UNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Agronomia Eliseu Maciel
Programa de Pós-Graduação em Agronomia

Thesis

Pre-harvest sprouting tolerance in wheat (*Triticum aestivum* L.): current status and analysis of a recombinant inbred line population

Ana Karina Frank Bastidas

Pelotas, 2025

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Tese apresentada ao Programa de Pós-Graduação Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Ciências Area de concentração: Fitomelhoramento

Supervisor: Dr. Antonio Costa de Oliveira

Dr. Eduardo Venske

Dr. Luciano Carlos Da Maia

Pelotas, 2025

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação da Publicação

B326p Bastidas, Ana Karina Frank

Pre-harvest sprouting tolerance in wheat (*Triticum aestivum* L.) [recurso eletrônico] : current status and analysis of a recombinant inbred line population / Ana Karina Frank Bastidas ; Antonio Costa de Oliveira, orientador ; Eduardo Venske, Luciano Carlos Da Maia, coorientadores. — Pelotas, 2025.
83 f. : il.

Tese (Doutorado) — Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, 2025.

1. Germinação pré-colheita. 2. Herdabilidade. 3. RIL. 4. Correlação. I. Oliveira, Antonio Costa de, orient. II. Venske, Eduardo, coorient. III. Maia, Luciano Carlos Da, coorient. IV. Título.

CDD 633.113

Elaborada por Ubirajara Buddin Cruz CRB: 10/901

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Data da Defesa: 31/03/2025

Banca examinadora:

Prof. Dr..Antonio Costa de Oliveira (Orientador)
Doutor em Genética pela Purdue University

Prof. Dr. Flavio Gilberto Herter
Doutor em Agronomia pela Universidade de Clermont-Ferrand

Prof. Dr. Rosa Lia Barbieri
Doutor em Genética e Biologia Molecular pela Universidade Federal do Rio Grande do Sul

Prof. Dr. Maicon Nardino
Doutor em Agronomia pela Universidade Federal de Pelotas

Acknowledgements

I am really grateful to all the people who contribute for the realization and success of this project, my sincere thanks for their valuable time, support and for belief in me all the time.

To my dear supervisor, Professor Antonio Costa, for his accompaniment, guidance and his invaluable great support given at the end of the road of this project. To my dear co-supervisors, Prof. Luciano Da Maia and Eduardo Venske, for the great support always, trust and friendship. All my affection, admiration and respect for all of them.

To my dear family, especially my mom and Gladys, for believing in me, their love, support and for encouraging me not to surrender, staying close even at a distance.

To my lovely friends for all the given support: Katy, Luis, Tania, Thairone & Gustavo, Karine, Cinthia, Valeria, Carito, Naye, Aquelarre, Juan, Lu, Mr Heron and family.

To EMBRAPA Trigo, the breeding area and grain quality laboratory, Ricardo Lima, Eduardo Caierão, Martha Miranda and Ellen Rogoski, and the students and staff who supported me.

To my colleagues from CIMMYT for their great support, Angela Pacheco, Cesar Petroli, and especially Kevin Pixley, thanks for belief in me.

And finally, I would like to thank Brazil, the Universidade Federal de Pelotas and CAPES for the opportunity and supporting this project.

My heartfelt thanks to all.

Quando a palavra impossível não está em nosso vocabulário, não existem limites.
ACO, 2025

Resumo

FRANK-BASTIDAS, Ana Karina. **Germinação pré-colheita na espiga de trigo (*Triticum aestivum* L.): status atual e análise de uma população endogâmica recombinante.** 2025. 83f. Tese (Doutorado em Ciências) - Programa de Pós-Graduação em Agronomia, Faculdade de Eliseu Maciel, Universidade Federal de Pelotas, Pelotas, 2025.

O trigo é um dos três principais cereais do mundo. Apesar de ser cultivado em muitos climas diferentes, os estresses abióticos, como a germinação pré-colheita, afetam o rendimento e a qualidade dos grãos em muitos países. O presente trabalho teve como objetivo identificar a herdabilidade da tolerância à germinação pré-colheita (PHS) em uma população RIL obtida do cruzamento ORS Quartzo x ORS Marfim. Um total de 150 linhagens foram cultivadas nos anos de 2017 e 2018, sendo fenotipadas para características de campo e de laboratório. Foi analisada a distribuição de características como dias para o espigamento (DTH), dias para a maturação (DTM), altura da planta (PTH), produtividade (YLD), peso de mil grãos (TKW), nota visual de germinação (GVS), comprimento da espiga (SL), peso da espiga (SW), número de espiguetas por espiga (NSS), peso de grãos por espiga (GWS) e número de grãos por espiga (NGS), porcentagem de germinação (GP), número de queda (FN). As análises BLUP mostraram um valor de herdabilidade de 0,55 para GP, um dos dois caracteres que indicam a resistência ao PHS. Para o FN, foi obtida uma herdabilidade de 0,64 com o coeficiente de repeteabilidade. A análise de uma população RIL do cruzamento ORS Quartzo x ORS Marfim revelou diferenças na progênie que sugerem segregantes transgressivos para várias características, formando cinco grupos principais. As correlações significativas mais altas foram detectadas entre DTH e DTM (0,84), GSW e SW (0,98), NGS e SW (0,87), NGS e GSW (0,88). A maior correlação negativa significativa foi obtida entre DTH e FN (-0,61).

Palavras-chave: germinação pré-colheita; herdabilidade; RIL; correlação

Abstract

FRANK-BASTIDAS, Ana Karina. **Pre-harvest sprouting tolerance in wheat (*Triticum aestivum* L.): current status and analysis of a recombinant inbred line population.** 2025. 83p. Thesis (Doctor degree in Sciences) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2025.

Wheat is one of the three major cereals worldwide. Despite its cultivation in many different climates, abiotic stresses such as pre-harvesting sprouting affect grain yield and quality in many Countries. The present work aimed at identifying the heritability of pre-harvest sprouting (PHS) tolerance in a RIL population obtained from the cross ORS Quartzo x ORS Marfim. A total of 150 lines were cultivated in the years of 2017 and 2018, being phenotyped for field and laboratory traits. The distribution of traits days to heading (DTH), days to maturation (DTM), plant height (PTH), yield (YLD), thousand kernel weight (TKW), germination visula score (GVS), spike length (SL), spike weight (SW), number of spikelets per spike (NSS), grain weight per spike (GWS) and number of grains per spike (NGS), germination percentage (GP), falling Number (FN) was analysed. The BLUP analyses showed a heritability value of 0.55 for GP, one of the two characters indicating PHS resistance. For FN, a heritability of 0.64 was obtained with the repeatability coefficient. The analysis of a RIL population from the cross ORS Quartzo x ORS Marfim revealed differences in the progeny that suggest transgressive segregants for many traits, forming 5 major groups. The higher significant correlations were detected between DTH and DTM (0.84), GSW and SW (0.98), NGS and SW (0.87), NGS and GSW (0.88). The highest negative significant correlation was obtained between DTH and FN (-0.61).

Keywords: pre-harvest sprouting; heritabiliy; RIL; correlation

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List of Abbreviations

CGIAR	Consultative Group on International Agricultural Research
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo
CONAB	Companhia Nacional de Abastecimento
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária
FAO	Food and Agriculture Organization of the United Nations
IWIN	International Wheat Improvement Network
PHS	Pre-harvest sprouting
RIL	Recombinant Inbreed Line
SisBi/UFPel	Sistema de Bibliotecas da Universidade Federal de Pelotas

Summary

1. INTRODUCTION GERAL

Wheat, rice and maize, make the three most widely grown and consumed grains globally, being, together with potato, the main sources of carbohydrates in the human diet. Although originally from a temperate climate, wheat, due to its polyploid nature, has been adapted to different latitudes of the globe. Brazil started producing wheat after the arrival of the Portuguese, in the 1500s. Later, the crop moved south and, due to the contribution of the Italians, spread in the States of Rio Grande do Sul, later Santa Catarina and Paraná. The expansion of wheat to the Cerrado areas has long been dreamed of. However, two major constraints have delayed the spread of wheat to the warmer areas. The occurrence of blast (*Piricularia oryzae* fs. Triticum) in the wet season and irrigation in the dry season. This deficiency in production is partly due to climatic conditions which do not favor the progress of the crop, and the lack of cultivars better adapted to these environments (CONAB, 2025; FAO, 2025). Although Rio Grande do Sul remains one of the largest wheat producer states in Brazil, every year, due to the occurrence of rains in the spring, several fields loose yield due to pre-harvest sprouting (PHS). Plant Breeding is one of the best weapons that humankind has to combat hunger. Today, near 114 years after the birth of Norman Borlaug, we have an understanding that, through breeding, billions of people can be taken out of famine. Breeders use several tools, including developing crosses and generating mapping populations to identify the genes controlling important traits. Likewise, there is a need for research aimed at identifying gene regions associated with the trait PHS to be used in wheat breeding programs and developing new cultivars that are resistant to this trait. Heritability, a fundamental concept in quantitative genetics, is of paramount importance in plant breeding, offering a measure of the proportion of phenotypic variance that can be attributed to genetic variance within a specific population and environment.

Understanding heritability is crucial for plant breeders as it dictates the potential for selection and improvement of desirable traits in crop plants. It serves as a predictive tool, estimating the extent to which progeny will resemble their parents in a given trait, thus informing decisions on breeding strategies and resource allocation (Narvariya & Singh, 2019). Plant breeding leverages the principles of heritability to identify and select plants exhibiting superior phenotypic traits, with the ultimate goal of generating

progeny that inherit these desirable characteristics, thereby enhancing crop performance and productivity.

The concept of heritability is intrinsically linked to the partitioning of phenotypic variance, which represents the total variability observed in a trait within a population, into its fundamental genetic and environmental components. Phenotypic variance is important for plant breeders for selecting the breeding methods, the locations for conducting yield tests and predicting selection gains. Environmental variations overshadow genetic variations, and the greater the proportion of environmental variability in relation to total variability, the more difficult it is to make an effective selection. Genetic variance, in turn, can be further dissected into additive, dominance, and epistatic variance, each reflecting different modes of gene action and inheritance patterns (Borém & Miranda, 2013). Additive genetic variance, representing the sum of the average effects of individual genes on the phenotype, is of particular significance to plant breeders, as it is the primary determinant of the response to selection (Hill et al., 2008).

The phenotype is the product of gene expression influenced by the environment. Variations in phenotypic values are shaped by genetic and environmental factors, as well as the interaction between genotypes and the environment. The variance component represents the uncontrollable sources of variation, commonly referred to as experimental error or environmental variance. This component reflects the differences in phenotypes arising from the genotype-environment interaction. The variance component is a consequence of genetic differences among individuals. When genes exhibit additive effects, the genotypic value of the trait can be enhanced or diminished by the substitution of an allele. In the broad sense, heritability can be defined as the ratio of genotypic variance to phenotypic variance. In the narrow sense, heritability can be defined as the ratio of additive variance to phenotypic variance. Heritability in the narrow sense is more informative, as it quantifies the relative importance of the additive portion of genetic variance that can be transmitted to the next generation (Borém & Miranda, 2013).

This work combines recent advances in understanding pre-harvest sprouting, providing a concise overview of the causes, consequences, potential mitigation strategies and offering valuable insights into the management and breeding for PHS regulation in bread wheat. Also, the phenotyping analysis of a Recombinant Inbred

Population obtained from the cross ORS-Quartzo x ORS-Marfim, contrasting parents for this trait.

1.1. General Objective

To assess the information regarding PHS in wheat production worldwide. determine the broad sense heritability for pre-harvest sprouting tolerance in wheat, of a RILs population belonging to the program of the Plant Genomics and Breeding Center, Faculdade de Agronomia Eliseu Maciel of the Universidade Federal Pelotas.

1.2. Specific objectives

1.2.1. To identify genotypes with greater tolerance to pre-harvest sprouting and lower alpha-amylase activity in the starch degradation process.

1.2.2. To adjust statistical models to analyze the population behavior in terms of tolerance to pre-harvest sprouting.

1.2.3. To estimate the variance components and calculate the broad heritability of the traits associated with pre-harvest sprouting.

2. REVIEW ON GENETIC IMPROVEMENT FOR PRE-HARVEST SPROUTING TOLERANCE IN WHEAT (*TRITICUM AESTIVUM L.*)

2.1 Introduction

Wheat is one of the three most widely grown and consumed grains globally, along with rice and maize, it is one of the main sources of carbohydrates in the human diet. Brazil has great potential for wheat production, even though the country's largest wheat-growing region is made up of the states of Paraná and Rio Grande do Sul, but also growing in the Cerrado areas. However, production is not sufficient to supply the country, being necessary to import more than half of the wheat consumed to meet national demand. This deficiency in production is partly due to climatic conditions which do not favor the progress of the crop, and the lack of cultivars better adapted to these environments (CONAB, 2025; FAO, 2025). Worldwide, abiotic stressors severely reduce wheat output, and in most wheat-growing regions, their frequency and severity are expected to rise. Humid regions prone to rainfall during the harvest season cause the seed to break dormancy, resulting in premature germination of the grains in the plant's head. This phenomenon, called pre-harvest sprouting (PHS), is a problem that has been reported in most regions of the world including Japan, China, India, the United States, Canada, Australia, North Africa, Europe, and Brazil. PHS can cause up to 30-50% damage to production when highly susceptible wheat cultivars are used, reducing yield potential and grain quality and, consequently, generating losses in the commercial value of the product. The degree of damage caused by germination in the ear, in addition to rainfall intensity, depends on various other factors such as temperature, grain maturity stage, ear morphology, seed dormancy and the presence/absence of PHS tolerance genes (Patwa & Penning, 2020; Schereen & Caierão, 2015). In this sense, this is a trait in which multiple quantitative genes are involved that are heritable and strongly influenced by the environment. Therefore, there is a need for research aimed at identifying gene regions associated with the trait to be used in wheat breeding programs and developing new cultivars that are tolerant to

PHS (Ali et al., 2019). Maintaining the stability of global wheat production is crucial for ensuring food security. To meet the rising food demand driven by a growing

global population, it is vital to improve wheat production through innovation and tackle climate change challenges such as PHS.

This review combines recent advances in understanding pre-harvest sprouting, providing a concise overview of the causes, consequences, potential mitigation strategies and offering valuable insights into the management and breeding for PHS regulation in bread wheat.

2.2 Importance of wheat production

For over 10,000 years, wheat has been a fundamental pillar of food and nutritional security, being one of the main sources of carbohydrates in the human diet. Moreover, along with providing calories, it is essential to ensure that other nutritional components of diets are met. Wheat is a key source of dietary fiber, minerals, B vitamins, and other micronutrients, and it also serves as an excellent source of plant-based protein (Figure 1A and 1B). It provides about 20% of human dietary protein and calories, surpassing maize and rice as a primary protein source and, ranking only behind rice for calories (FAO, 2025; Reynolds & Braun, 2022).

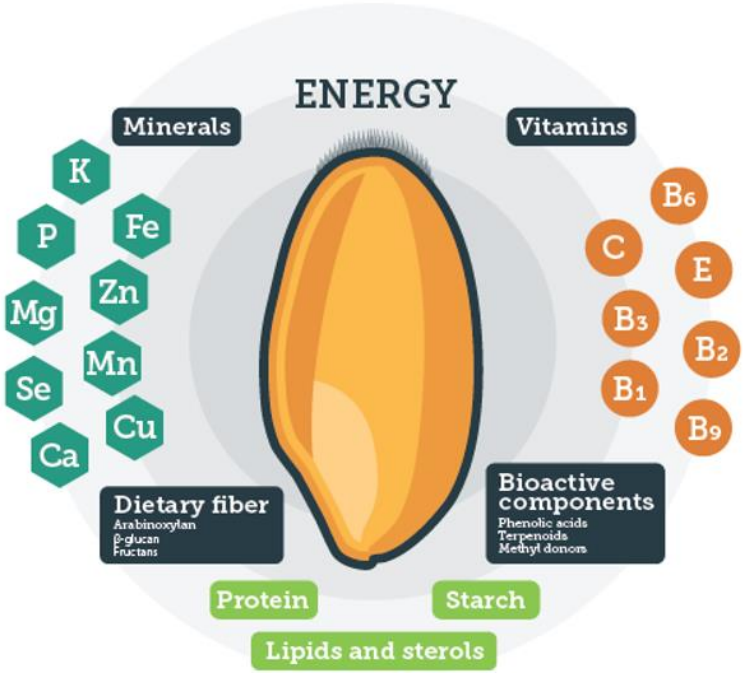

Figure 1. Wheat nutritional composition (CIMMYT.org)

Nutritional value of wheat		
Energy content	222.29	Kcal/100g
Protein	7.86	g/100g
Fat	1.47	g/100g
Carbohydrate, available	40.31	g/100g
Dietary fiber	8.13	g/100g
Calcium	21.32	mg/100g
Iron	2.67	mg/100g
Magnesium	74.63	mg/100g
Phosphorus	229.89	mg/100g
Potassium	259.87	mg/100g
Zinc	1.91	mg/100g
Vitamin A (RE)	0.67	mg/100g
Thiamin	0.29	mg/100g
Riboflavin	0.07	mg/100g

Table 1. Nutritional value of wheat (FAO, 2025)

Wheat is one of the three most widely grown cereals in the world, along with maize and rice (Figure 2), only these three cereals summarize 91% of the total cereal crop production globally (Table 2).

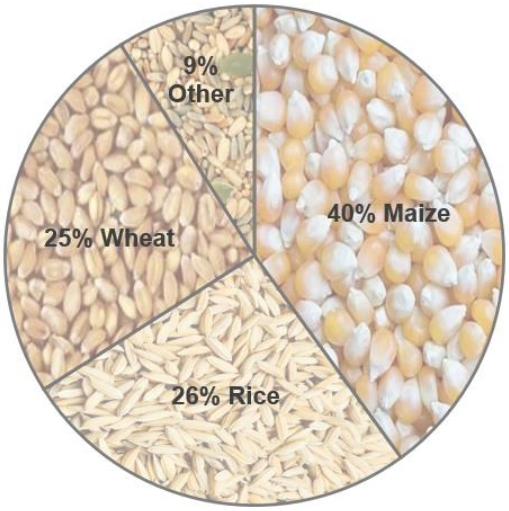

Figure 2. The proportions of global cereal crop production by 2023 (Figure drawn by Karina Frank with data from FAOSTAT 2023 (FAO, 2025)

Table 2. Global cereal crop production by 2023 (FAO, 2025)

Cereal crop	Production (t)	%Global
Maize	1,241,557,811	40
Rice	799,999,505	26
Wheat	798,975,306	25
Others	293,761,275	9
Total	3,134,293,897	100

91%

Cultivated on 215 million hectares annually, wheat is the most widely grown staple crop globally. With a trade value of nearly US $50 billion each year, wheat is a crucial food source for 2.5 billion people across 89 countries, feeding about 40% of the world's population. Projections suggest that by 2050, wheat demand will grow by 60%. Meeting this demand presents significant challenges, as it must be achieved without expanding arable land, relying instead on more efficient use of fertilizers, water and labor (Chang et al., 2023; wheat.org, 2025).

Production and yield must be increasing together to guarantee global food security, without increasing land use. In the last 30 years, production has been increased in less harvested area, according to FAOSTAT 2025 data (Figure 4).

