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An F_2 Barley Population as a Tool for Teaching Mendelian Genetics †

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Abstract: In the context of a general Genetics course, the mathematical descriptions of Mendelian inheritance and population genetics are sometimes discouraging, and students often have serious misconceptions. Innovative strategies in expositive classes can clearly encourage student's motivation and participation, but laboratories and practical classes are generally the students' most favourite academic activities. The design of lab practices focused on the learning of abstract concepts such as genetic interaction, genetic linkage, genetic recombination, gene mapping, or molecular markers, is a complex task that requires a suitable segregant material. The most optimal population for pedagogical purposes is an F_2 population, which results extremely useful not only to explain different key concepts of genetics (as dominance, epistasis, and linkage) but also to introduce additional curricular tools, particularly, concerning statistical analysis. Among various model organisms available, barley possesses several unique features for demonstrating genetic principles. Therefore, we have generated a barley F_2 population from the parental lines of the Oregon Wolfe Barley collection. The objective of this work is to present this F_2 population as a model to teach Mendelian genetics in a medium-high level Genetics course. We provide an exhaustive phenotypic and genotypic description of this plant material that, together with the description of the specific methodologies and practical exercises, can be helpful for transferring our fruitful experience to anyone interested in implementing this educational resource in his/her teaching.

Keywords: genetics education; mendelian genetics; F_2 population

1. Introduction

Given the undoubted importance of genetics in relevant aspects of human lives and activities (medicine or agriculture, among others), an enhanced understanding of its fundamental pillars is necessary to prepare the next generation of scientists and ensure that life-science students acquire a solid knowledge of basic genetic concepts. That is needed for a mindful interpretation of the continuous advances in this field and for the appropriate use of genetic applications [1]. An education based on memorization of facts and methods is insufficient in a society and economy based on knowledge. Moreover, several evidences indicate that the information itself is insufficient as educational objective, and current society requires the use of alternative learning pathways with the

aim that people understand complex concepts, and are able to work and generate new theories, ideas and products [2].

There are several difficulties in genetics education. Besides the abstract nature of the subject and the specific terminology, the mathematical descriptions of Mendelian inheritance and population genetics are sometimes discouraging, which leads to the acquisition of misconceptions [3,4]. Improvement strategies in expositive classes can encourage student participation and motivation but presentation of concepts only through lectures gives many students a superficial understanding of the subject [5]. Following Dopico and co-workers, research in real contexts and environments is a highly motivating and educationally responsible resource for students' formation in modern education [6]. Laboratories, where hands-on experiments can be performed, are not only one of the preferred academic activities for students but a fruitful learning environment that can be used, beyond text-books and lectures, as a teaching element of methodological change and educational innovation [7–9].

Genetic practices are usually employed with the aim to teach experimental methods such as polymerase chain reaction (PCR and RT-PCR), nucleic acids and protein analysis, etc. However, the inclusion of complex concepts that are common in basic and applied research (i.e., genetic interaction, quantitative inheritance, genetic linkage, statistic in inheritance studies, molecular markers…) is a hard task, and a suitable segregant population is required to address and strengthen those concepts. Several types of plant populations can be used: F_2, BC, RILs, DH…. In a general course in Genetics, the most optimal population to carry out a genetic study would be an F_2 population, which is extremely useful to explain different key concepts of genetics (as dominance and epistasis) to students and for including teaching additional aspects, particularly, concerning statistical analysis (i.e., [9]). However, such a goal makes it necessary to develop an F_2 consisting of a large number of individuals, which might require a huge space, either in a greenhouse or in the field. It would also imply having the plants ready to be examined by the students at the right time as the theory classes are developed. This can be specially hard to fit into the academic calendar, in addition to require a high endowment of material and human resources. To be able to solve all these problems, a cereal species is the most viable option, as the dry ears can be stored and maintained for successive student's generations, allowing phenotypic studies without the need to cultivate the lines yearly. In this sense, some interesting resources have been developed in maize for teaching purposes [10].

