective learning environment (Capdevila-Gutiérrez & Rodríguez-Valls, 2018) and helps to close the achievement gap for Spanish-speaking students who often encounter difficulties in accessing high-quality education resources in their native language (Uzzell & Ayscue, 2021). The K–12 lesson plans are publicly available

on the OAITC website (<https://oregonaitc.org/lessonplan/>) and the OWB website (<https://barleyworld.org/education>).

## 4 | CONCLUSIONS

From a scientific perspective, it is noteworthy that the majority of the single-locus dominant and recessive genes that were painstakingly assembled by Dr. Wolfe into the OWB-D and OWB-R marker stocks have now been genetically mapped. Segregating independently in the OWB DH populations, the responsible genes have now been identified at the molecular level. Most of these—including hooded/awned florets (*BKN3*, i.e., *Kap*; Müller et al., 1995), hulled/hulless seed (*NUD*; Taketa et al., 2008), two-row/six-row inflorescence (*VRS1*; Komatsuda et al., 2007), long/short awns (*LKS2*; Yuo et al., 2012), dwarf plant with compact head/normal height and head length (*ZEO1*, Houston et al., 2013), green/albino lemma and nodes (*ALMI*; Taketa et al., 2021), and black/yellow lemma and pericarp (*BLP*; Li et al., 2024)—are responsible for striking variation in the visual appearance of the inflorescence. Among students, these loci have invoked a profound scientific fascination and curiosity around the biology of reproductive plant development and its underlying importance for crop improvement. In contrast, while most of the as-yet unidentified genes generally confer less obvious phenotypic traits, we predict that most will be identified in the near future as gene identification becomes ever more routine through continually evolving and immensely powerful genetic and/or genomic approaches (e.g., Druka et al., 2011; Hansson et al., 2024). Collectively, our increasing knowledge of the specific molecular variants responsible for generating the variation illustrated in the OWB population has provided opportunities to explore beyond the causal variants themselves into the molecular networks and epigenetic and epistatic interactions that, together, contribute to a complex visual phenotype. Beyond scientific inquiry, the OWBs have stimulated the development of an international community of users who continue to generate new and innovative materials and translate them into publicly available, hands-on instructional resources. Here, we present the OWBs as a graphic illustration of “genetics in action” and encourage educators at all levels to explore the multi-dimensional collection of teaching resources we have assembled and make freely available to the scientific community.

## 5 | AVAILABILITY

The barley breeding and genetics program at OSU has maintained the complete OWB HB and AC populations since the original DH lines were developed in the late 1980s and early 2000s, respectively. Over the decades, several seed increases

were performed in a greenhouse in Corvallis, OR, to provide interested researchers and educators with sets of each population. A seed increase of all of the OWB HB and AC lines was performed in a greenhouse in Corvallis in 2024 using a seed source that was generated in 2023. One plant of each line was grown to maturity, and the resulting spikes were threshed by hand to avoid loss or breakage of the seed.

From this increase, seed samples of 81 and 93 lines from the OWB HB and AC populations, respectively, were sent for deposition into the USDA-Agricultural Research Service National Center for Genetic Resources Preservation (NCGRP). Small quantities of seed for research purposes may be obtained from NCGRP upon request. Alternatively, M. Krause of OSU ([margaret.krause@oregonstate.edu](mailto:margaret.krause@oregonstate.edu)), M. Lillemo of the Norwegian University of Life Sciences ([morten.lillemo@nmbu.no](mailto:morten.lillemo@nmbu.no)), S. Salvi of the University of Bologna ([silvio.salvi@unibo.it](mailto:silvio.salvi@unibo.it)), and M. von Korff of the University of Düsseldorf ([maria.korff.schmising@hhu.de](mailto:maria.korff.schmising@hhu.de)) maintain seed of the OWB populations and may be able to provide samples upon request.

For long-term security, the OWB HB and AC populations are currently stored in the Svalbard Seed Vault (<https://www.croptrust.org/work/svalbard-global-seed-vault/>) thanks to the efforts of K. Sato, H. Hisano, and Å. Asdal. Backup samples of 654 barley accessions including the OWBs, conserved in the genebank at the Barley and Wild Plant Resources Center at Okayama University, were deposited into the Svalbard Seed Vault on Wednesday, May 29, 2024.

Lesson plans, datasets, and other educational resources related to the OWB populations can be found at <https://barleyworld.org/education>.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.


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