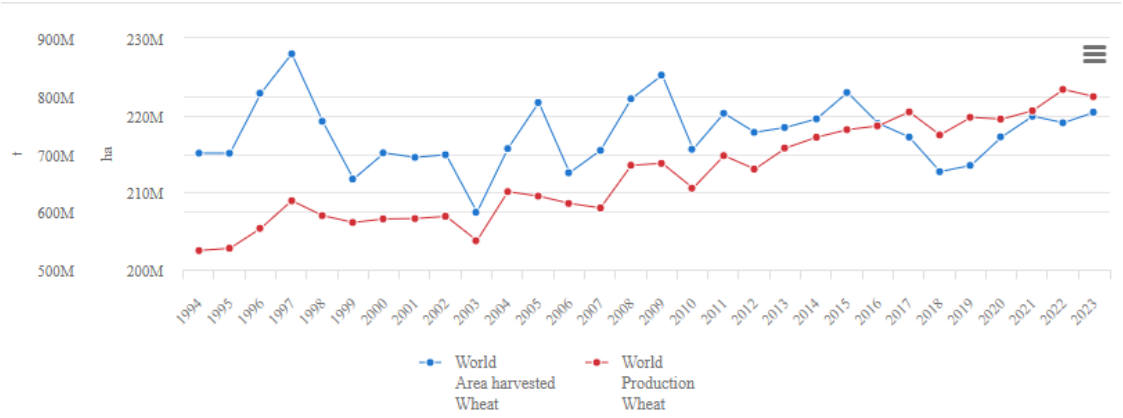

Figure 3. Production/Yield quantities of Wheat in World + (Total) 1994 – 2023 (FAO, 2025)

If we look at the main countries producing wheat globally, these are China, India, Russia, France, Ukraine, Germany and Australia, and, from America those are United States, Canada, Argentina and Brazil (Figure 5). If considered as a unit, the European Union becomes the second largest wheat producer, with ca. of 140 million tons.

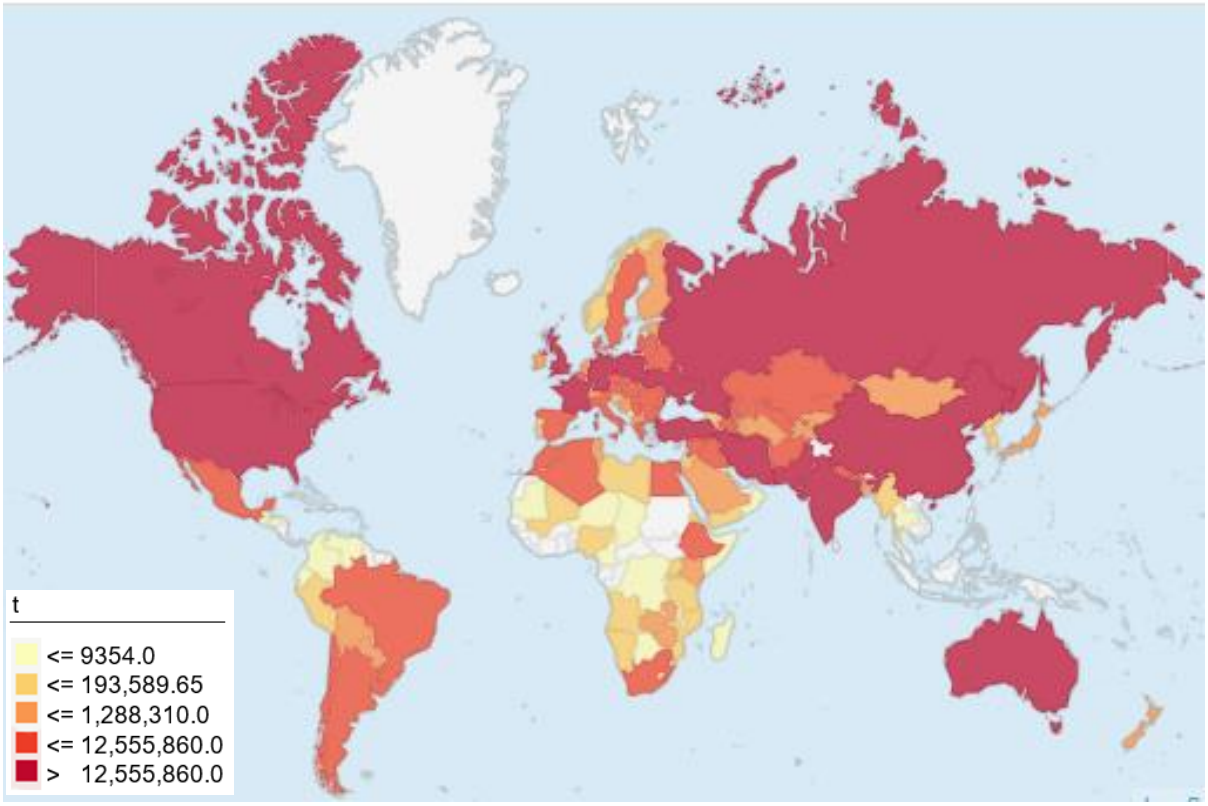

Figure 4. Global wheat production (FAO, 2025)

In the Americas, Brazil has a great potential for wheat production, going beyond the country's largest wheat-growing region, i.e., the states of Paraná and Rio Grande do Sul, reaching the cerrado areas. However, production is not sufficient to supply the country, so much so that it is necessary to import more than half of the wheat consumed to meet national demand, and this deficiency in production is partly due to climatic conditions which do not favor the progress of the crop, and the lack of cultivars better adapted to these environments (CONAB, 2019; FAO, 2019).

In the 2024 season, Brazil's wheat production reached 9,117,900 tons, with a yield of 3.04 kg ha^{-1}, marking increases of 15.6% and 18%, respectively, compared to the previous year (Table 4). This was grown on an area of 2,995 thousand hectares, which is 2.1% smaller than the previous year, representing a significant achievement of producing more on a reduced cultivated area (CONAB, 2025).

Table 3. Brazil wheat production from 2019-2025 (CONAB, 2025)

Season	Area (ha)	Yield (t ha^{-1})	Production (t)
2019	2040.5	2.5	5154700
2020	2341.5	2.7	6234600
2021	2739.3	2.8	7679400
2022	3086.2	3.4	10554400
2023	3473.4	2.3	8096800
2024	3058.7	2.6	7889300

It is interesting to mention that the data of 2023 wheat production in the Americas highlight Brazil as the 4th country with larger wheat production, reaching 7.7 million tons and a harvested area of 3.3 million hectares, only below the US, Canada and Argentina.

On the other hand, regarding yields, Brazil ranks as t 6th with a value around 2.3 kg ha^{-1}. Therefore, considering the great extension of arable lands in the country and the high potential of production, improving wheat yield Brazil has a great potential to launch it to be one of the main wheat producers (Fig.8A-8C).

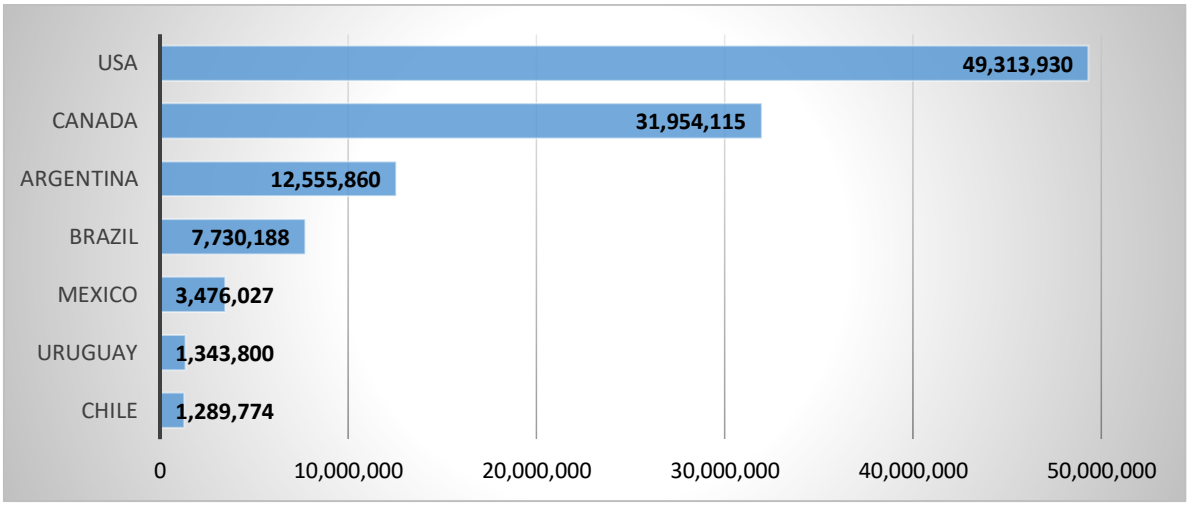

Figure 5A. American countries with major wheat production (t) in 2023 (FAO, 2025)

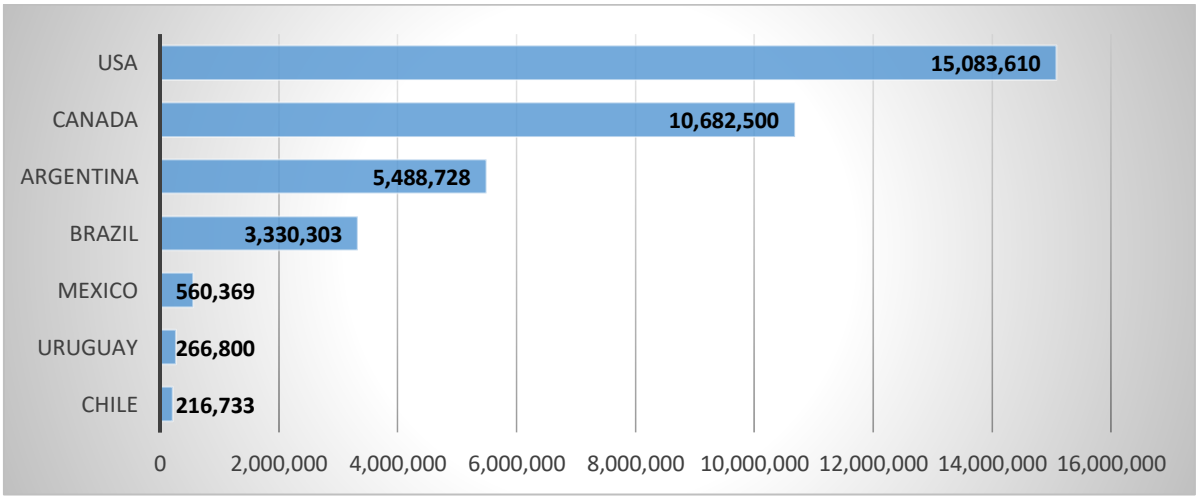

Figure 5B. American countries with major wheat harvested area (ha) in 2023 (FAO, 2025)

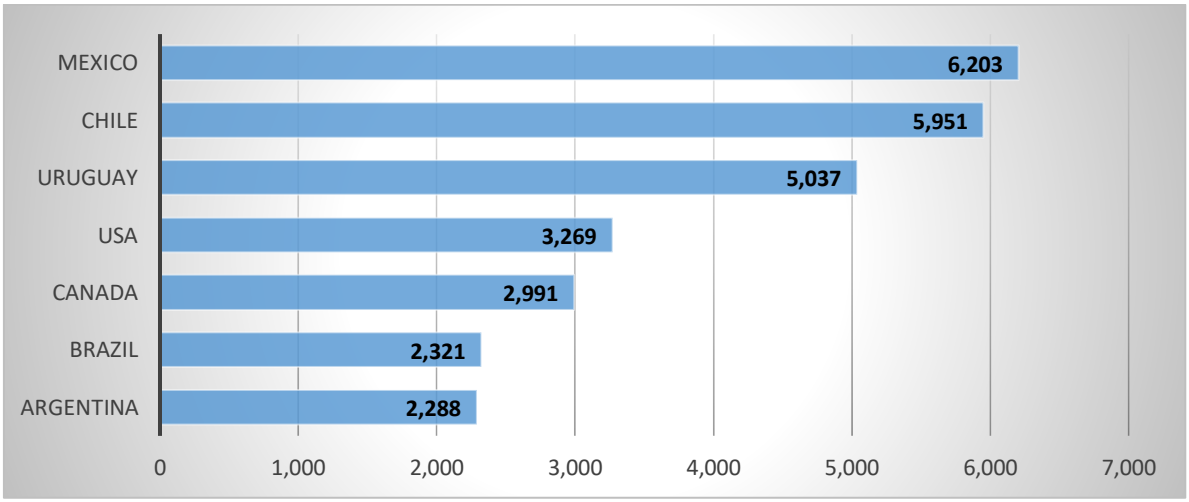

Figure 5C. American countries with major wheat yield (kg ha^{-1}) in 2023 (FAO, 2025)

2.3 Understanding pre-harvest sprouting (PHS)

As a crop cultivated across all five continents and adapted to a wider range of environments than any other, wheat is susceptible to a broad variety of transboundary diseases and abiotic stresses that limit wheat yield stability. Climate change represent huge challenges, with increases in temperature and humidity variability, we face several abiotic stresses on wheat crop, one of them is the phenomenon called pre-harvest sprouting (Reynolds & Braun, 2022).

2.3.1 Pre-harvest sprouting definition

Pre-harvest sprouting is the early germination of grains while they are still on the spike of the mother plant, with breakage of dormancy after maturity but before harvest. This phenomenon affects various cereals, mainly wheat (Figure 6), barley, rye and triticale. It has the effect of reducing the commercial value of the grains, representing a serious global problem for agricultural production (Patwa & Penning, 2020; Tai et al., 2021). Pre-harvest sprouting occurred recurrently in many major wheat producing areas of the world, including China, USA, Japan, Canada, Australia, Europe and Brazil (Ali et al., 2019).

Figure 6. Photo PHS. Courtesy by Thomas Lumpkin/CIMMYT 2008

A series of factors and biochemical reactions are necessary for this event to occur, but mainly two conditions: the breaking of seed dormancy during the grain filling phase, plus the occurrence of rainfall in the pre-harvest period when the germination genes are activated (Cunha & Pires, 2004; Guarienti et al., 2017).

Excessive precipitation during the wheat harvest period cause seed to absorb water, initiating the germination process (Figure 7). This physiological change in the grain's composition occurs when the crop remains unharvested under such wet conditions. Pre-harvest sprouting results from increased alpha-amylase activity, that initiates the process of converting the starch in the endosperm into more easily digestible carbohydrates that feed the growing sprout and provide energy for germination. The starch degradation at this stage is undesirable as it is directly associated with reduced yield and it can diminish grain quality and consequently affect crop values by 20-50%, and in extreme scenarios, render them unfit for human consumption, suitable only for use as animal feed (Ali et al., 2019; Patwa & Penning, 2020; Vetch et al., 2019).

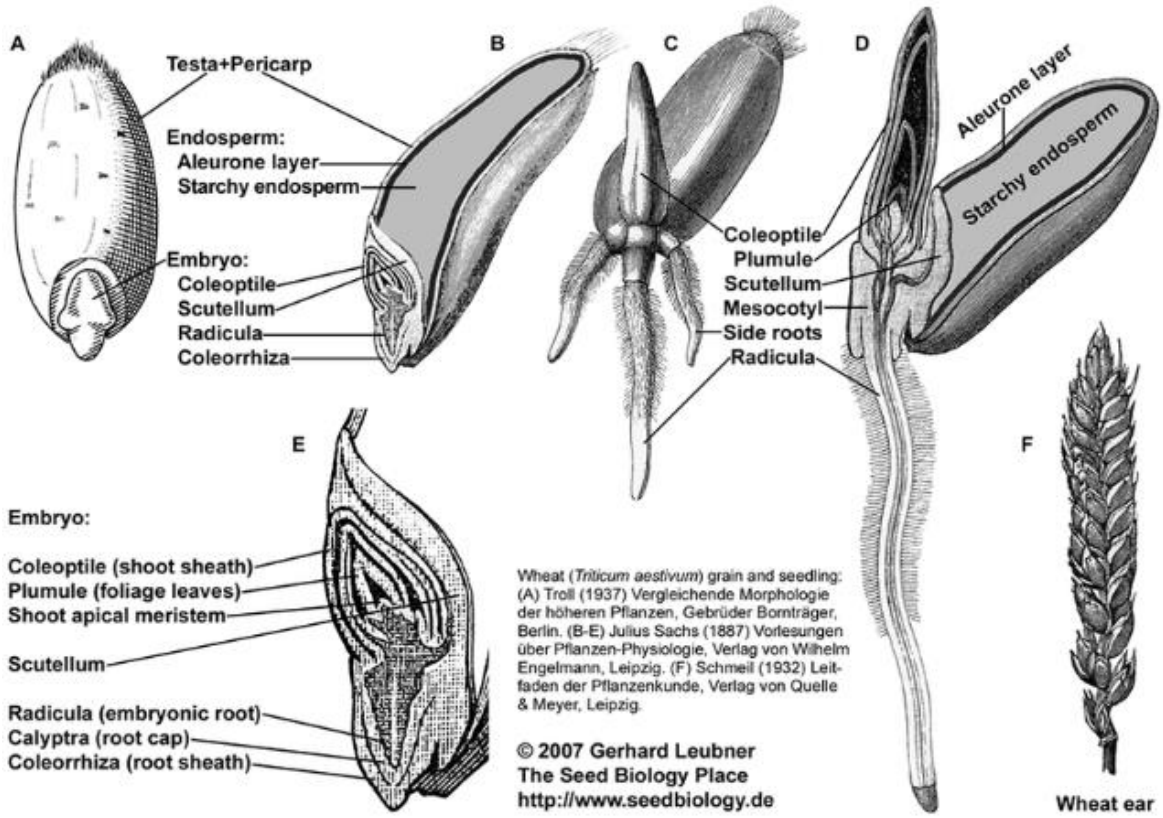

Figure 7. The anatomical sketch of wheat grain: A) posterior view, B) Longitudinal section, C&D) germinating seeds, E) Longitudinal view of the embryo and F) wheat ear. Figure courtesy by Gerhard Leubner @ 2007; The seed biology place.

2.3.2 Mechanisms of Pre-Harvest Sprouting

Preharvest germination in wheat is influenced by a complex interplay between genetic and environmental factors. The environmental conditions that have the most significant impact on the occurrence of premature germination include humidity and temperature during the final stage of the plant's cycle, as well as the morphological characteristics of the ear and the physiological factors involved in the seed germination process (Ali et al., 2019; Chang et al., 2023)

2.3.2.1 Environmental factors

Among the many environmental factors that are related to pre-harvest germination, high relative humidity, heavy rainfall conditions close to harvest, and, temperature (high or low) in the final stages of grain development, all can influence dormancy levels (Figure 11). In this sense, cold temperatures between 10 and 15 °C during grain filling, or hot temperatures above 30°C during grain soaking, are associated with increased dormancy (Ali et al., 2019; Reynolds & Braun, 2022; Vetch et al., 2019).