Among cereals, barley possesses several unique features for demonstrating genetic principles: i) It is a diploid species (2n = 14), with a small genome easy to handle [11]; ii) Barley genome sequence is available from time ago [12] and numerous genetic maps and genomic resources are accessible [13]; iii) It possesses a wide range of phenotypic variation for various traits, particularly grain and spike traits, that are easily scored on dry material and, iv) It is easy to cross and grown in the green house or in field. There is a well-known barley collection, the Oregon Wolfe Barleys (OWB) (https://barleyworld.org/owb), developed several years ago as a teaching resource for understanding the importance and uses of genetic diversity in plants. It was launched at Oregon State University by Dr. Bob Wolfe who developed the parental lines by systematically crossing recessive alleles into one parent and dominant alleles into the other parent [14,15]. From these parental lines, two different OWB doubled-haploid populations were developed [16,17]. These barley lines provide a highly segregant resource for the construction of genetic maps [18–20] and a unique genetic background for the mapping of complex traits [21,22]. The OWB populations have been extensively used for teaching aims and several lesson plans are available at https://barleyworld.org/main/education. We have enlarged the pedagogical toolbox by generating an F_2 population from the cross of the parental lines. The objective of this paper is to present this population as an impeccable model to teach Mendelian genetics. The exhaustive description of the material as well as the specific methodologies and practical exercises carried out in Genetics courses at the Universidad Politécnica de Madrid (UPM) aims to transfer our experience to anyone interested in implementing this resource in his/her teaching.

2. Methods

2.1. Barley F_2 Generation

2.1.1. The Plant Material

An F_2 population was generated from the cross of the parental lines OWB-D and OWB-R and self-pollination of the F_1 (Figure 1). Crosses were made in May 2014, seven F_1 seeds were obtained and sown in November 2014 in a greenhouse (1 seed/pot) of the School of Agricultural, Food and Biosystems Engineering of UPM. After self-pollination, more than 500 F_2 seeds were obtained in summer 2015. From autumn 2015 to summer 2016, 303 F_2 plants were grown in the greenhouse. Flag leaf samples were taken for DNA extraction, and 6–8 mature spikes of each plant were collected and stored in labelled plastic bags which also contained a few harvested grains of the corresponding F_2 plant. The remaining F_2 seeds are kept in a dry environment at 4 °C and can replace the actual set when needed.

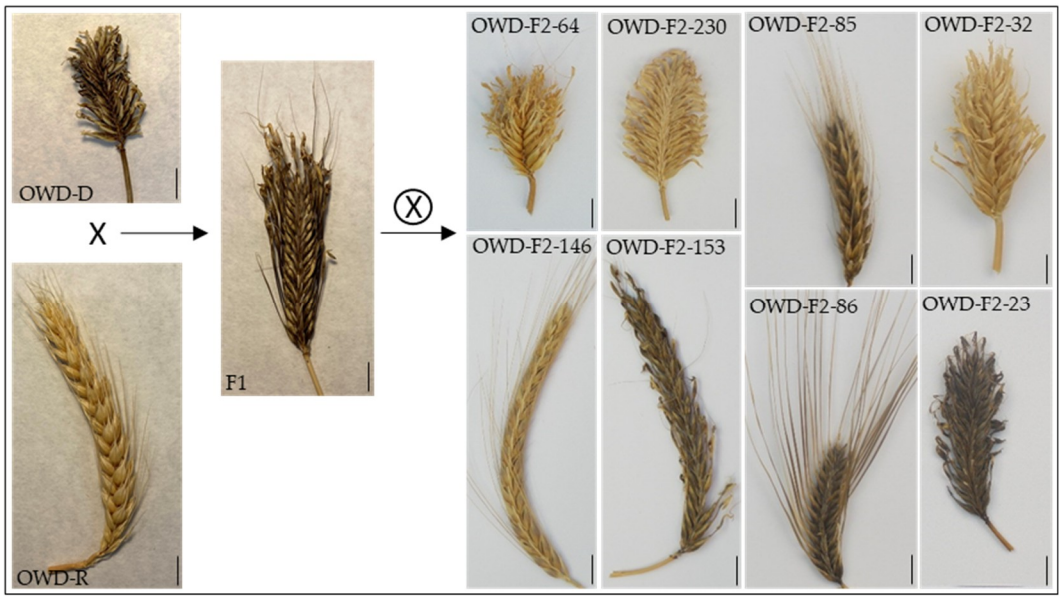

Figure 1. Parental lines (OWB-D and OWB-R), F_1 and some F_2 individuals showing the wide range of variability of this population. Scale bars represent 1 cm.