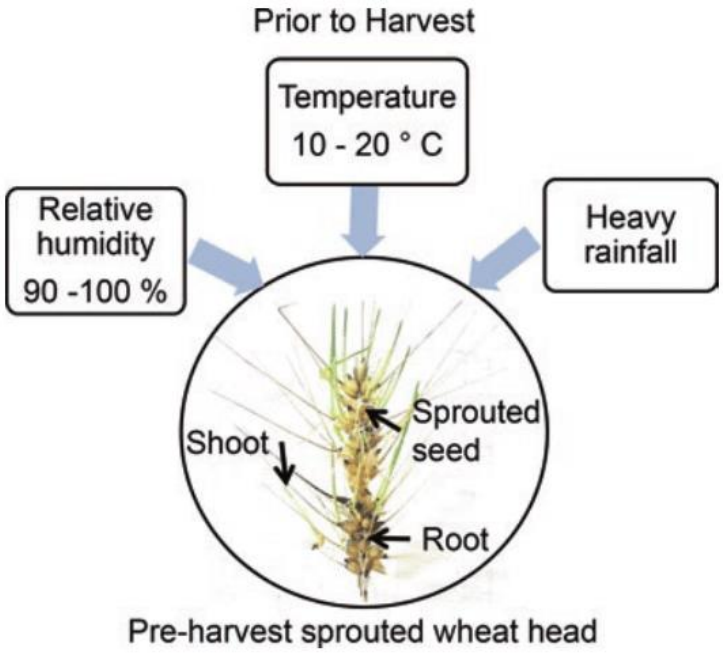

Figure 8. Environmental conditions affecting pre-harvest sprouting. Image of a heavily sprouted soft winter wheat spike with many roots and shoots extended out from germinating seeds. Rain, humidity, and temperature can all contribute to sprouting before harvest. (Patwa & Penning, 2020)

2.3.2.2 Crop morphology and biological factors

PHS resistance is associated with several developmental, physiological, and morphological features of the spike and seed. One can include seed coat (pericarp) color and permeability, seed dormancy, α-amylase activity, and levels of plant growth hormones (abscisic acid, gibberellin and auxin).

Morphology

Several morphological characteristics such as awn length, head angle, spike shape, and glume tenacity can affect the amount of moisture in the grain head and its absorption. Wheat lines with heads that remain upright tend to shed more moisture than those that bend parallel with the ground or turn downward, resulting in less sprouting (Wade Thomason et. al., 2024). Another factor that can influence early germination is the structure and morphology of the ear in relation to water absorption, where longer and more open ridges maximize the surface area exposed in the ear for water collection (Vetch et al., 2019). On the other hand, the impermeability of the seed's protective structure and the presence of inhibitory substances in the structure of the ear may also be directly related to the incidence of this phenomenon (Peske et al., 2012).

Other factors have also been linked with PHS resistance, like waxiness, hairiness, ear morphology, and germination-inhibitory compounds produced in bracts surrounding the grains. Among them, seed dormancy is the major genetic factor controlling PHS resistance, thus requiring much attention from investigators in order to understand the molecular mechanism of seed dormancy and its link to PHS resistance breeding.

Seed embryo-imposed dormancy is linked to seed survival mechanism of several species. The seed coat is a barrier to radicle protrusion and is impermeable to water and/or oxygen causing restrictions to germination (Ali et al., 2019).

Grain color

PHS is partially controlled by genetics. The color of the seed coat seems to influence sensitivity to PHS. Lines with white seed-coat tend to be sensitive while those with red seed coats tend to be resistant. Red and white refer to the color of the seed pericarp (seed coat), which is controlled by three independent genes (Figure 9). The

genes that control PHS are located nearby on the chromosome to – the genes for seed-coat color. Therefore, selection for white seed coats, leads to indirectly selecting for lower inherent dormancy. All three separate genes for seed-coat color must be homozygous for white to produce wheat with a white seed coat. The dominant gene codes for red color, resulting in darker shades of red when dominant genes are added, with a continuum ranging from white (0 dominant genes) to dark red (three dominant alleles). There is an association of higher dormancy levels in these lines with higher numbers of red seed-coat genes. Some variation is found regarding to the different dominant alleles in each locus. There are also genes controlling dormancy that are independent of the color genes, for example the Brazilian wheat cultivar BRS Parrudo that present red grain and yet is susceptible to pre-harvest sprouting (EMBRAPA TRIGO, 2017; Wade Thomason et. al., 2024).

The red pigment in the testa of plant grains comes from different compounds: catechin and proanthocyanidins (PA) that are produced in the flavonoid biosynthesis pathway and synthesized by different enzymes. These enzymes are dihydroflavonol-4-reductase (DFR), chalcone flavanone isomerase (CHI), flavanone 3-hydroxylase (F3H), and chalcone synthase (CHS). These enzymes are expressed only in immature red grains and can be close to completely repressed in the grains of white wheat. (Ali et al., 2019).

Figure 9. White and red color kernel wheat (T. Pearson, 2010)

Seed dormancy

The inhibition of germination of ripe and healthy seeds is known as dormancy, acting independently of optimum conditions of light, moisture, and temperature. The dormancy initiation and maintenance can be affected by genetic and environmental factors. The dormancy can be based on seed coat, when inhibitory compounds crosstalk, ie., phytohormones, such as abscisic acid (ABA), gibberellin (GA), and auxin, are involved in embryo-imposed dormancy (Ali et al., 2019; Chang et al., 2023).

Dormancy mechanisms are evolutionary adaptations necessary for the survival of species, a safety mechanism that prevents seeds from germinating under unfavorable conditions for seedling development (Peske et al., 2012). Dormancy is a trait controlled by multiple genes, characterizing a complex inheritance, being a quantitatively inherited trait that is strongly influenced by environmental variables. In addition, genetic improvement processes, with advances in generation, tend to select genotypes against dormancy (Guarienti et al., 2017). Thus, breaking dormancy before harvest results in unwanted germination of the grains in the ear.

The presence of low dormancy in wheat leads to significant losses in yield and quality in wheat. Therefore, it is essential to induce an adequate level of seed dormancy to prevent PHS.

2.3.2.3 Genes associated with pre-harvest sprouting

The genetic predisposition of wheat cultivars to pre-harvest sprouting has been extensively studied, as it is a typical quantitative trait. Numerous QTLs and genes conferring PHS tolerance have been identified in wheat, including *TaSdr*, *TaPHS1*, *TaMFT*, *TaVp-1*, *Tamyb10*, and *TaMKK3-A*. These QTL/genes are valuable for gene pyramiding in breeding programs. The genetic control of pre-harvest sprouting tolerance involves both epistatic and additive genetic effects, which are modulated by environmental factors. Researchers have examined the interplay between quantitative trait locus epistasis and the environment to delineate the complex genetic underpinnings of pre-harvest sprouting tolerance. In wheat, PHS tolerance is controlled jointly by multiple QTLs located in almost 21 chromosomes (1A, 1B, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 5B, 5D, 6A, 6B, 6D, 7A, 7B and 7D). Among them, the most relevant are *TaMFT-3A*, which is attributed 58% of the phenotypic variation. *TaMKK3* as the

second with the highest impact with 43% and, the *R-A1*, *R-B1* and *R-D1* genes that control the red color of the grain in addition to dormancy, attributed between 26% and 44% of the phenotypic variation (Ali et al., 2019; Vetch et al., 2019).

Abscisic acid is a key regulator of seed dormancy, playing a significant role in inducing and maintaining dormancy during seed development and imbibition. Numerous genes, including *TaPHS1* (a *TaMFT-like* gene), *TaCYP707A1*, and *TaDOG1*, have been identified as involved in seed dormancy and the biosynthesis and signaling of abscisic acid. The cloned genes *TaPHS1/TaMFT*, *TaSdr*, *PM19-A1/A2*, and *TaMKK3-A* have been found to control seed dormancy and pre-harvest sprouting resistance in wheat. The *TaSdr* genes *TaSdr-A1*, *TaSdr-B1*, and *TaSdr-D1* are implicated in seed dormancy, with *TaSdr-B1* on chromosome 2B playing a vital regulatory role. Additionally, *PM19-A1* and *PM19-A2* have been identified as positive regulators of seed dormancy, with *PM19-A1* highly expressed in dormant genotypes during grain maturation and *PM19-A2* showing sequence variations between non-dormant and dormant genotypes. The gene *MKK3-A*, also known as *TaMKK3-A*, has been identified on chromosome 4AL as a candidate gene of the *Phs-A1* locus, which is associated with the duration of seed dormancy (Ali et al., 2019).

Wheat grain color is an important genetic trait that influences the brightness of flour and is also associated with seed dormancy and pre-harvest sprouting tolerance. This trait is controlled by the *R-1* gene series, which is located on the long arms of chromosomes 3A, 3B, and 3D. Dominant R-1 alleles, denoted as *R-A1b*, *R-B1b*, and *R-D1b*, confer red grain color, while recessive alleles, *R-A1a*, *R-B1a*, and *R-D1a*, contribute to white grain color. For the dominant *R-1* alleles, the expression of red color is dose-dependent, with a single allele being sufficient to produce red color, and the intensity increasing with the number of dominant alleles. The R genes function as transcriptional activators of flavonoid synthesis genes and are positioned in the same chromosomal regions as *Myb*-type transcription factor loci (*Tamyb10-A1, Tamyb10-B1,* and *Tamyb10-D1*). Studies have confirmed that the three *Tamyb10-1* genes on chromosomes 3AL, 3BL, and 3DL are candidate genes underlying the *R-1* loci for wheat grain color.

In order to improve quality, understanding the heritability of each trait, its genetic underpinnings, and the extent to which environmental factors influence its variation is crucial for effectively breeding. Among the key elements influencing wheat quality,

grain hardness, gluten quality, flour color, and starch properties have been extensively studied. As a result, substantial information is available on the genetic and environmental factors that affect their variation (Table 4) (Reynolds & Braun, 2022).

Table 4. Genes associated with major influences on wheat quality traits (Reynolds & Braun, 2022)

Trait	Chromosomes	Locus/gene	Protein/enzyme
Grain hardness	5DS	*Hardness*	Puroindoline a, b
Gluten quality	1AS, 1BS, 1DS	*Glu3*	Low-molecular-weight glutenins
	1AL, 1BL, 1DL	*Glu1*	High-molecular-weight glutenins
	1AS, 1BS, 1DS	*Gli1*	γ and ω-gliadins
	6AL, 6BL, 6DL	*Gli2*	α/β-gliadins
Yellow pigment accumulation	7AL, 7BL, 7DL	*Psy1*	Phytoene synthase
	4AL, 4BL, 4DL	*Pds1*	Phytoene desaturase
	2AS, 2BS, 2DS	*Zds1*	ζ-carotene desaturase
	3A, 3B, 3D	*ε-LCY*	Lycopene ε-cyclase
Yellow pigment degradation	4AS, 4BS, 4DS	*Lox1.1*	Lipoxygenase
Flour discoloration	2AL, 2BL, 2DL	*Ppo1*	Polyphenol oxidase
Starch functionality	7AS, 4AL, 7DS	*Wx1*	Granule bound starch synthase I
	7AS, 7BS, 7DS	*Ss1*	Starch synthase I
	7AS, 7BS, 7DS	*Ss2*	Starch synthase IIa
	1AS, 1BS, 1DS	*Ss3*	Starch synthase III
	7AL, 7BL, 7DL	*SbeI*	Starch branching enzyme I
	2AL, 2BL, 2DL	*SbeIIa*	Starch branching enzyme IIa
	2AL, 2BL, 2DL	*SbeIIb*	Starch branching enzyme IIb

The QTLs that influence PHS tolerance can be modulated by environmental factors and by the genotype × environment interaction. The adaptation of wheat cultivars to these environmental conditions can influence the expression of QTLs and, consequently, the effectiveness of PHS tolerance. Genotypes that show resistance to PHS in a specific region may not have the same performance or resistance in another region with different environmental conditions. The scientific literature provides examples that corroborate this interaction, emphasizing the need to consider local environmental conditions when studying and applying information on QTLs in breeding programs (Reynolds & Braun, 2022).

The identification of molecular markers strongly associated with quantitative trait loci governing germination in wheat ears offers the potential for marker-assisted selection to be applied in breeding programs.

2.3.2.4. Impact of pre-harvest sprouting on yield and wheat quality

PHS can reduce the commercial value of the grains, since the flour made from sprouted grains result in bread with reduced volume, more compact and having a dark rind. Therefore, reductions in yield and industrial quality are seen (Guarienti et al., 2017).

One of the initial outcomes of the process of germination is the production of alpha-amylase, followed by other hormones. Proteins are degraded, including gluten, which is key in bread structure (Okuyama et al., 2020). The hydrolysis of endosperm storage materials (such as starch and protein), the 1000-grain weight and bulk weight of germinated seeds decrease, therefore resulting in yield reduction (Chang et al., 2023). Also, besides the decrease in test weight, which leads to reductions in yield, products made with sprouted grain are undesirable because they may be off color, porous, and sticky. It adversely affects the baking quality of flour leading to bread loaves with large holes, sticky crumb, and dark-colored crusts or sticky noodles and pasta. This decrease on quality could be easy measured through a lab test called Falling Number (FN), where a low FN value results from a high alpha-amylase activity and, indicating a high occurrence of PHS (Figure 10) (Patwa & Penning, 2020; Vetch et al., 2019).

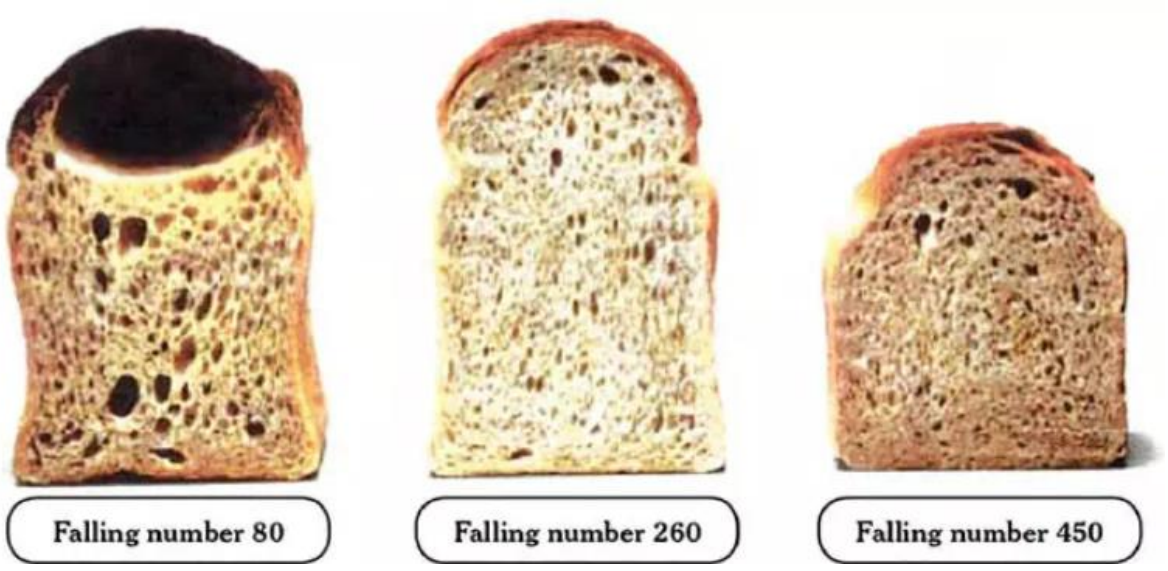

Figure 10. Baking results related to Falling Number

2.3.2.5. Economic Consequences of pre-harvest sprouting

PHS occurs in almost every wheat growing region in the world, being responsible for up to $1 billion in annual losses. (Vetch et al., 2019; Ali et al., 2019). The price of sprouted grain is decreased by 20–50% and is unacceptable for human food if it contains more than 4% sprouted grains.

PHS incidence varies by region and is influenced by climatic conditions. For instance, in China, PHS affects 83% of the country's wheat-growing area, or approximately 25 million hectares of wheat. The annual loss in Canada is also high, estimated to exceed USD 100 million. In the United States, PHS is a problem when there is late-season rainfall. Australia, United Kingdom and France also have reported big losses in wet years (Chang et al., 2023).

Wheat variety and region can influence the severity of PHS, as well as harvest timing. Ongoing research aims to develop wheat varieties with enhanced resistance to PHS to mitigate these losses (Haleck et al., 2022).

2.4 Wheat breeding goals for pre-harvest sprouting tolerance

Although some understanding of the genetic control of PHS does exist, breeders have to take into account tolerance, market preferences and yield of germplasm.

2.4.1 Origins of the pre-harvest sprouting problem

Wheat is among the world’s oldest and most widely used food crops, domesticated more than 10,000 years ago in the Near East’s Fertile Crescent (Reynolds & Braun, 2022). Its domestication has significantly influenced seed dormancy and pre-harvest sprouting (PHS). Some traits, such as uniform germination and rapid growth targeted by selection, inadvertently reduced seed dormancy, leading to PHS sensitivity. Integrate PHS screening early in breeding programs and maintaining genetic diversity for PHS-relevant traits like seed coat color, embryo dormancy, and spike morphology, could be good strategies to overcome this problem.

2.4.2. Management of pre-harvest sprouting

In order to prevent the occurrence of pre-harvest germination, cultural control strategies are the most commonly used, however, the aim is to make the process more efficient by preferring genetic control.

2.4.2.1 Cultural management

The alternatives for cultural control of the problem of pre-harvest germination in wheat, are the choice of cultivars, staggering of sowing times and organization of early or staggered harvesting, as well as management strategies. The use of mechanical (use of mowing) and chemical (use of herbicides and/or growth regulators) cultural practices are also alternatives (Guarienti et al., 2017). In Brazil, the implementation of these strategies for mitigating the risk of PHS is suggested:

1) Cultivar choice. Use PHS-tolerant varieties (Frontana, Celebra, CD 1440, DNAT Prisma, Jadeíte 11, Quartzo, TBIO Astro, TBIO Alvorada, TBIO Mestre, Fundacep Raízes, Fundacep Cristalino, BRS Guamirim). Frontana cultivar has historically been recognized as a benchmark for PHS.
1) Sowing timing. Sowing earlier or later windows (depending on region) to avoid grain maturation during the rainy season.
2) Harvest early, as soon as physiological maturity is reached to avoid late-season rain exposure, even with higher moisture if drying is possible.
3) Drainage. Choose well-drained soils or improve field drainage to reduce prolonged moisture near the wheat heads.
4) Spacing. Optimize row spacing and plant density to enhance airflow and reduce humidity around the spike during maturation.
5) Nitrogen timing. Apply split nitrogen doses early and mid-season rather than late, high nitrogen near maturity can lead to delayed senescence and increased canopy humidity, raising PHS risk.
6) Implement field monitoring visual inspections and sprouting lab tests
7) Regional collaboration with local cooperatives, research institutes (e.g., Embrapa), and extension agents to get real-time alerts and share PHS tolerant cultivars

9) Genetic tools. Encourage use of molecular markers and QTL-informed breeding (de Franceschi et al., 2009; Lima De Castro et al., 2014; Nörnberg et al., 2015)

2.4.2.2 Genetic management

While environmental effects cannot be controlled in the field, genetic resistance/tolerance is possible. Genetic control of PHS and late maturity alpha-amylase is complicated by the numerous factors involved in modulating alpha-amylase which ultimately leads to starch degradation. This includes multiple enzymatic pathways, multiple plant hormones, a complex signaling pathway, several physical factors, and potential protein inhibitors that have been found to mitigate alpha-amylase (Patwa & Penning, 2020).

The use of cultivars tolerant to pre-harvest sprouting allows wheat to be grown in regions not favored by the environment, but there is a constant need for new cultivars developed by breeding programs in order to diversify the range of alternatives for use by producers. In the breeding process, choosing genitors with a higher level of dormancy and lower alpha-amylase enzyme activity is fundamental for obtaining desirable genetic materials (Okuyama, 2013). Regarding germplasm resources, PHS-resistant, red-grained semi-spring varieties are relatively rich germplasm resources (Chang et al., 2023).

The most popular wheat cultivars recommended to be sowed in Rio Grande do Sul, Brazil because of it high PHS-tolerance are showed in the Table 5 (BIOTRIGO GENÉTICA, 2023; EMBRAPA TRIGO, 2023; OR SEMENTES, 2023).

Table 5. Top 10 PHS-Tolerant wheat cultivars in Rio Grande do Sul, Brazil (2024)

Cultivar	PHS Classification	Developer	Release Year	Notes
TBIO Audaz	MR	Biotrigo Genética	2019	Most widely used cultivar; high yield; good sprouting and disease tolerance.
TBIO Toruk	R	Biotrigo Genética	2022	Gaining ground rapidly; excellent PHS and yellow rust resistance.
BRS Reponte	R	Embrapa	2014	Popular for bread making; consistent yield and stable PHS resistance.
BRS Gralha-Azul	R	Embrapa	2002	Well-known for high gluten strength and reliable PHS resistance.
TBIO Mestre	R	Biotrigo Genética	2021	High baking quality; early maturity; increasingly adopted.
TBIO Alvorada	MR	Biotrigo Genética	2016	Widely used in previous years; still relevant in some regions.
TBIO Astro	MR	Biotrigo Genética	2013	Older cultivar; adapted to multiple environments; moderate PHS control.
Fundacep Raízes	R	Fundacep/ Fecotrigo	2020	Solid option for disease control and sprouting tolerance.
BRS 327	R	Embrapa	2017	More adopted in Paraná, but adapted to southern Brazil; solid PHS and yield.

PHS Resistance Classification: R = Resistant, MR = Moderately Resistant

Note: Popularity based on recent use in RS (2023–2024), agronomic bulletins, and breeding programs

2.4.2.3 Genetic improvement and reducing PHS Susceptibility

Genetic improvement for pre-harvest sprouting (PHS) tolerance in wheat is a critical area of agricultural research aimed at enhancing crop resilience and grain quality. Based on the characteristics passed down through history and in comparison, to wild ancestors, early plant breeders and farmers selected for three main trait

classes: 1) Prioritizing the growth of edible plant structures to maximize yield; 2) Improving palatability and nutritional content; 3) Enhancing adaptation to a variety of biotic and abiotic stresses, an ongoing challenge for breeders.

In essence, contemporary breeding practices are qualitatively similar to those employed by our ancestors, as the core selection objectives have largely remained unchanged, despite the advancements in breeding technologies.

Crop management strategies can optimize the plant's environment to some degree, including through the provision of adequate nutrients, management of biotic threats, and the selection of appropriate sowing dates, crop rotations, and irrigation practices where feasible. However, the persistent presence of significant yield gaps in most annual cropping systems underscores the critical importance of selecting for heritable traits through plant breeding efforts (Reynolds & Braun, 2022).

Wallace et al. and Fernie and Yan divided the evolution of breeding into four stages: 1)The first stage of crop improvement was phenotypic selection by farmers, who chose desirable traits based on visual observation; 2) The second stage ushered in the era of hybridization, where breeders began crossing different plant varieties; 3) Currently, most breeding programs are in the third stage, utilizing biotechnologies such as marker-assisted breeding, genomic selection, transgenics, and bioinformatics; 4) We are now entering the fourth stage, known as "breeding by design," which involves genome editing and precision breeding supported by big data analysis. This stage aims to develop crops that meet the expectations of farmers and consumers in terms of yield, yield stability, tolerance to biotic and abiotic stresses, as well as improved nutrition and quality. (Fernie & Yan, 2019; Wallace et al., 2018).

Crop improvement depend on integration and application of many disciplines and it has been essential for global food security since the Green Revolution, when the human population more than doubled. Over the past half-century, cereal yields have tripled even as the area sown has remained relatively constant. This demonstrates the significant impact of crop research on breeding and crop management. However, the challenges now are not just feeding nearly 10 billion people by 2050, but doing so sustainably in a warmer, more unpredictable climate (Reynolds & Braun, 2022).

The CGIAR Research Program on Wheat, an international research collaboration to foster a sustainable, food-secure future, focusing on one of the world's

most important staple crops: wheat. From 2012-2022, wheat research led to the development and deployment of more than 951 wheat varieties in 60 countries. For this objective, there is a combined contribution from the International Maize and Wheat Improvement Center (CIMMYT), the International Center for Agricultural Research in the Dry Areas (ICARDA) and International Winter Wheat Improvement Program (IWWIP).

2.5 Breeding Strategies and Techniques

Breeding wheat with high PHS resistance has always been one of the main goals in regions affected by this phenomenon.

To date, most breeding methods for PHS resistance involve traditional hybridization between the parents instead of molecular breeding. Previous studies have shown that molecular marker-assisted selection can effectively improve the efficiency and accuracy of breeding PHS-resistant wheat varieties. Other studies have also shown that transplanting genes and gene editing can significantly improve seed dormancy and PHS resistance. The identification of more genes for PHS resistance can broaden the scope of molecular breeding techniques (such as transgenic and gene editing techniques) in breeding wheat varieties. Therefore, it is particularly important to develop and identify excellent germplasm resources with high PHS resistance and to clone some functional genes with significant effects on PHS resistance during molecular breeding of wheat. (Chang et al., 2023)

2.5.1. Traditional Breeding Methods

Traditional breeding approaches, such as crossing and backcrossing between wheat parents, have been the primary methods employed, particularly in China. Under strict selection pressure (long-term natural and artificial selection), many wheat varieties with good PHS resistance have been identified and bred by the conventional hybridization method in areas prone to this abiotic stress. The majority of the PHS-resistant wheat varieties are red-grained spring types, encompassing landraces, local cultivars, and recently developed modern varieties. However, most of the varieties with favorable PHS resistance are red-grained, while white-grained wheat varieties with

comparable PHS tolerance are limited in cultivation and utilization within these regions. The resistance mechanism is closely associated with grain color, the red-grained varieties have more advantages in terms of PHS resistance, compared with the white-grained varieties, an advantage that has persisted over time (Chang et al., 2023).

2.5.2. Biotechnology tools for pre-harvest sprouting tolerance

Several biotechnological tools can be of use to the improvement of PHS tolerance. However, since the stacking of resistant genes can be obtained by genetic modification (GM) technology, policy makers and consumers must first accept such products. Therefore, the use of marker-assisted selection and genomic selection are viable alternatives to efficiently change ineffective alleles for more effective ones. Hence genomic selection for yield is based on the modelling of largely random markers to train QTL-based models of yield prediction, it overlooks the importance of genetic background and environment in determining which alleles may impact crop performance. One can argue that before reaching a deterministic stage where models can effectively predict population performance, phenomics, genomics, in silico breeding, technologies required the addition of a broader range of disciplines combining biological basis and mathematical algorithms (Ali et al., 2019; Reynolds & Braun, 2022).

2.5.2.1 Quantitative Trait Loci (QTL) Mapping

The distribution of QTLs for PHS resistance cover almost all 21 chromosomes of wheat. A total of seven regulatory genes were identified and several molecular markers have been described according to their potential use in the selection of PHS resistant plants. Additionally, loci on chromosomes 3A, 3B, and 4A, have already been used in PHS resistance breeding. Some studies have shown that the genes/loci used for pyramiding PHS tolerance, are still limited to 3AS (*Barc321*/*TaMFT-3A*), 3BL (*TaVp-1B*) and 4AL (*TaMKK3-A*), while other reported genes/loci are rarely used. The reason for the lack of use of other sources may be that the functions are still not well verified, or their effect on PHS resistance is weak and lack effectiveness. However, as more and more candidate genes/loci are mined and identified, more and more genes will be used in wheat PHS resistance breeding (Chang et al., 2023)

2.5.2.2 Transgenic and Gene Editing Breeding

The Green Revolution in the 1960s, based in wheat on *Rht1* and *Rht 2* dwarfing genes and breeding genetic backgrounds to suit them, have delivered more sophisticated methodologies for crop improvement. In the 1980s, a biotechnology revolution has enabled one to efficiently meet the demands of a fast-growing global population through steady genetic gains and broad-spectrum resistance to pests and diseases in wheat and other staple crops, with exceptionally high returns on investment documented (Reynolds & Braun, 2022).

Gene editing technology has been shown to be a powerful tool to incorporate novel and effective alleles in many crop species. Clustered regularly interspaced palindromic repeats (CRISPR), a gene editing technology, has made rapid developments in the past few years and has become an important tool for plant functional gene research and crop genetic improvement. Therefore, the application of gene-editing technology can effectively improve breeding progress of PHS resistance in wheat..The genes that have been used for transgenic and gene editing approaches are seven. The maize maize gene *Vp-1*, including promoter and coding regions, has been inserted into the wheat variety Zhengmai 9023, and the seed GI value of T3–T5 of the transgenic generations decreased by 79%, 80%, and 82% compared with the wild type, respectively (Huang et al., 2012). The *Qsd1* gene controlling dormancy in barley grains encodes an alanine aminotransferase. Its homologous in wheat, *TaQsd1* was cloned and its involvement in the regulation of seed dormancy was confirmed. In a previous study, CRISPR-Cas9 technology was used to edit *TaQsd1*, and the genetically edited wheat had a significantly longer dormancy period and improved PHS resistance compared with the wild type.

Sequencing of the wheat genome, in conjunction with phenotyping cultivars in different environments will shed light on the most important alleles for each trait in a given environment. This information will help to obtain PHS tolerant genotypes for each specific cultivation region, refining breeding strategies (Reynolds & Braun, 2022).

2.5.3 Methods for assessing pre-harvest sprouting

Pre-harvest sprouting is most often evaluated through visual observation and by the Hagberg-Perten Falling Numbers (FN) test (Figure 11). Visual assessment involves directly observing the presence of radicles or shoots, which indicates the grain has progressed past the initial germination stage and is considered severely damaged, suitable only for animal feed. In the absence of visible signs of germination but suspected PHS-affected grain, the Falling Numbers assay is employed to determine if sprouting has commenced. This test is utilized as a quality factor during grain receival and trading. The assay consists in a measurement of an increase on alpha-amylase activity, an enzyme involved in the spouting process through the starch degradation, which when present can significantly reduce grain quality. A low falling number value is an indirect indicator of low seed dormancy and low PHS resistance (Ali et al., 2019).

Figure 11. Hagberg-Perten equipment for Falling Number determination. Embrapa Trigo, Passo Fundo, 2019

Falling number assay determines if starch degradation has occurred by thoroughly mixing the whole meal flour with water, heating the solution and then letting a stirring paddle drop through the resulting gel. The length of time it takes the paddle to fall is indicative of starch structure. The longer time for the plunger takes to fall, higher the falling number means. A low falling number indicates the enzymatic process of the starch breaking down has begun to occur. So, the lower the number, the more

enzymatic activity has occurred (high flour alpha amylase level), indicative of the starch degradation and consequently, incidence of pre-harvest sprouting and low quality. Flour from PHS contaminated grain has a less viscous gel allowing the stir bar to drop faster than in a sound sample. For wheat, a typical range for falling number value required for most milling grades would be 300-400 seconds (Vetch et al., 2019).

To study preharvest sprouting in wheat, researchers often need to artificially induce this phenomenon, as many growing regions do not consistently experience favorable conditions for its natural occurrence every year. In a field setting, PHS can be stimulated through the application of overhead irrigation to mimic rainfall near harvest time. Two common laboratory techniques for evaluating PHS susceptibility are the use of misting chambers with intact wheat heads and the weighted germination index method. For both approaches, wheat heads are typically collected at physiological maturity, dried for 1-2 days at 37°C, and then stored frozen until analysis. The misting chamber method involves placing the heads in a controlled environment chamber for a predetermined duration (often seven days), after which the degree of sprouting is assessed using subjective scales or direct measurement of head area changes. The misting chamber is the preferred technique for screening large numbers of genotypes for breeding and genetic studies. Alternatively, the weighted germination index method involves collecting heads at physiological maturity, germinating the seeds in a temperature-controlled chamber, and recording the germination dynamics over time, with earlier germinating grains weighted more heavily than later-maturing ones (Vetch et al., 2019).

Preharvest sprouting in wheat grains can significantly reduce the overall quality of the grain. Wheat flour is a crucial ingredient in various baked goods, and maintaining the appropriate starch balance is essential for optimal product performance. When grains undergo preharvest sprouting, the starches are converted into simpler carbohydrates like glucose, which can disrupt the fermentation process during baking, leading to issues with dough rise and overall product quality. To mitigate the impact of low-quality wheat with reduced falling numbers, mills often blend wheat with varying falling number levels to achieve the desired balance for each batch. However, this blending strategy is typically not feasible for individual farmers, as it requires a substantial quantity of high-quality wheat to offset the effects of even a small percentage (as low as 5%) of wheat with low falling number.

2.6 Challenges in Wheat Breeding for pre-harvest sprouting tolerance

Breeding wheat for pre-harvest sprouting (PHS) tolerance is a complex endeavor due to the involvement of multiple biological, environmental, and genetic factors. One of the primary challenges lies in the polygenic nature of PHS tolerance. The trait is controlled by several quantitative trait loci (QTLs) located on various chromosomes, such as 3A, 4A, 5B, and 7D, making selection and introgression into elite cultivars difficult.

A critical issue in this context is the trade-off between seed dormancy and germination performance. High levels of seed dormancy provide PHS resistance by preventing premature germination, but they may also reduce germination uniformity and seedling vigor, which are essential for successful crop establishment. Thus, breeders must strike a balance between enhancing dormancy and ensuring good field emergence.

Environmental influence is another significant challenge. PHS is highly affected by weather conditions, particularly rainfall and humidity during grain maturation, which can vary significantly from year to year and across locations. This leads to instability in the expression of resistance and makes reliable phenotyping difficult and resource-intensive. Standardized phenotyping protocols are often lacking or difficult to apply at large scale.

Furthermore, PHS resistance frequently interacts with other agronomic traits, such as plant height, maturity timing, and glume tenacity. These interactions can complicate breeding because improving one trait may inadvertently affect another. For example, increasing dormancy to improve PHS resistance might delay uniform germination or affect yield stability.

White-grained wheat cultivars present an additional layer of complexity. While they are preferred for certain markets such as noodles and specific types of bread, they are inherently more susceptible to PHS compared to red-grained cultivars. This creates a market-versus-resistance dilemma for breeders.

From a molecular breeding perspective, although several genes and markers have been identified—such as Vp-1, TaMFT, and QPhs loci—there is still a limited availability and application of reliable molecular markers for large-scale marker-

assisted selection (MAS). Many breeding programs in developing regions may lack the infrastructure to deploy these tools effectively.

Additionally, the genotype-by-environment interaction (G×E) in PHS tolerance is substantial. A cultivar that shows good resistance in one region or season may not perform as well under different conditions. This variability requires multi-environment trials and careful statistical analysis to identify stable resistance.

Finally, there is the concern of resistance durability. Environmental shifts or genetic erosion may lead to the eventual breakdown of dormancy mechanisms, reducing the effectiveness of previously resistant cultivars over time. Continuous monitoring and incorporation of new resistance sources are thus essential.

In conclusion, the development of PHS-tolerant wheat cultivars is a multidisciplinary challenge that requires integrating classical breeding, molecular genetics, and agronomic knowledge. Overcoming the trade-offs between seed dormancy and germination, improving phenotyping tools, and accounting for genotype-by-environment interactions are all essential components of an effective breeding strategy. With continued research and investment in genetic and genomic resources, it is possible to achieve more stable and broadly adapted PHS resistance in wheat, helping safeguard grain quality and yield in increasingly variable climatic conditions.

2.7 Impact of PHS resistance on wheat production systems

PHS-resistant varieties can reduce the need for chemical treatments (e.g., sprout inhibitors), which is beneficial to farmers and consumers, reducing costs and being more environment-friendly. Also, by improving harvest timings, PHS resistance is beneficial to the farmer, releasing constraints regarding urgency of harvesting. PHS-resistant wheat can also be of benefit to the industry sector, leading to lower risk of sprouting during post-harvest. Therefore, there are sustainability and economic benefits, with potential cost savings for farmers and millers, reducing crop losses and enhancing food security.

2.8 Cooperative breeding initiatives in Wheat Breeding for PHS

The classification of wheat cultivars in terms of pre-harvest germination is essential for farmers to be able to choose the most suitable cultivars. Much can still be done in relation to pre-harvest germination, but the use of molecular markers in selective breeding processes can help and speed up the development of new cultivars that are tolerant and better adapted to different growing conditions. Consequently, in order to achieve gains in productivity and industrial quality, genetic improvement programs and the use of more efficient cultural practices need to be further promoted. Adding biotechnological tools, such as forward and reverse genetics, bioinformatics, transcriptome analyses, and other approaches to detect major genes/loci for PHS resistance. (Chang et al., 2023)

2.9 Global Collaboration and Knowledge Sharing

An international collaboration in wheat breeding research and sharing resources, germplasm, and knowledge to combat PHS is of paramount importance. Since many challenges to wheat production are experienced across continents (Figure 12), global collaboration is essential to improve efficiency and avoid effort duplication. This can be achieved by coordinating efforts across a range of stakeholders, sharing know-how and tailoring specific needs (Reynolds & Braun, 2022).

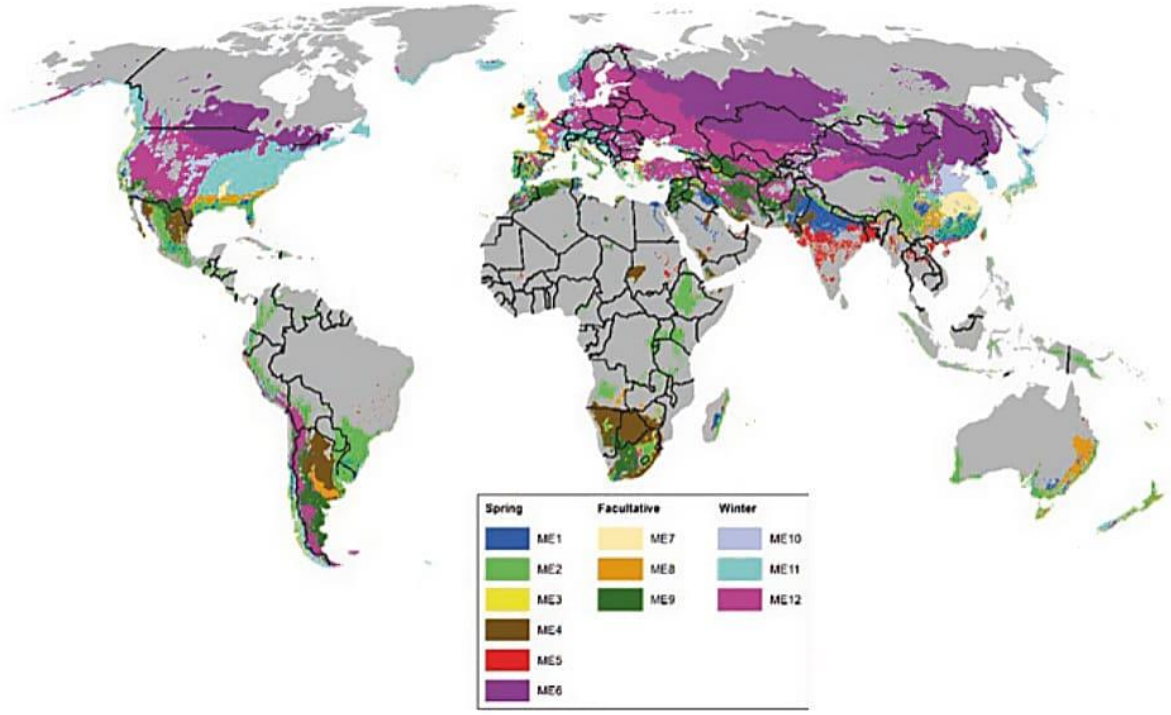

Figure 12. The International Wheat Improvement Network (IWIN) embraces a global collaboration of wheat scientists testing approximately 1,000 new high yielding, stress adapted, disease resistant wheat lines each year. Breeding is directed towards 12 different ME, representing a range of temperature, moisture, and disease profiles. Spring wheat: ME1 irrigated, high yield, ME2 high rainfall disease prone environments, ME3 acid soils, ME4 water limitation, ME5 heat stress, ME6 temperate, high latitude; Facultative wheat: ME7 irrigated, moderate cold, ME8 high rainfall, moderate cold, ME9 low rainfall, moderate cold.; Winter wheat: ME10 irrigated severe cold, ME11 high rainfall/irrigated, severe cold, ME12 low rainfall, severe cold. (Reynolds & Braun, 2022)

CIMMYT and ICARDA's wheat breeding programs have remained public since their inception around half a century ago, with support from many governments, non-profit organizations and institutions. These two centers supply advanced breeding lines and continue to play a vital role in facilitating collaboration between wheat breeders around the world. The germplasm exchange and performance results are distributed free of change to all bona fide breeders, whether public or private.