2.1.2. Phenotyping of Spike and Grain Traits

In this kind of exercise, it is very important to use a coherent set of traits easy to handle by the students. As mentioned before, the OWB-D and OWB-R lines differ from each other for many morphological characters whose segregation can be easily monitored in a segregant population. From all the possible traits, we selected seven that are described in Table 1. Five traits can be directly scored in dry spikes by students, while the remaining two must be scored in growing plants.

Table 1. Description of qualitative morphological traits selected to be analysed. The morphological markers were designated following the nomenclature for barley genes described by Francowiak [23].

Material	Trait	OWB-D Phenotype	OWB-R Phenotype
Dry spikes	Type of spike	Zeo = dense spike	zeo = lax spike
	Number of rows	Vrs1 = two-rowed spike	vrs1 = six-rowed spike
	Type of awn	Kap = hooded awn	Kap = normal awn
	Length of awn	Lks2 = long awn	lks2 = short awn
	Type of grain	Nud = covered caryopsis	Nud = naked caryopsis
Growing plants	Leaf variegation	Wst = non variegated leaf	wst = variegated leaf
	Stem pubescence	Hsh = hairy leaf sheath	hsh = non hairy leaf sheath

Growing plant traits were scored in the green house by the students attending our courses during the academic year 2015–16, and then supervised by the teachers' team. In the following courses, these data are given to the students to complete the data set for further analyses.

2.1.3. Genotyping of Barley F_2 Population

There is a lot of information about Simple Sequence Repeats (SSR) and Single-Nucleotide Polymorphisms (SNPs) markers in the OWB population (https://barleyworld.org/owb/data). For didactic purposes, three SSR markers selected from the literature and one PCR-based dominant marker were chosen. The dominant marker Knox-dup was in-house developed for the allelic discrimination of the *Hvknox3* gene, that is located on the short arm of barley chromosome 4 [24]. The dominant allele (*Kap*) of this gene is responsible for the hooded phenotype and differs from the recessive allele (*kap*) in a tandem duplication of 305 bp located in intron IV of this allele. The inclusion of a dominant marker is useful to explain the differences in the analysis of both type of molecular markers (dominant and codominant) and helps the student to understand the advantages and disadvantages of their use. Three out of the four markers employed are mapped in chromosome 4H which is relevant for further linkage analysis, and genetic dissection of the epitasis (Table 2).

Table 2. Molecular markers selected for genotyping of the barley F_2 population.

Marker	Oligo Forward	Oligo Reverse	Tm	D Allele	R Allele	Class	Chr
Bmac 0310	CTACCTCTGAGATATCATGCC	ATCTAGTGTGTGTTGCTTCCT	55 °C	180 pb	140 pb	SSR	4
Bmag 0211	ATTCATCGATCTTGTATTAGTCC	ACATCATGTCGATCAAAGC	55 °C	187 pb	198 pb	SSR	1
HVM40	CGATTCCCCTTTTCCCAC	ATTCTCCGCCGTCCACTC	55 °C	175 pb	146 pb	SSR	4
Knox-dup	ATGTTGCTGTATTTTGCG	ACTGCACTGCAACTGGTCAG	60 °C	325 pb	-	PCR	4

Chr: chromosome, D allele: allele present in the OWB-D parental line, R allele: allele present in the OWB-R parental line.

3. Results and Discussion

3.1. Segregation of Morphological Traits in the Barley F_2 Population

Segregation analysis of morphological traits is presented in Table 3.

Table 3. Segregation of morphological traits in the barley F_2 population.

Trait	Gene	Dominant	Recessive	N	χ^2 3:1		χ^2 9:7	
Type of spike	*Zeo*	229	74	303	0.05	n.s.		
Number of rows	*Vrs1*	230	73	303	0.13	n.s.		
Type of awn	*Kap*	169	133	302	58.39	***	0.01	n.s.
Length of awn	*Lks2*	56	77	133	76,75	***		
Type of grain	*Nud*	237	65	302	1.95	n.s.		
Leaf variegation	*Wst*	237	66	303	1.67	n.s.		
Stem pubescence	*Hsh*	186	70	256	0.75	n.s.		