The national seed systems for getting new varieties to farmers remain the final step in the process. In most countries, publicly controlled independent testing of candidate varieties for yield and other important attributes, and the associated

registration of new varieties is the path followed by breeder's seeds to the farmers (Reynolds & Braun, 2022).

An important part of this process is the training of new scientists. From 2012-2022, WHEAT worked in partnership with 491 institutions in 89 countries, training from 2012-2022, over 360 wheat scientists and students from 54 countries (wheat.org, 2025).

2.10. Concluding Remarks

PHS resistant varieties are the best way to eliminate the harmful effects of PHS. Breeding for this trait can now have the additional help of novel techniques, such as marked-assisted selection, genomic selection, transgenic and gene editing approaches. These technologies can be integrated to faster deliver PHS resistant genotypes and contribute to world food security.

3. MATERIALS AND METHODS

3.1 Genetic constitutions

The study population consisted of 150 recombinant inbred lines (*RILs*) and their parents, derived from the crossing of the commercial cultivars ORS Quartzo x ORS Marfim, tolerant and susceptible to PHS- pre-harvest sprouting, respectively. According to Lima De Castro et al., 2014, Quartzo and Marfim were identified as contrasting cultivars for PHS-tolerance, with values of Falling Number of 394 and 299, and 11% and 32.3% of germinated grains after the rain simulation test.

3.2 Experimental conditions

The experiments were carried out in the 2017 and 2018 harvests in the experimental field of the Center for Genomics and Plant Breeding (CGF), located at the Palma Agricultural Center (CAP), belonging to the Eliseu Maciel Faculty of Agronomy of the Federal University of Pelotas (UFPel), municipality of Capão do Leão – RS, located at 31º 52' 00'' south latitude and 52º 21' 24'' west longitude; at an altitude of 13.24 m, with an average rainfall of 2,209 mm. The evaluations of sampled plants will be carried out at the CGF Laboratory, UFPel and at the Post-Harvest Quality Laboratory of Embrapa Wheat, in Passo Fundo, RS.

The experimental design was enlarged blocks, with 17 control blocks randomly arranged. The size of the experimental unit per genotype was a 1-meter-long line. Planting was carried out manually and the sowing density was 300 seeds per m^2, with 60 seeds per linear meter; with a row spacing of 20 cm and an approximate depth of 5 cm. The management was carried out according to the technical recommendations of the Meeting of the Brazilian Commission for Research on Wheat and Triticale (RBPTT, 2017, 2018).

3.3 Phenotypic evaluation of yield components

A sample of ten main ears harvested in the 2017 and 2018 harvests, when the total number of plants in the plot reached the physiological maturity phase, was used for the evaluation of yield components.

The yield components evaluated were: spike length (SL), spike weight (SW), number of spikelets per spike (NSS), grain weight per spike (GWS) and number of grains per spike (NGS). These evaluations were carried out in the CGF Laboratory.

The spike length (SL), was measured in centimeters with the aid of a graduated ruler, measuring from the base to the tip of the spike, excluding the awn. Spike weight (SW) and grain weight per spike (GWS) were determined in grams using an analytical balance. The number of spikelets per spike (NSS) was measured by manually counting the spikelets, disregarding infertile spikelets (at the apex and base of the spike). The number of grains per spike (NGS) was calculated after the manual threshing of the spike, by manually counting the grains considering their total. First, a sample of grains was obtained from the total harvested from the plot, then the sample was cleaned to remove impurities using an electric fan (FAO, 1991), after which the grains were counted in an automatic counting equipment. Finally, samples of 100 grains of each genotype were weighed in triplicate, and the averages extrapolated to a mass of one thousand kernel grains (TKW), expressed in grams, according to the Seed Analysis Rules (MAPA, 2009).

3.4 Phenotyping evaluation for pre-harvest sprouting

A sample of ten ears at the physiological maturity stage was randomly collected from the main stem and tillers in the 2017 and 2018 harvests. The samples were collected in cardboard bags and stored at -20°C until the analysis was performed. Before subjecting the ears to the germination test, a disinfestation was carried out with a 70% alcohol bath for 5 seconds, washing with distilled water, immersion in 2% sodium hypochlorite for 5 minutes and finally three washes with distilled water. Soon after, the ears were immersed in distilled water at room temperature for 8 hours, and after that period removed and placed on paper towels to dry the excess water. The ears were placed on germination paper previously soaked in distilled water 2.5 times the total weight of the paper, according to the Rules for Seed

Analysis (MAPA, 2009), rolled on the paper holding the ends of the roll with elastic bands and placed inside plastic bags to better preserve moisture. Then they were incubated in a germination chamber under controlled conditions, at a temperature of 20°C ±1°C, photoperiod 12 hours light – 12 hours dark, for 7 days, and then drying the rolls at 50°C in a forced circulation oven for 72 h. After this process, the visual reading of germination according to the proposed scale of visual notes from 1 to 3 was performed, where 1 represents the lowest value and 3 the highest. Finally, the tracking of the ears and count the germinated and total grains were performed, to obtain the value of the percentage of germinated grains.

3.5 *Falling Number* analysis

The *Falling Number* is a method of indirect evaluation of germination in the ear, by determining the intensity of the activity of the enzyme alpha-amylase, which expresses the intensity of germinated grains before they emit the radicle, or present pericarp rupture. The result of the enzyme activity is expressed in seconds, high values indicate low enzyme activity, while low values indicate high activity, a situation that commonly results from the pre-harvest germination process. Breads made with high-activity flour (NQ <200s) tend to have a dark and sticky crumb (Miranda et al., 2009). The determination of the enzymatic activity was determined by the Hagberg method in the "*Falling Number*" apparatus. For sample preparation, the grains were previously ground in an experimental mill (Lab Mill 3100, Perten brand) to produce whole meal flour. To carry out the analyses, samples of 7g of flour will be taken and evaluated in the *Falling Number* 1500 automatic device, Perten brand.

3.6 Statistical analyses

For the statistical analyses DTH, DTM, PHT, YLD, TKW, SL, NSS, SW, GSW, NGS, GVS, GP and FN das RILs, two measurements were used, one originating from the phenotyping in 2017 and the second in 2018. For the parents, 17 replications were used (each one coming from one block) in each year, totaling 34 replications per parent.

3.6.1 Analysis of mean amplitude and standard deviation values of RILs

In order to obtain means, amplitude, standard deviation and graphs containing histograms, data distribution and box-plot, comparting two seasons (2017 and 2018), the data was analysed using the “proc ttest” procedure in the SAS software.

3.6.2 Repeatability analysis

The repeatability values were obtained from the mean square values of ANOVA using “proc glm” from SAS. The following model y = block(year) + genotype, being:

y = response variable (DTH, DTM, PHT, PROD, TKW, SL, NSS, SW, GSW, NGS, GVS, GP and FN);
block(year) = the values of each block are nested within the years. The blocks are not the same in the diferente years, even when the same values are obtained.
Genotype = RILs + parentes

From the results obtained from the Anova, the values of VC%, CVG% and repeatability were obtained according to Figure 13, described by (Sade et al., 2022):

Source	Mean Square
Block(year)	
Genotype	MSg
Erro	MSe

$$\sigma_g^2 = \frac{MSg - MSe}{r} \qquad \sigma_e^2 = MSe$$

$$r = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$$

$$cv\% = \frac{\sqrt{\sigma_e^2}}{\bar{y}}$$

$$cvg\% = \frac{\sqrt{\sigma_g^2}}{\bar{y}}$$

Figure 13. Scheme for obtaining the repeatability, cv% and cvg% from the ANOVA table.

3.6.3 Correlation analyses

The Pearson’s correlation matrix was obtained for the characters using the mean of two Years for each Variable, using the “proc corr” from SAS.

3.4 RESULTS

3.4.1 Phenotyping evaluation of yield components

The yield components evaluated were: days to heading (DTH), days to maturation (DTM), spike length (SL), spike weight (SW), number of spikelets per spike (NSS), grain weight per spike (GWS) and number of grains per spike (NGS).

Table 6. Analysis of equality of variances in the distribution of 150 wheat RILs from the cross ORS Quartzo x ORS Marfim in the years 2018 and 2019.

Equality of Variances				
Method	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Folded F (DTH)	149	149	2.42	<.0001
Folded F (DTM)	149	149	3.01	<.0001
Folded F (PTH)	149	149	1.54	0.0092
Folded F(YLD)	149	149	1.05	0.7618
Folded F(TKW)	149	149	1.03	0.8628
Folded F (SL)	149	149	1.34	0.0725
Folded F(NSS)	149	149	1.06	0.7409
Folded F(SW)	148	149	1.69	0.0015
Folded F(GWS)	149	149	1.55	0.0077
Folded F(NGS)	149	149	1.06	0.7129
Folded F(GVS)	149	149	1.10	0.5540
Folded F(PG)	149	148	1.41	0.0371
Folded F(FN)	183	182	2.05	<.0001

According to table 4 the variances of the two distributions of wheat RIL lines were different for the variables DTH, DTM, PHT, SW, GWS, PG and FN ($p \leq 0.05$).

geno	N Obs	Variable	N	Mean	Minimum	Maximum	Std Dev	Coeff of Variation	Skewness	Kurtosis
marfi	17	dae	17	105.00	102.00	111.00	2.42	2.31	1.01	0.71
		dam	17	145.12	143.00	151.00	2.50	1.72	1.35	0.92
		est	17	67.53	56.80	77.80	5.67	8.40	0.21	-0.04
		prod	17	2.12	0.41	4.94	1.11	52.32	0.77	1.33
		pmg	17	33.22	28.57	35.83	1.82	5.49	-0.70	1.51
		cesp	17	7.22	6.60	7.90	0.29	3.95	0.02	2.15
		nesp	17	14.23	13.33	15.80	0.66	4.62	0.89	1.08
		mesp	17	1.24	0.43	1.54	0.24	19.15	-2.50	9.16
		mge	17	0.86	0.29	1.05	0.17	19.47	-2.55	8.97
		ngesp	17	29.27	23.75	34.50	2.80	9.56	0.00	0.20
		ngerm	17	1.24	1.00	2.00	0.44	35.40	1.37	-0.15
		porcgerm	17	28.41	16.58	39.74	5.69	20.04	-0.24	0.71
		nqfn	0	.	.	.	.	.	.	.
quar	17	dae	17	109.88	101.00	114.00	2.91	2.65	-1.66	5.08
		dam	17	150.00	147.00	154.00	2.09	1.39	-0.05	-0.87
		est	17	71.84	58.00	80.60	6.98	9.71	-0.39	-0.96
		prod	17	2.43	0.58	4.28	1.03	42.50	0.10	-0.91
		pmg	17	32.11	27.70	33.90	1.43	4.45	-1.79	5.23
		cesp	17	7.90	6.45	8.30	0.41	5.22	-2.91	10.47
		nesp	17	14.69	11.60	16.40	1.04	7.06	-1.46	4.75
		mesp	17	1.60	1.31	1.87	0.15	9.11	0.17	0.22
		mge	17	1.18	0.97	1.46	0.13	11.12	0.55	0.57
		ngesp	17	37.97	31.10	44.90	3.33	8.78	0.10	1.02
		ngerm	17	1.18	1.00	2.00	0.39	33.40	1.87	1.67
		porcgerm	17	29.29	9.33	45.91	8.37	28.57	-0.05	1.77
		nqfn	0	.	.	.	.	.	.	.

Figura 14. Média dos pais ORS Quartzo e ORS Marfim no ano de 2017.

geno	N Obs	Variable	N	Mean	Minimum	Maximum	Std Dev	Coeff of Variation	Skewness	Kurtosis
marfi	17	dae	17	90.29	86.00	98.00	3.06	3.39	0.96	1.10
		dam	17	134.76	130.00	140.00	2.88	2.14	0.39	-0.42
		est	17	63.54	56.00	71.00	4.55	7.16	-0.06	-0.88
		prod	17	2.72	1.10	4.63	0.83	30.40	0.52	0.92
		pmg	17	34.53	26.93	38.33	3.09	8.96	-0.83	0.85
		cesp	17	7.87	6.33	8.90	0.65	8.21	-0.51	0.55
		nesp	17	16.49	14.67	20.00	1.36	8.22	1.00	1.43
		mesp	17	2.13	1.54	2.94	0.39	18.28	0.49	-0.11
		mge	17	1.56	1.13	2.25	0.33	21.16	0.63	-0.06
		ngesp	17	42.49	29.80	57.00	6.79	15.99	0.10	0.51
		ngerm	17	2.29	1.00	3.00	0.59	25.62	-0.11	-0.33
		porcgerm	17	52.94	22.95	70.68	12.59	23.78	-0.81	0.55
		nqfn	17	312.30	239.00	426.00	43.92	14.06	0.77	2.05
quar	17	dae	17	94.88	82.00	108.00	5.84	6.16	-0.01	1.30
		dam	17	141.35	135.00	149.00	3.77	2.67	0.38	0.04
		est	17	65.09	50.50	74.00	6.21	9.54	-0.72	0.26
		prod	17	3.02	1.22	5.71	1.40	46.26	0.69	-0.82
		pmg	17	36.33	29.60	41.53	3.08	8.49	-0.41	0.31
		cesp	17	8.83	7.30	10.10	0.89	10.11	-0.27	-0.99
		nesp	17	15.64	12.80	17.60	1.44	9.22	-0.43	-0.76
		mesp	17	2.12	1.25	3.03	0.50	23.51	-0.44	-0.41
		mge	17	1.54	0.82	2.32	0.40	25.95	-0.25	-0.25
		ngesp	17	40.33	23.50	57.00	9.26	22.97	-0.11	-0.55
		ngerm	17	1.82	1.00	3.00	0.53	28.99	-0.26	0.74
		porcgerm	17	46.80	30.22	60.95	9.68	20.69	-0.13	-0.98
		nqfn	17	255.00	115.00	356.00	60.25	23.63	-0.69	1.33

Figura 15. Média dos pais ORS Quartzo e ORS Marfim no ano de 2018.

For DTH (Table 7), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 101.0 to 115.0, and a mean equal to 108.0. For 2018, the 150 RILs showed an amplitude ranging from 82.0 to 109.0, with a mean of 92.37.

Table 7. Values of mean amplitude and standard deviation of RILs for DTH.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	108.0	2.9182	0.2383	101.0	115.0
a18		150	92.3667	4.5413	0.3708	82.0000	109.0
Diff (1-2)	**Pooled**		15.6000	3.8170	0.4407		
Diff (1-2)	**Satterthwaite**		15.6000		0.4407		

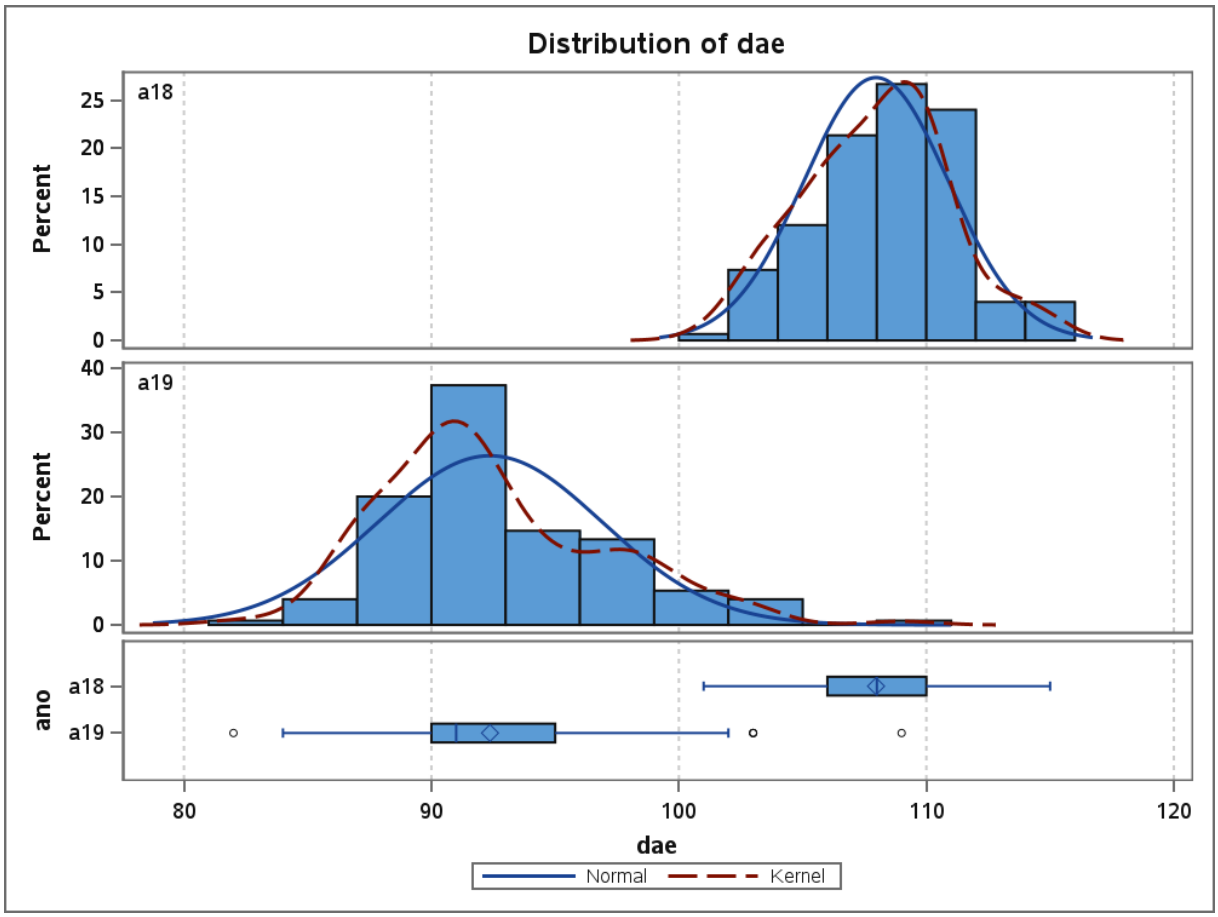

Figure 16. DTH (days to heading) distribution for 150 RILs in two years.

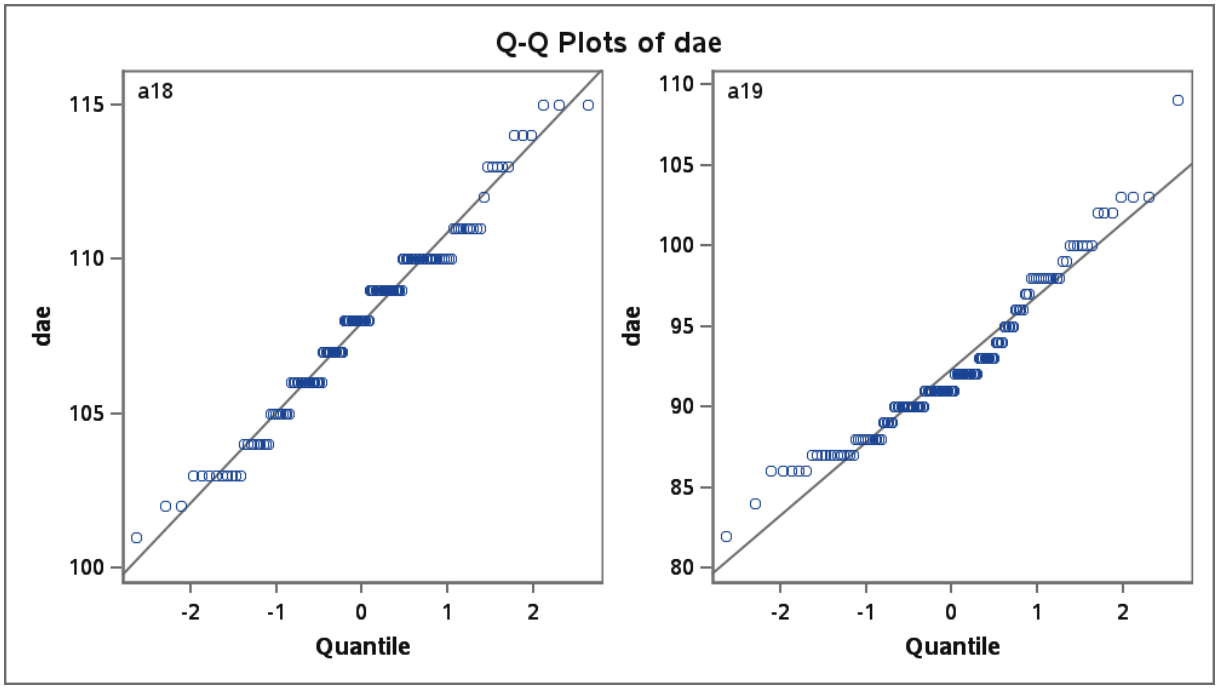

Figure 17. Q-Q Plots for the DTH (days to heading) of 150 RILs in two years.

For DTM (Table 8), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 142.0 to 157.0, and a mean equal to 147.1. For 2018, the 150 RILs showed an amplitude ranging from 130.0 to 152.0, with a mean of 138.6.

Table 8. Values of mean amplitude and standard deviation of RILs for DTM.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	147.1	2.9325	0.2394	142.0	157.0
a18		150	138.6	5.0902	0.4156	130.0	152.0
Diff (1-2)	**Pooled**		8.5533	4.1539	0.4796		
Diff (1-2)	**Satterthwaite**		8.5533		0.4796		

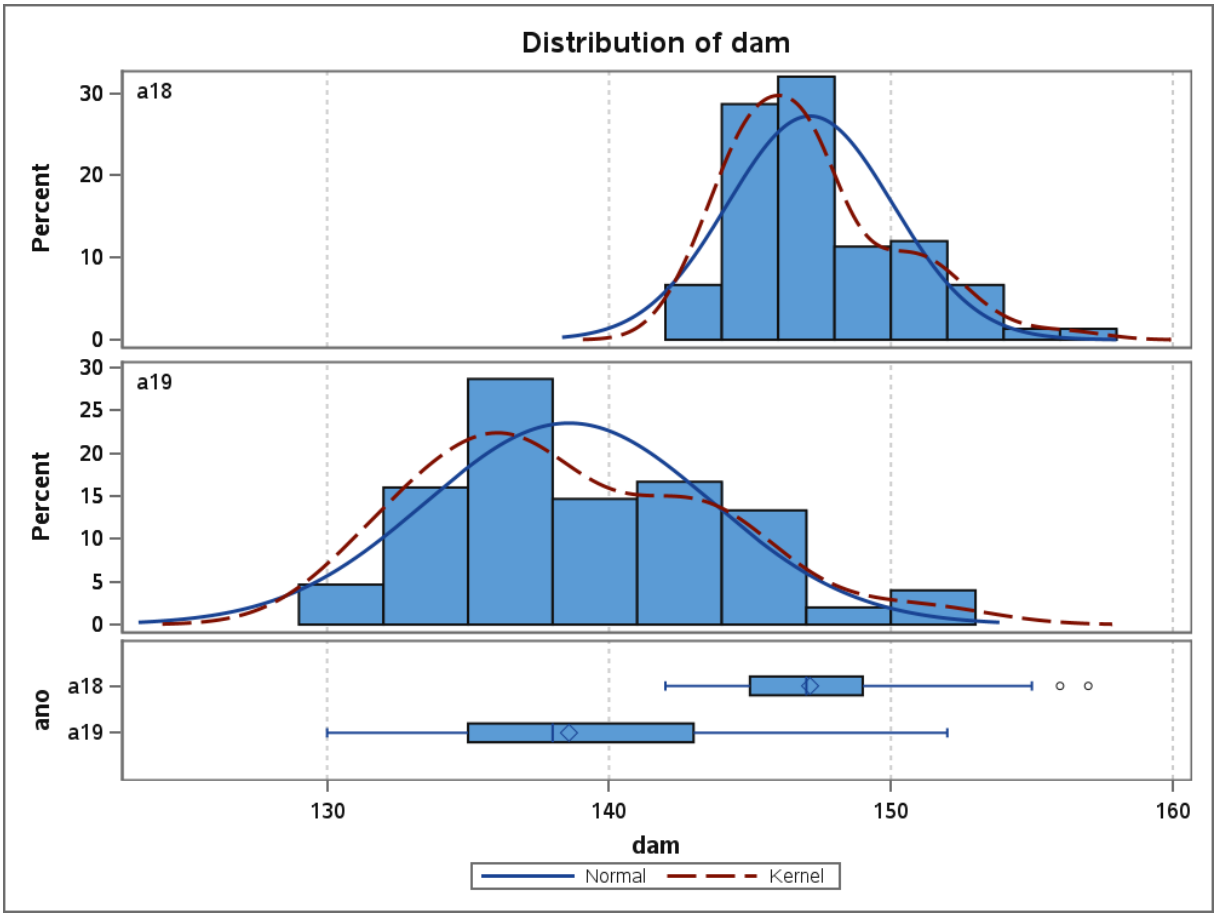

Figure 18. DTM (days to maturation) distribution for 150 RILs in two years.

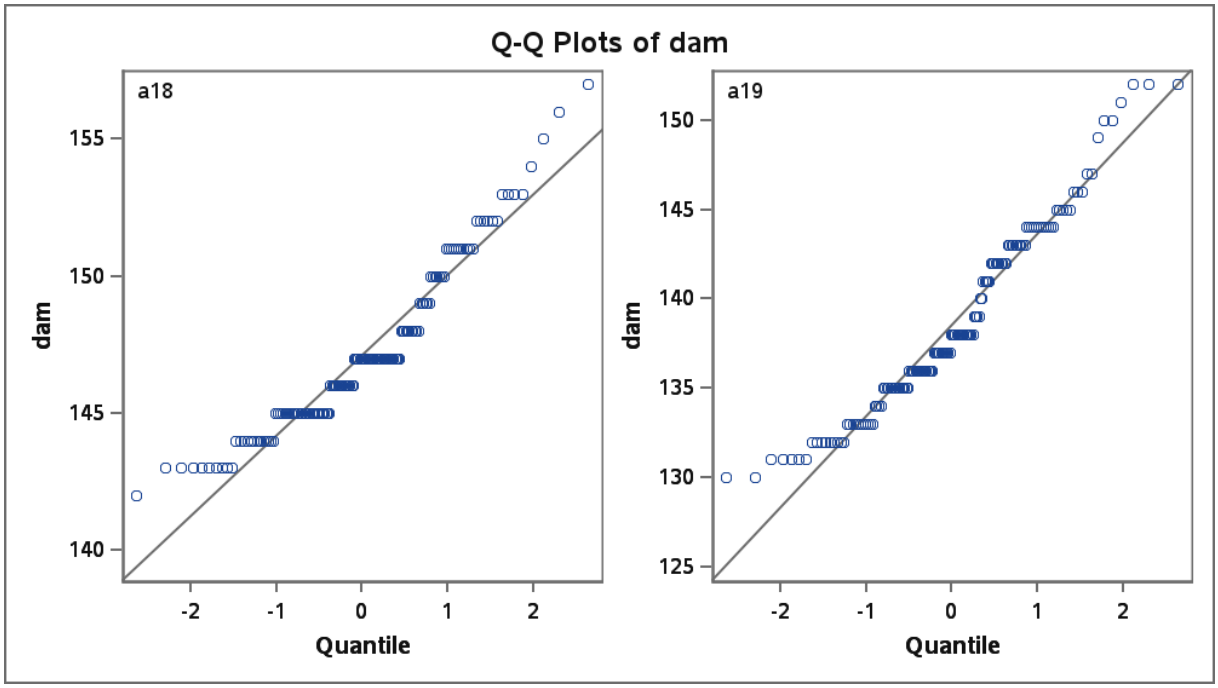

Figure 19. Q-Q Plots for the DTM (days to maturation) of 150 RILs in two years.

For PHT (plant height) (Table 9), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 54.4 to 86.2, and a mean equal to 72.7. In 2018, the 150 RILs showed an amplitude ranging from 57.0 to 81.4, with a mean of 68.5.

Table 9. Values of mean amplitude and standard deviation of RILs for PHT.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	72.7377	5.3294	0.4351	54.4000	86.2000
a18		150	68.4917	4.3005	0.3511	57.0000	81.4000
Diff (1-2)	**Pooled**		4.2461	4.8423	0.5591		
Diff (1-2)	**Satterthwaite**		4.2461		0.5591		

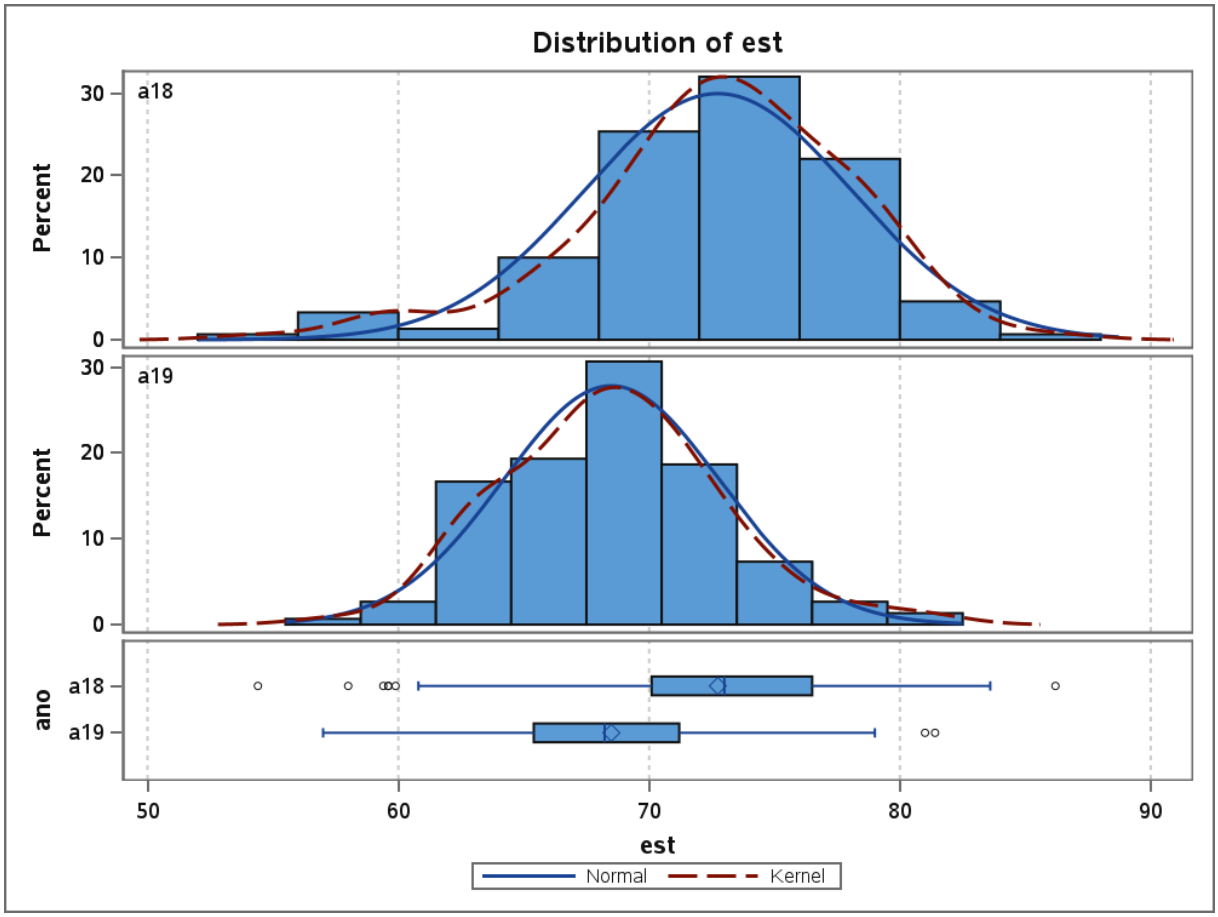

Figure 20. PHT (plant height) distribution for 150 RILs in two years.

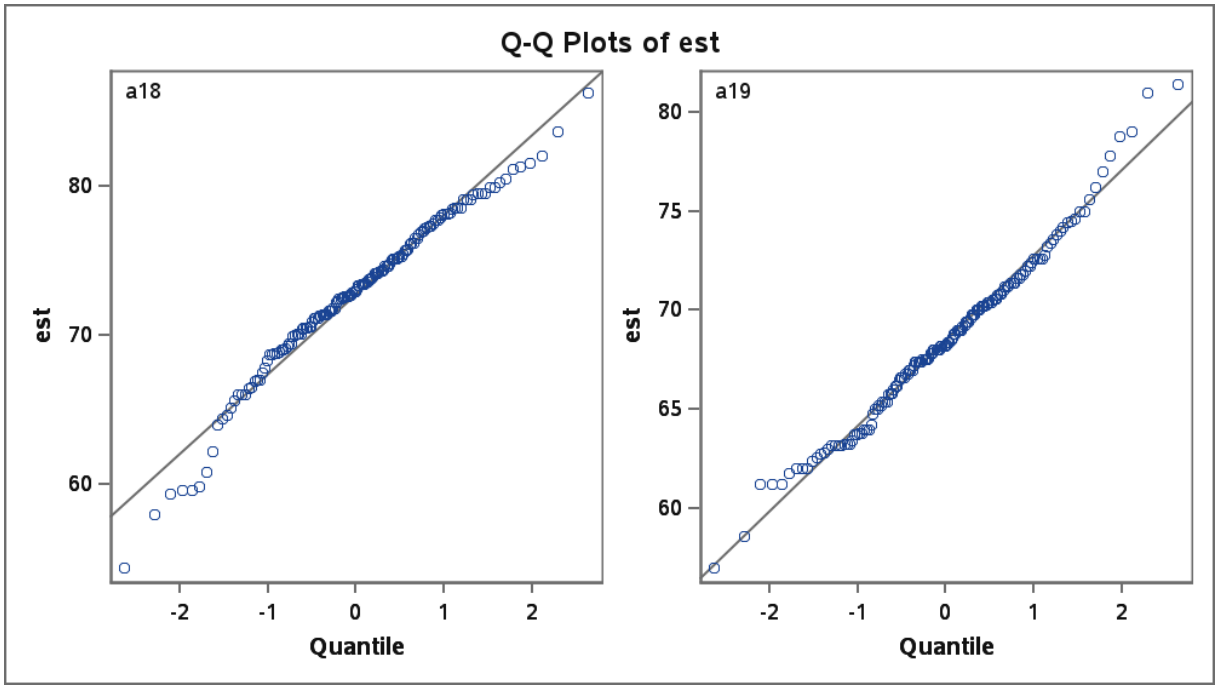

Figure 21. Q-Q Plots for the PHT (plant height) of 150 RILs in two years.

For YLD (yield) (Table 10), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 0.93 to 7.01, and a mean equal to 3.3. In 2018, the 150 RILs showed an amplitude ranging from 3.23 to 8.42, with a mean of 5.2.

Table 10. Values of mean amplitude and standard deviation of RILs for YLD.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	3.3012	1.0568	0.0863	0.9300	7.0100
a18		150	5.2185	1.0308	0.0842	3.2300	8.4200
Diff (1-2)	**Pooled**		-1.9173	1.0439	0.1205		
Diff (1-2)	**Satterthwaite**		-1.9173		0.1205		

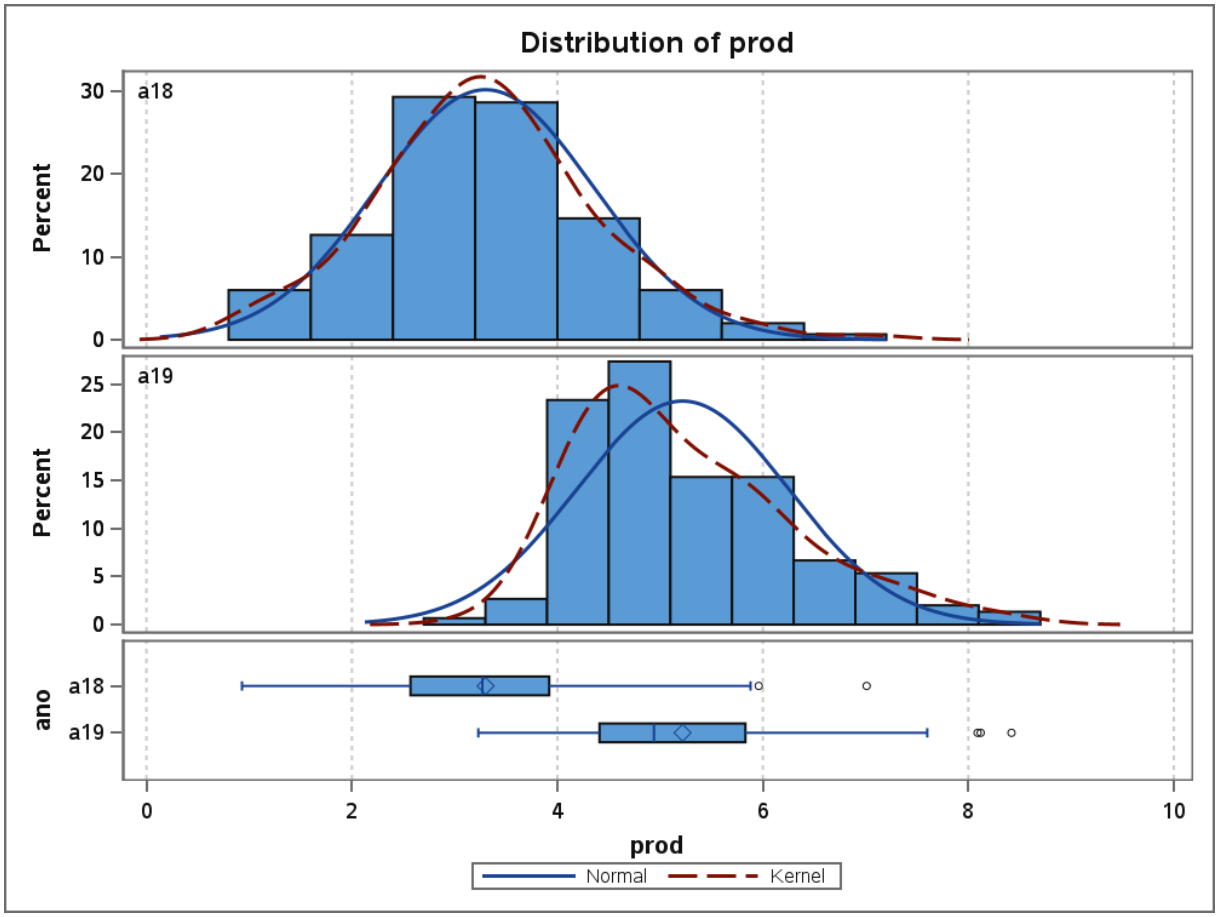

Figure 22. YLD (yield) distribution for 150 RILs in two years.