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, n.s.: non-significant, N: total number of individuals.

The traits controlled by *Vrs1*, *Nud*, *Zeo*, *Wst*, and *Hsh* behave as expected for a mendelian monogenic dominant inheritance, segregating in a 3:1 ratio in the population. However, the traits controlled by *Lks2* and *Kap* (length and type of awn) do not follow the expected 3:1 segregation, the fitting of the type of awn (hooded *versus* normal) to a 9:7 segregation suggesting a more complex genetic control. These traits, related to the awn morphology, provide an excellent example to introduce the students in non-mendelian segregations. It is known that the hooded barley phenotype is caused by a mutation in the *Hvknox3* gene (in chromosome 4H), involved in floral evocation [24], but there are epistatic effects from other genes as *Lks2* (7H), which codes for a transcription factor of the SHI family that regulates awn elongation and pistil morphology [25]. In the case of *Lks2*, the classification of awns as long or short can only be made in normal awned spikes (*kapkap*). Hence, the analysis of the character "type of awn" must be re-done with both characteristics (type and length)

which classifies the spikes in three phenotypic classes: hooded, normal short and normal long. This analysis allows to confirm the segregation 9:3:4 as corresponding to a recessive epistasis (Table 4).

Table 4. Segregation of type of awn in the barley F_2 population.

Kap/Lks2						
Phenotype	**Hooded Awn**	**Normal, Long Awn**	**Normal, Short Awn**	**N**	χ^2 9:3:4	
Individuals	169	56	77	302	0.14	n.s.

n.s.: non-significant, N: total number of individuals.

3.2. Barley F_2 Genotypic Description

The molecular markers segregation analysis is presented in Table 5. The SSRs Bmag 0211 y HVM40 behaved as expected for a mendelian codominant inheritance, while Bmac 0310 showed a slightly deviation from the expected for a codominant marker. The Knox-dup marker showed a 3:1 segregation as expected for a dominant marker.

Table 5. Segregation of selected molecular markers in the barley F_2 population.

Marker	**Homozygous OWB-D Allele**	**Heterozygous**	**Homozygous OWB-R Allele**	**N**	χ^2 1:2:1		χ^2 3:1	
Bmac 0310	57	167	76	300	6.26	*		
Bmag 0211	80	130	88	298	5.28	n.s.		
HVM40	63	160	78	301	2.69	n.s.		
Knox-dup	230		73	303			0.13	n.s.

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, n.s.: non-significant, N: total number of individuals.

As the Knox-dup marker is completely linked to *Kap* gene, the dissection of the genetic interaction can be attempted by the classification of the F_2 individuals not only as hooded, short or long, but also as dominant or recessive for this gene. The combined analysis is presented in Table 6.

Table 6. Segregation of awn phenotypes combined with molecular marker Knox-dup.

Genotype	***Kap_Lks2_***	***kapkapLks2_***	***Kap_lks2lks2***	***kapkaplks2lks2***	**N**	χ^2 9:3:3:1	
Awn phenotype	Hooded/Normal		Normal				
		Long	Short	Short			
Individuals	169 + 10 #	46	50	27	302	6.76	n.s.

See text. n.s.: non-significant, N: total number of individuals.

F_2 individuals carrying the epistatic recessive allele in homozygosis, *lks2lks2,* develop always a short type awn with independence of *Kap* genotype. Individuals homozygous recessive *kapkap* carrying a dominant allele *Lks2* develop a long type awn. The individuals carrying one dominant allele in each locus develop mostly a hooded awn, but a small proportion (10 out of 179) develop a normal long awn. It suggests that some additional loci can be modulating this complex phenotype.

3.3. Linkage Analysis

With all the data for qualitative traits and molecular markers we performed linkage analyses. The *Kap* morphological marker was not included due to the epistasis but was supplemented with Knox-dup molecular marker. *Lks2* could be analyzed according to the genetic model previously stablished (Table 6). The chi-square values for independence are presented in Table 7. All two-by-two tests including a codominant marker are based in 2 × 3 linkage contingency tables instead of the typical 2 × 2 contingency tables used for pairs of dominant traits.

Table 7. Linkage analysis of molecular and morphological markers in the barley F_2 population.

Gene/Marker	*Lks2*	*Nud*	*Zeo*	*Wst*	*Hsh*	Knox-Dup	Bmac 0310	Bmag 0211	HVM40
Vrs1	n.s.	n.s.	**6.53** *[a]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Lks2		**162.93** ***[a]	n.s.	n.s.	n.s.	**6.69** *[a]	n.s.	n.s.	n.s.
Nud			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Zeo				**50.25** ***[a]	n.s.	n.s.	n.s.	n.s.	n.s.
Wst					n.s.	n.s.	n.s.	n.s.	n.s.
Hsh						**5.42** *[a]	n.s.	n.s.	n.s.
Knox-dup							**15.47** ***[b]	n.s.	**21.92** ***[b]
Bmac 0310								n.s.	n.s.
Bmag 0211									n.s.

Chi-square test indicating significant differences with the expected values assuming independent inheritance are marked in bold. * = $p < 00.5$, ** = $p < 0.01$, *** = $p < 0.001$, [a]: 1 degree of freedom, [b]: 2 degrees of freedom. n.s.: non-significant.

Several genetic linkages were detected for morphological markers. The best estimation of r in a F_2 can be obtained from the second-grade equation $Nx^2 + (-a_1 + 2a_2 + 2a_3 + a_4)X - 2a_4$, where a_1, a_2, a_3 and a_4 stands for the four categories of the 2 × 2 F_2 contingency table, and r calculates as $1 - \sqrt{x}$. These estimations gave a value of r = 0.398 between *Vrs1* and *Zeo* loci, and r = 0.251 between *Zeo* and *Wst* loci. In this population no linkage has been detected between *Wst* and *Vrs1* which points out *Zeo* as the central locus (Figure 2). A closely linkage between *Lks2* and *Nud* (r = 0.10) was also detected.

Regarding the molecular markers, r can be estimated between the marker pairs HVM40 and Knox-dup (r = 0.329) and Knox-dup and Bmac 0310 (r = 0.375). To calculate these r values, codominant molecular markers can be converted into a dominant marker, thus the students can use the same equation for all the traits. No linkage could be detected between HVM40 and Bmac 0310 which, following the same reasoning as before, indicates that Knox-dup is the central locus (Figure 2). Our data support genetic linkage between Knox-dup and the *Hsh* locus, with a r value of 0.403. Although these two loci are actually in chromosome 4H, their linkage was unexpected because Bmac 0310, that locates between them, segregates as not linked with *Hsh*. This result could be related with the segregation distortion detected for Bmac 0310 (Table 5). However, there is also an unexpected linkage relationship between Knox-dup (4H) and *Lks2* (7H) which might be derived from the existence of some additional loci modulating awn phenotype as already discussed. It should be noted that all unexpected linkage results involve Knox-dup marker.