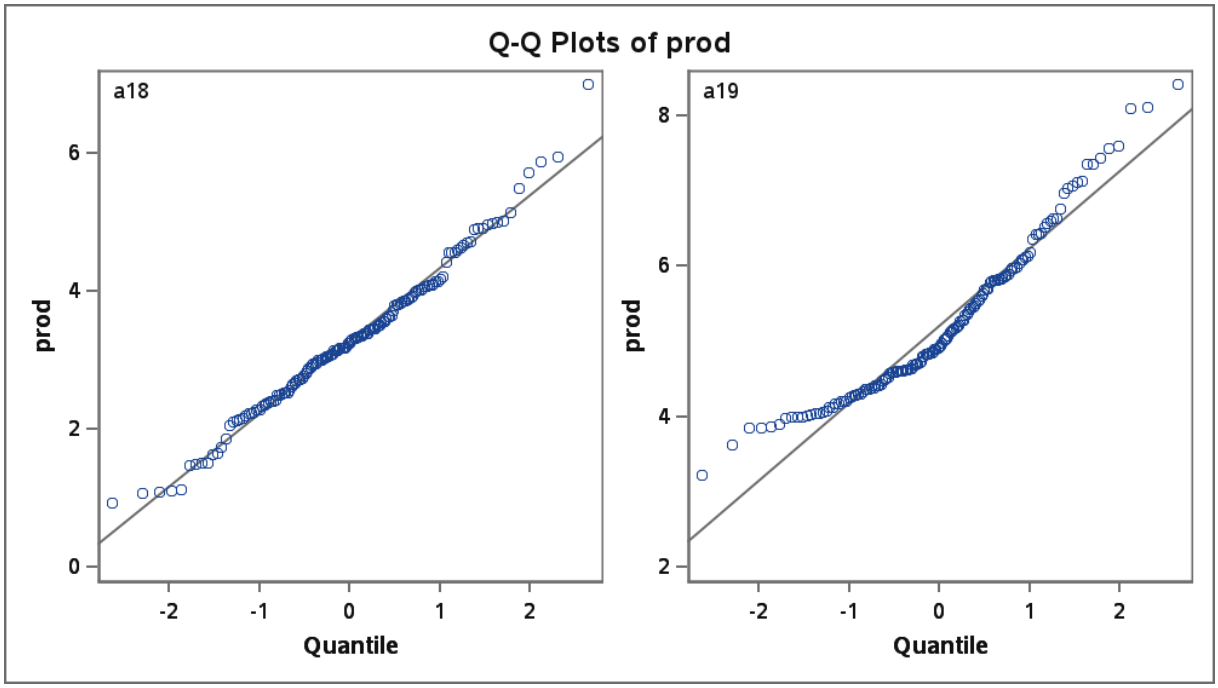

Figure 23. Q-Q Plots for the YLD (yield) of 150 RILs in two years.

For TKW (thousand kernel weight) (Table 11), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 23.2 to 40.1, and a mean equal to 31.4. In 2018, the 150 RILs showed an amplitude ranging from 27.1 to 44.1, with a mean of 35.9.

Table 11. Values of mean amplitude and standard deviation of RILs for TKW.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	31.4225	3.3509	0.2736	23.2100	40.1400
a18		150	35.8786	3.3988	0.2775	27.1000	44.1700
Diff (1-2)	**Pooled**		-4.4561	3.3749	0.3897		
Diff (1-2)	**Satterthwaite**		-4.4561		0.3897		

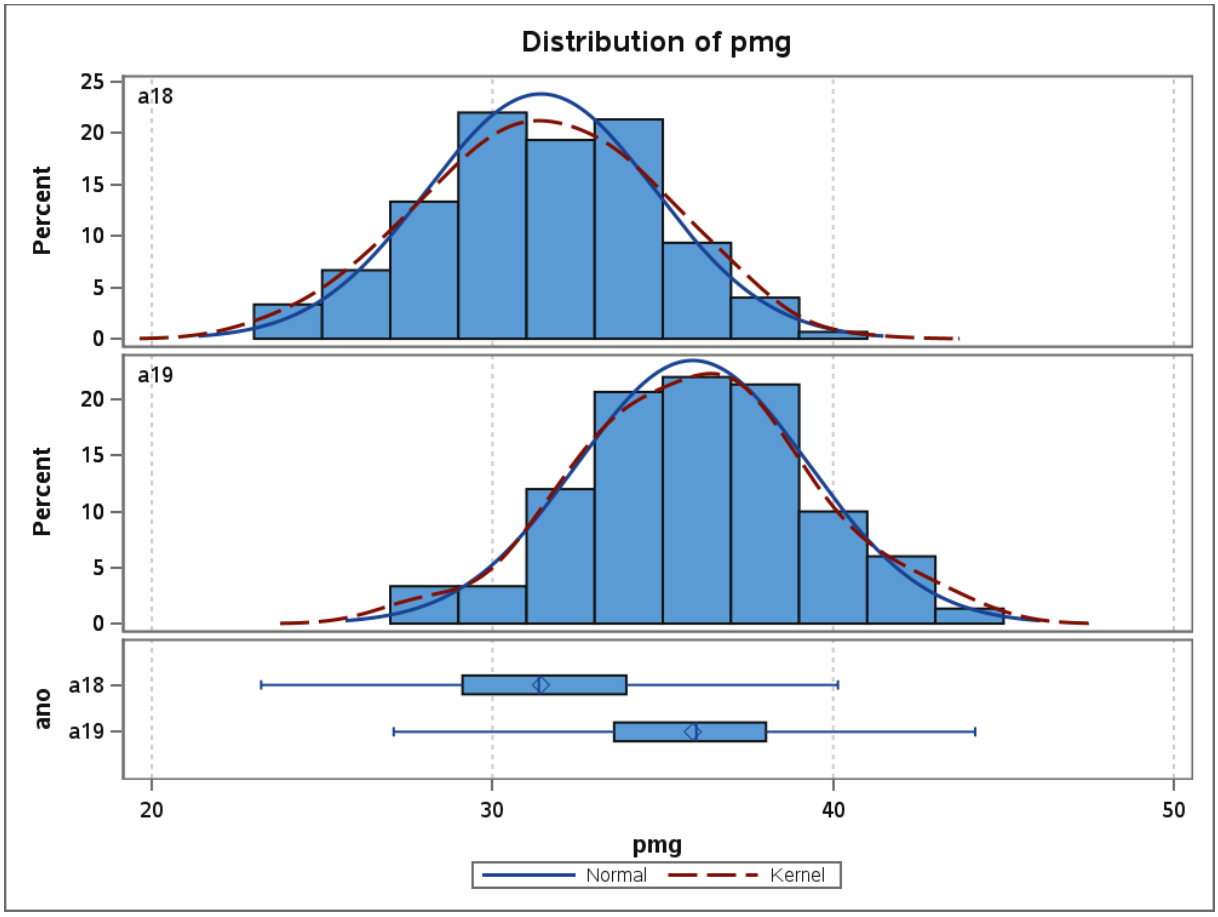

Figure 24. TKM (thousand kernel weight) distribution for 150 RILs in two years.

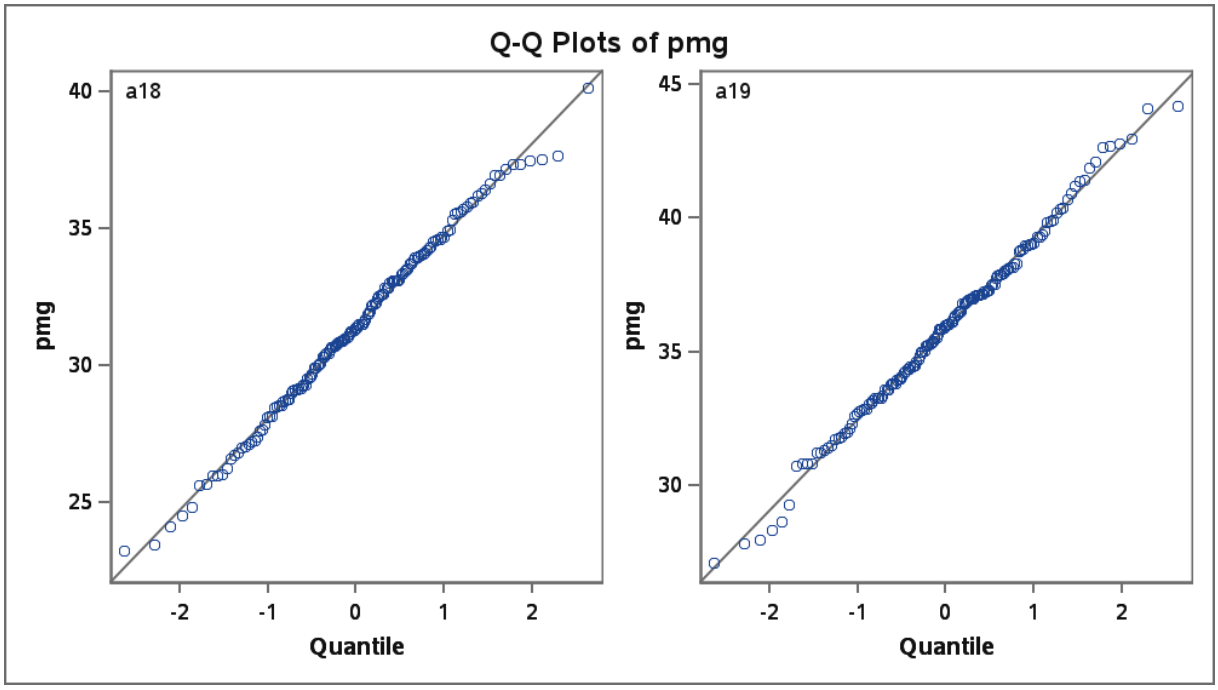

Figure 25. Q-Q Plots for the TKW (thousand kernel weight) distribution of 150 RILs in two years.

For SL (spike length) (Table 6), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 6.45 to 10.45, and a mean equal to 8.00. In 2018, the 150 RILs showed an amplitude ranging from 5.90 to 10.40, with a mean of 8.34.

Table 12. Values of mean amplitude and standard deviation of RILs for SL.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	8.0002	0.7101	0.0580	6.4500	10.4500
a18		150	8.3437	0.8231	0.0672	5.9000	10.4000
Diff (1-2)	**Pooled**		-0.3435	0.7687	0.0888		
Diff (1-2)	**Satterthwaite**		-0.3435		0.0888		

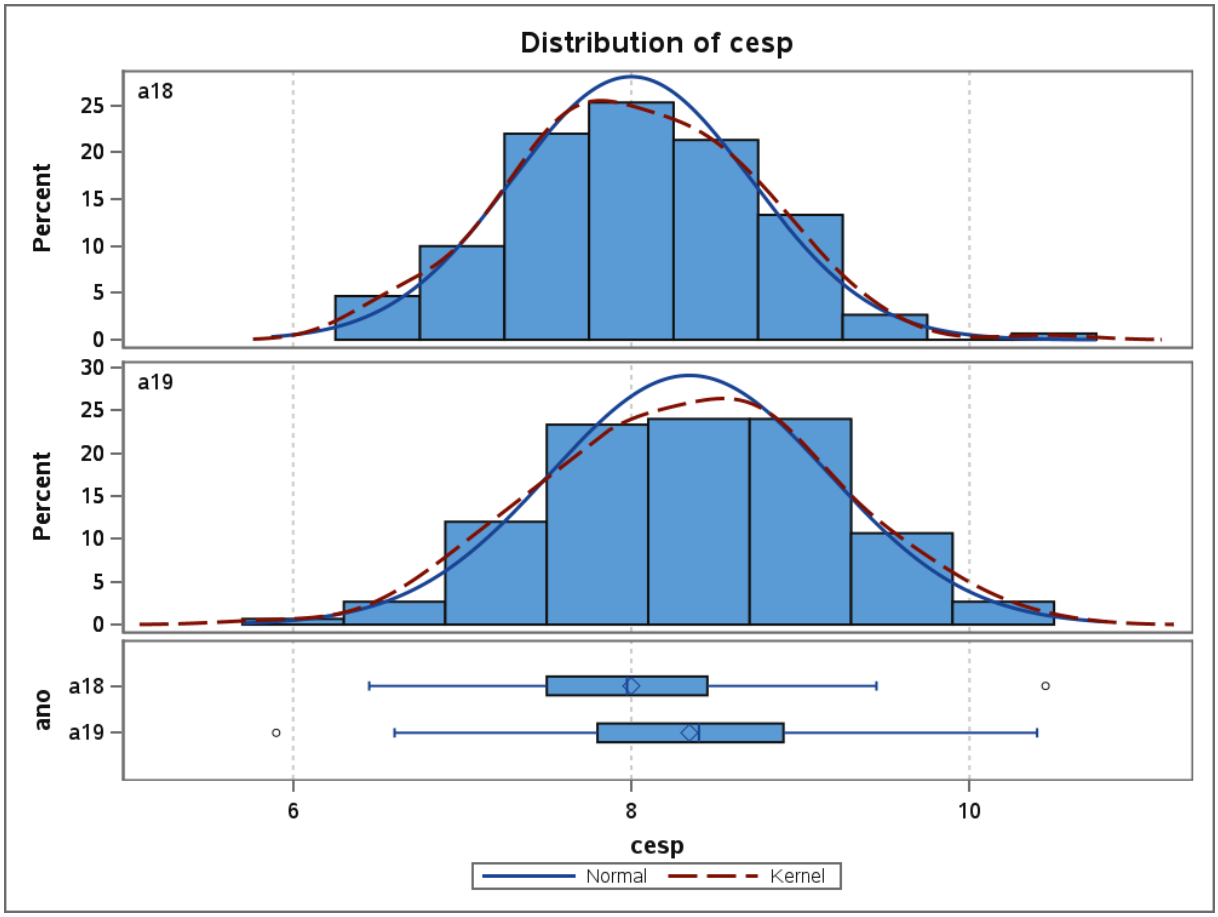

Figure 26. SL (spike length) distribution for 150 RILs in two years.

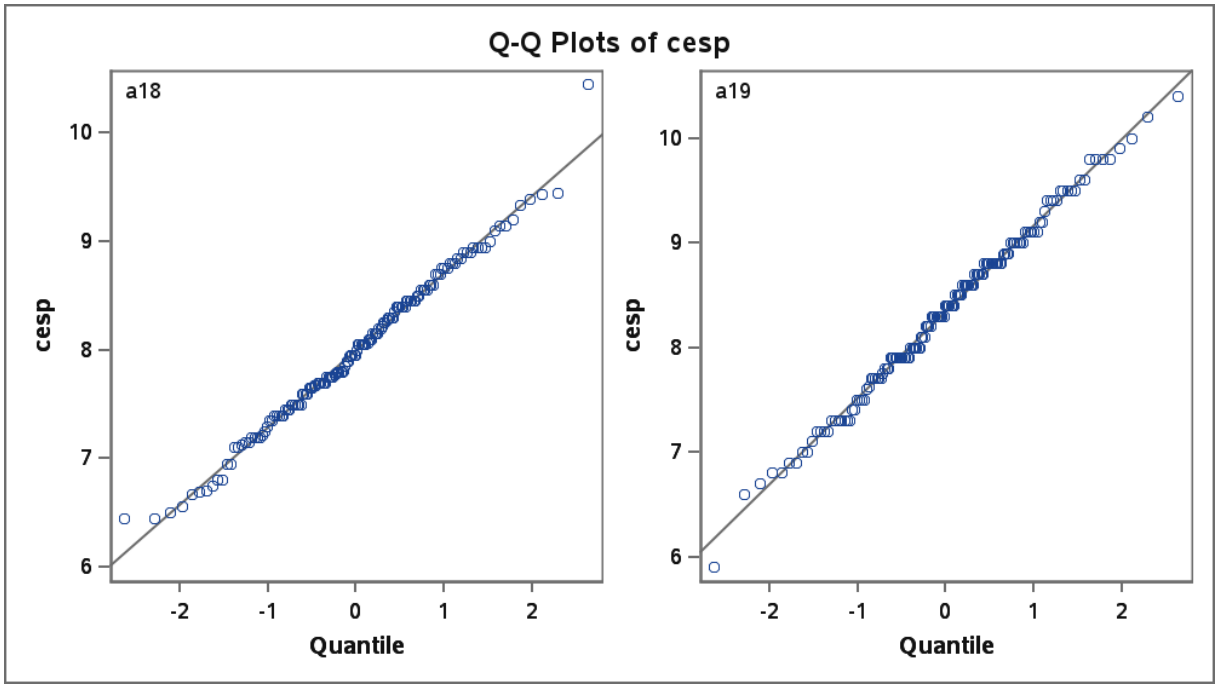

Figure 27. Q-Q Plots for the SL (spike length) of 150 RILs in two years.

For NSS (number of spikelets per spike) (Table 13), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 11.2 to 19.2, and a mean equal to 15.22. In 2018, the 150 RILs showed an amplitude ranging from 12.0 to 20.20, with a mean of 15.89.