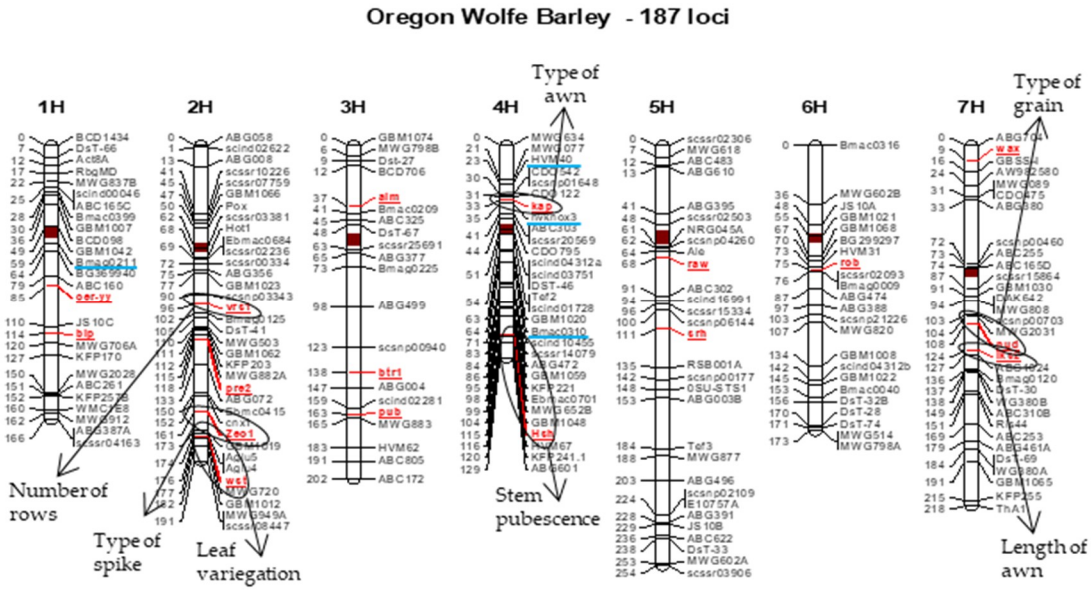

Figure 2. Location of the molecular markers (underlined in blue) and genes controlling the phenotypic traits used in this work on the genetic map of the Oregon Wolfe Barley population available at https://barleyworld.org/owb. In chromosome 4H, HvKnox3 stands for the Knox-dup molecular marker.

3.4. Teaching Experience

This is one type of exercise that can be developed from the previously described material and data. It is designed for students of a "Genetics" general course in a BSc level, but can be adapted for other courses/levels. The exercise must be scheduled once the topics of transmission genetics and molecular markers needed for completing the assignment have been covered at the course.

The exercise is organized in groups of around 20 students that will attend 4 sessions of 2–3 h each.

Session 1: Phenotyping (2 h). This session can be easily set up in a regular classroom. First, the professor will explain in detail the characters to be scored in the plant material (dry spikes and grains) by the students. The collection of spikes will be split in subsets so that the data for the whole F_2 population are got by combining all the subsets' data. The characters recorded by the students are: Number of rows (2 vs. 6), Type of grain (covered vs. naked), Type of spike (dense vs. lax) and Type of awn (hooded vs. normal). The students, in pairs, must do the phenotypic characterization of the F_2 spikes assigned (in our case, 40–50 F_2 individuals) and must record the observations in an excel datasheet. At the end of session 1, the professor must have a file with the phenotype records of the complete collection.

Session 2: Genetic analysis (3 h). In this session, that must be held in a computer room, the professor will guide the students in genetic analysis. This training is essential for successfully completion of the final report. Several points will be covered and discussed:

1. Data acquisition: The phenotype profiles recorded by students will be compared with the recorded by the teaching team ("official phenotyping").
2. Segregation analysis of traits: For this and further analyses, the professor will provide data for the two additional traits measured in grown plants (stem pubescence and leaf variegation). Students will check if the traits behave as expected assuming a model of genetic control by one locus with two alleles and complete dominance. They must employ Excel for data management and χ^2 analysis. There is only one trait, type of awn, that does not behave as expected. All the group will discuss about what can be happening with this trait. The professor will lead students to understand and to conclude that its genetic control may be an epistasis and will provide the data for the length of the spike. At this moment, it can be useful to give again the spikes to the student so they can see that the "normal awn" phenotype can be subclassified in long awn and short awn. This trait is not easy to score in F_2 plants; so, in our experience is more convenient to give the data fill up to the students. With the combined data, the students must check if the segregation observed now really correspond or not with an epistasis.
3. Linkage analysis: Students, by pairs, will perform the two-by-two linkage analysis for the seven traits, and will estimate the recombination fraction and create a genetic map. The professor will lead students to understand that despite no linkage is detected between *Wst* and *Vrs1* genes, both show linkage with *Zeo1*, which indicates that these three genes are placed in the same linkage group. In this case, students could calculate not only the recombination fraction but also interference and coincidence coefficients. Likewise, it is important to discuss why they cannot estimate the r with *Kap* gene, even if they detect genetic linkage with other genes. Students' conclusions can be also used to contrast with those based on official phenotyping, which is specially useful if phenotyping errors may have resulted in misleading outcomes.

Session 3: Molecular markers I (2 h). This session must be performed in a lab. The students will amplify by PCR two molecular markers, Knox-dup and Bmac 0310. First, the professor will explain the fundament of PCR, and how the reaction works. Then, each pair of students will be provided with DNA from 10 F_2 individuals and from the parental lines, and all the reagents and materials needed for the experiment. In order to promote autonomous work, the students must design the experiment, including the calculation of reagents' volumes in the PCR mix, and perform it on their own.

Session 4: Molecular markers II (3 h). Students will analyze the result of the PCR by agarose gel electrophoresis. The inclusion of a dominant and a codominant marker will allow to discuss the

differences in the results. Genotyping data must be included in the excel datasheet. At the end of session 4, the professor must have a file with the genotype data for all the collection that will make available to all the students. For a more complete analysis, the genotyping of the F_2 individuals for two additional SSR markers (HVM40 and Bmag 0211) will be included in the datasheet.

Results report (3 h personal work): Once completed the sessions, the students, in pairs, must fill out a report. In this document they must present: 1. The study of individual segregation of the four molecular markers, 2. The two-by-two linkage analysis for the four molecular markers, 3. The two-by-two linkage analysis between the spike and grain traits and the molecular markers, 4. The conclusion of the analyses. The reports delivered after the lab sessions are not based on analyses performed with their data but with the data given by the professor.

Extra session: Class discussion (1 h): Once all the reports have been submitted and reviewed, the professor may schedule an extra session in which the more common troubles faced by the students can be discussed.

During the past 5 years about 500 students have completed this practical activity. Student accuracy in phenotyping is low, with 70–80% of the raw forms needing correction. On the contrary, molecular markers practices and genotyping are usually easier than phenotyping for students. Most of students carry out PCRs adequately, without contamination or false-negative results.

Personalized discussion with each student during the first practical session helps to reduce the error rate. Number of rows is the easiest trait to be assessed by students, with the lowest rate of mistakes. Type of grain and Type of awn usually show more errors, however the mistakes are generally small and do not affect the results obtained in segregation and linkage analyses. Type of spike is the most difficult trait to be scored for students, and the number of mistakes can be large enough to significantly modify the results of the genetic analyses. This point allows to discuss with the students on the importance of finely performing the phenotypic studies.

4. Conclusions

In this study, an F_2 population of barley was generated from the two parental lines of the OWB collection. An F_2 is the most suitable and complete population to perform the study of complex genetic concepts, such as dominance, epistasis and linkage, and to carry out segregation, linkage and genetic interaction analysis. Many educational institutions maintain *Drosophila melanogaster* mutant stocks to develop F_2 populations for genetics practical teaching [26,27]. In our view, utilizing a cereal species as working organism represents several advantages, including the possibility to isolate DNA for genotyping of the F_2 individuals during the plant growing cycle. Dry material can be kept during long periods of time and traits easily phenotyped in dry ears and grains can be selected in order to design the experiments to be performed by students. Among cereals, barley is the best candidate because has a small genome and numerous genetic resources are accessible. The generation of the barley F_2 population presented here has allowed the implementation of this practical exercise during several years. Furthermore, a collection of images of the stored spikes and grains of each F_2 individual has recently served as on-line phenotyping resource for a group of students that had to follow the sessions 1 and 2 from home because of the Covid-19 pandemic.

With the accomplishment of the full exercise, the students have achieved the following goals: (i) Acquire the methodologies for data collection, treatment and analysis to study the genetic control of qualitative traits and analyze the existence of genetic linkage between two loci; (ii) Acquire the basic knowledge to analyze molecular markers in the laboratory (amplification and electrophoretic analysis of DNA sequences) and (iii) Understand the different types of molecular markers and their use in genetic linkage analysis.

The development of the practice exercise described above has led to improved learning of complex genetic concepts by the students, being placed in a context similar to a real research project. However, a survey to the student that followed the practice in 2019–20 (data not shown) has indicated that some of them neither understood the importance of using an F_2 population nor were able to successfully associate the exercise performed with some concepts that are studied in theoretical

classes of genetics. Clearly, professors must continue making educational efforts with the aim to connect both kinds of teaching.

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