Table 13. Values of mean amplitude and standard deviation of RILs for NSS.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	15.2176	1.4553	0.1188	11.2000	19.2000
a18		150	15.8923	1.4163	0.1156	12.0000	20.2000
Diff (1-2)	**Pooled**		-0.6747	1.4359	0.1658		
Diff (1-2)	**Satterthwaite**		-0.6747		0.1658		

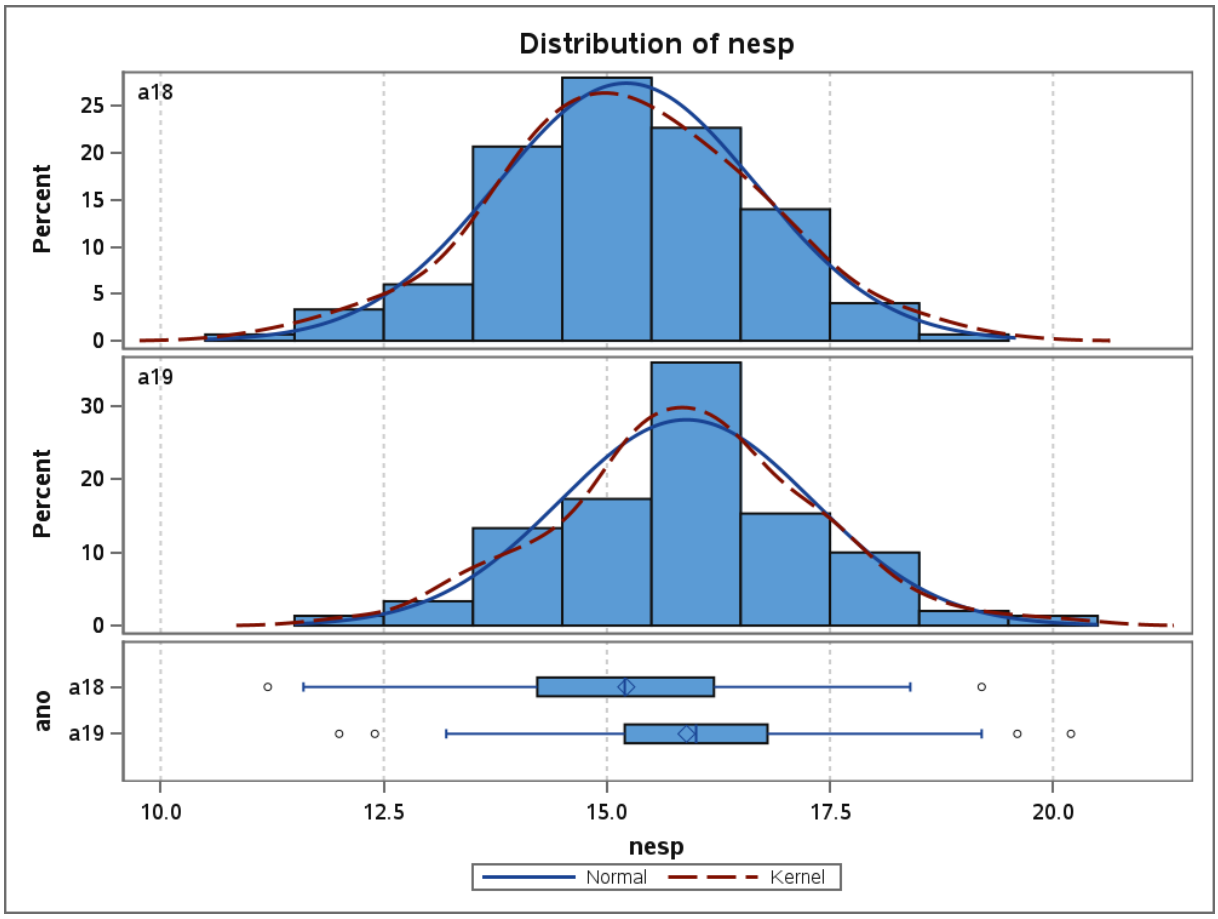

Figure 28. NSS (number of spikelets per spike) distribution for 150 RILs in two years.

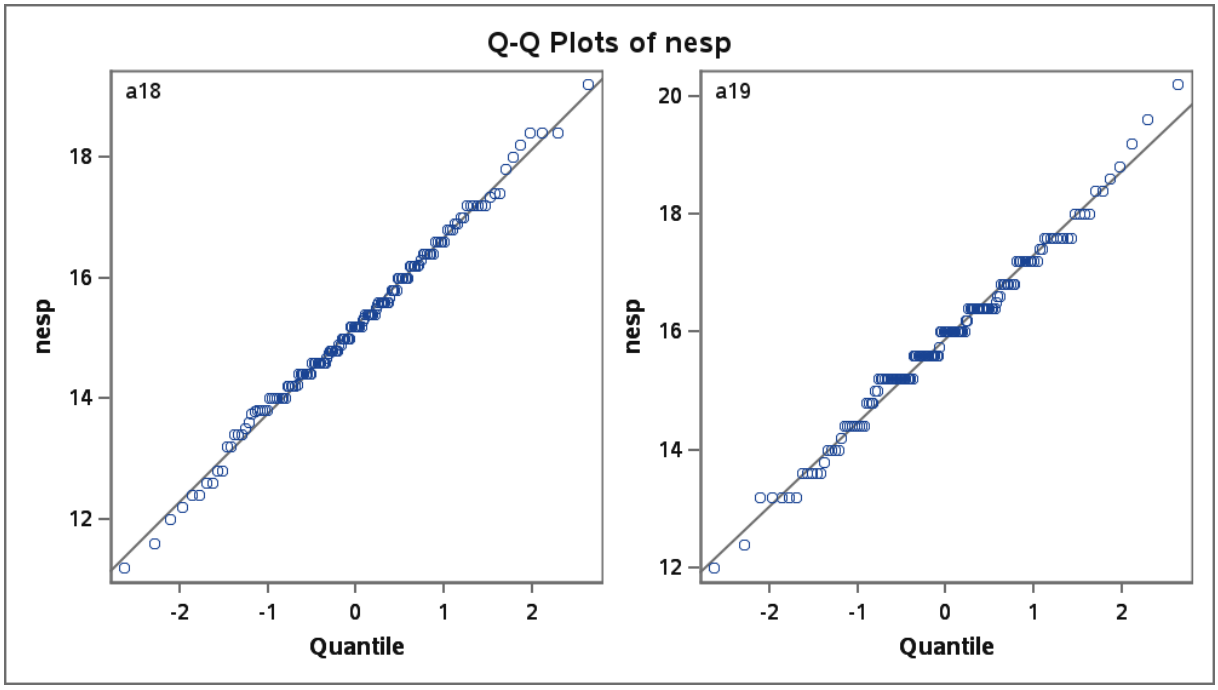

Figure 29. Q-Q Plots for the NSS (number of spikelets per spike) of 150 RILs in two years.

For SW (spike weight) (Table 14), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 0.92 to 2.59, and a mean equal to 1.64. In 2018, the 150 RILs showed an amplitude ranging from 1.07 to 3.27, with a mean of 2.14.

Table 14. Values of mean, amplitude and standard deviation of RILs for SW.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	1.6394	0.3156	0.0258	0.9200	2.5900
a18		149	2.1381	0.4102	0.0336	1.0700	3.2700
Diff (1-2)	**Pooled**		-0.4987	0.3658	0.0423		
Diff (1-2)	**Satterthwaite**		-0.4987		0.0423		

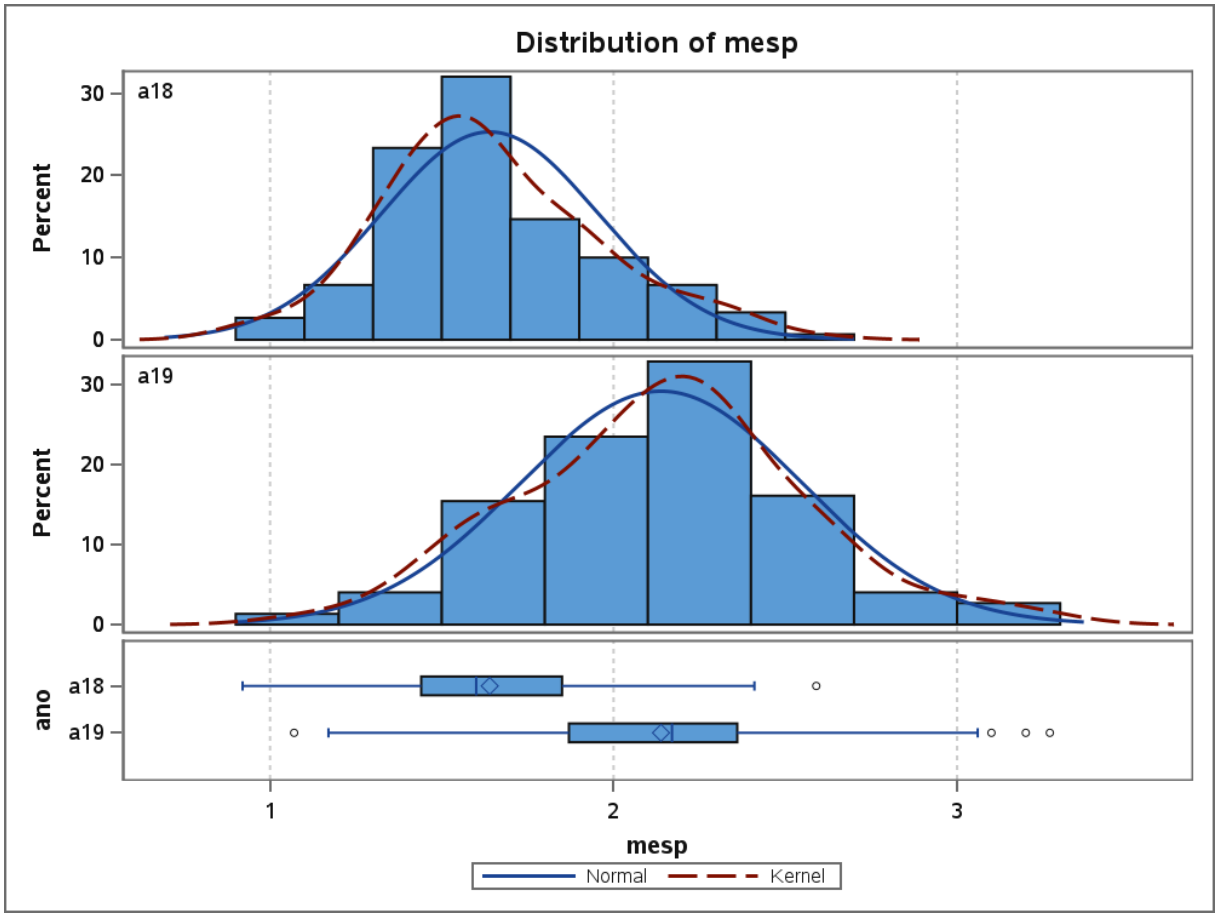

Figure 30. SW (spike weight) distribution for 150 RILs in two years.

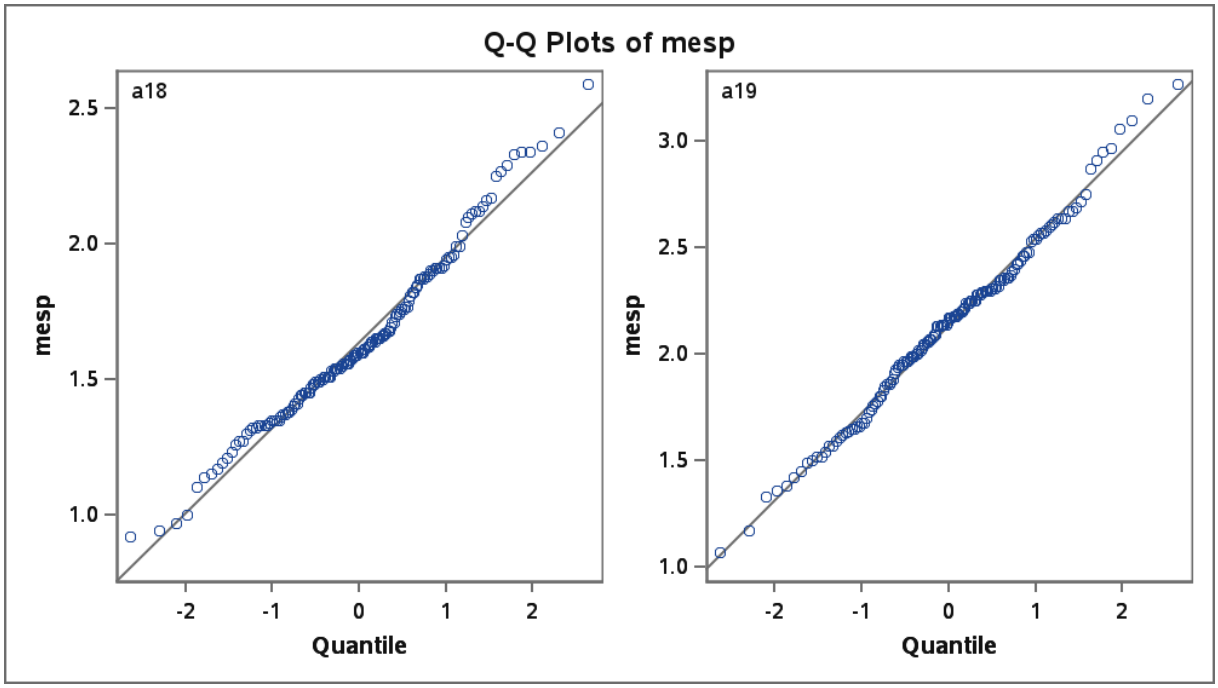

Figure 31. Q-Q Plots for the SW (spike weight) of 150 RILs in two years.

For GWS (grain weight per spike) (Table 15), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 0.59 to 1.92, and a mean equal to 1.16. In 2018, the 150 RILs showed an amplitude ranging from 0.72 to 2.46, with a mean of 1.59.

Table 15. Values of mean, amplitude and standard deviation of RILs for GSW.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	1.1649	0.2638	0.0215	0.5900	1.9200
a18		150	1.5885	0.3286	0.0268	0.7200	2.4600
Diff (1-2)	**Pooled**		-0.4236	0.2980	0.0344		
Diff (1-2)	**Satterthwaite**		-0.4236		0.0344		

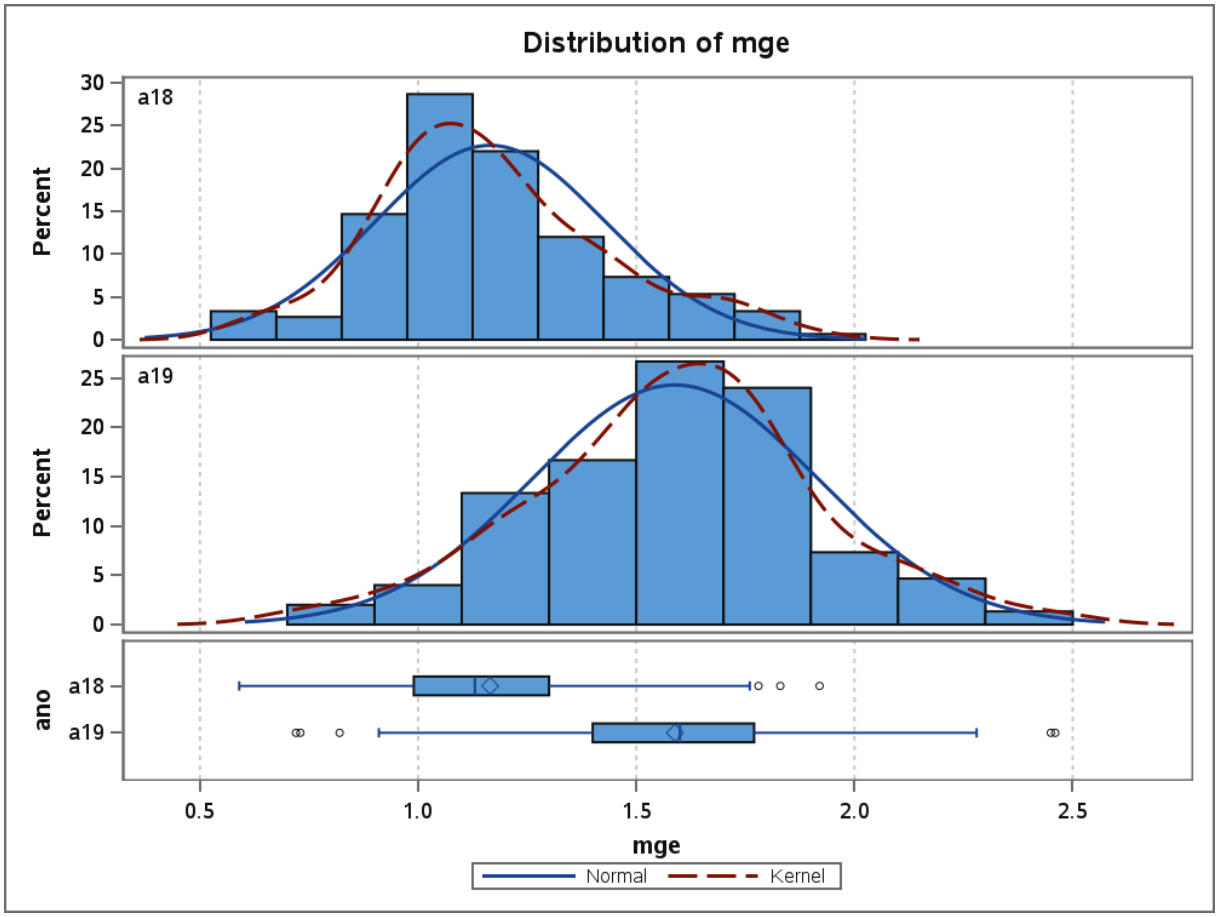

Figure 32. GWS (grain weight per spike) distribution for 150 RILs in two years.

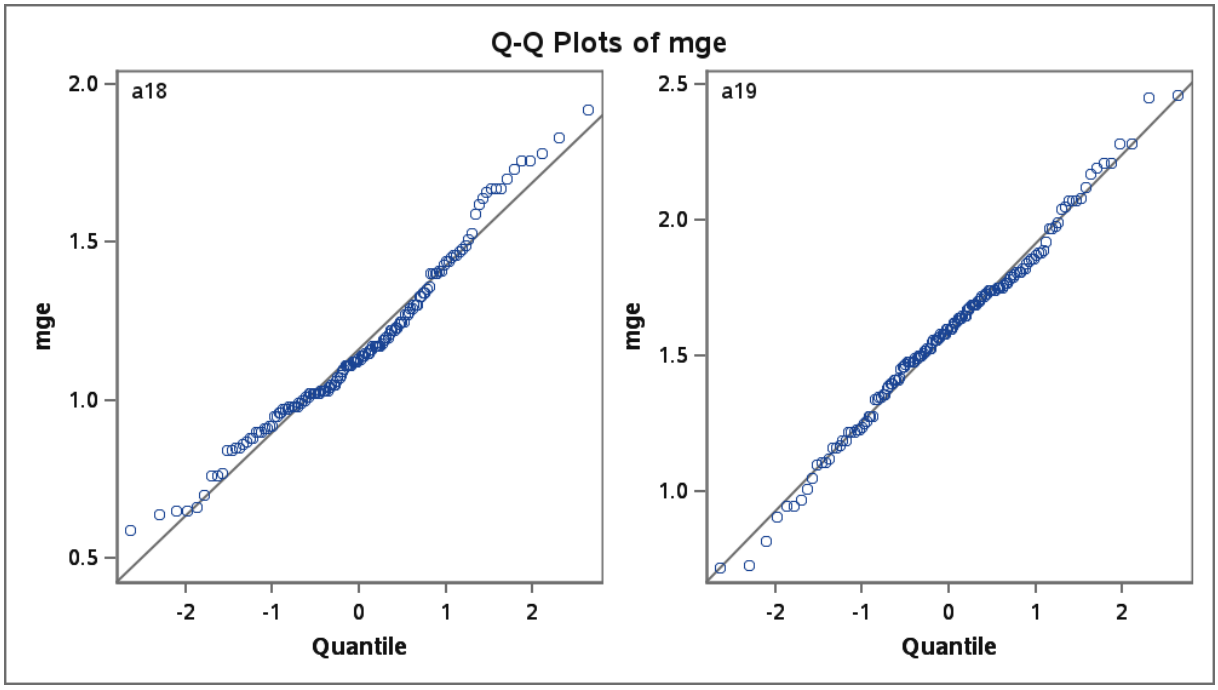

Figure 33. Q-Q Plots for the GWS (grain weight per spike) of 150 RILs in two years.

For (number of grains per spike) (Table 16), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 22.3 to 55.8, and a mean equal to 37.65. In 2018, the 150 RILs showed an amplitude ranging from 23.60 to 63.20, with a mean of 44.17.

Table 16. Values of mean, amplitude and standard deviation of RILs for NGS.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	37.6527	6.9433	0.5669	22.3000	55.8000
a18		150	44.1677	7.1562	0.5843	23.6000	63.2000
Diff (1-2)	**Pooled**		-6.5149	7.0506	0.8141		
Diff (1-2)	**Satterthwaite**		-6.5149		0.8141		

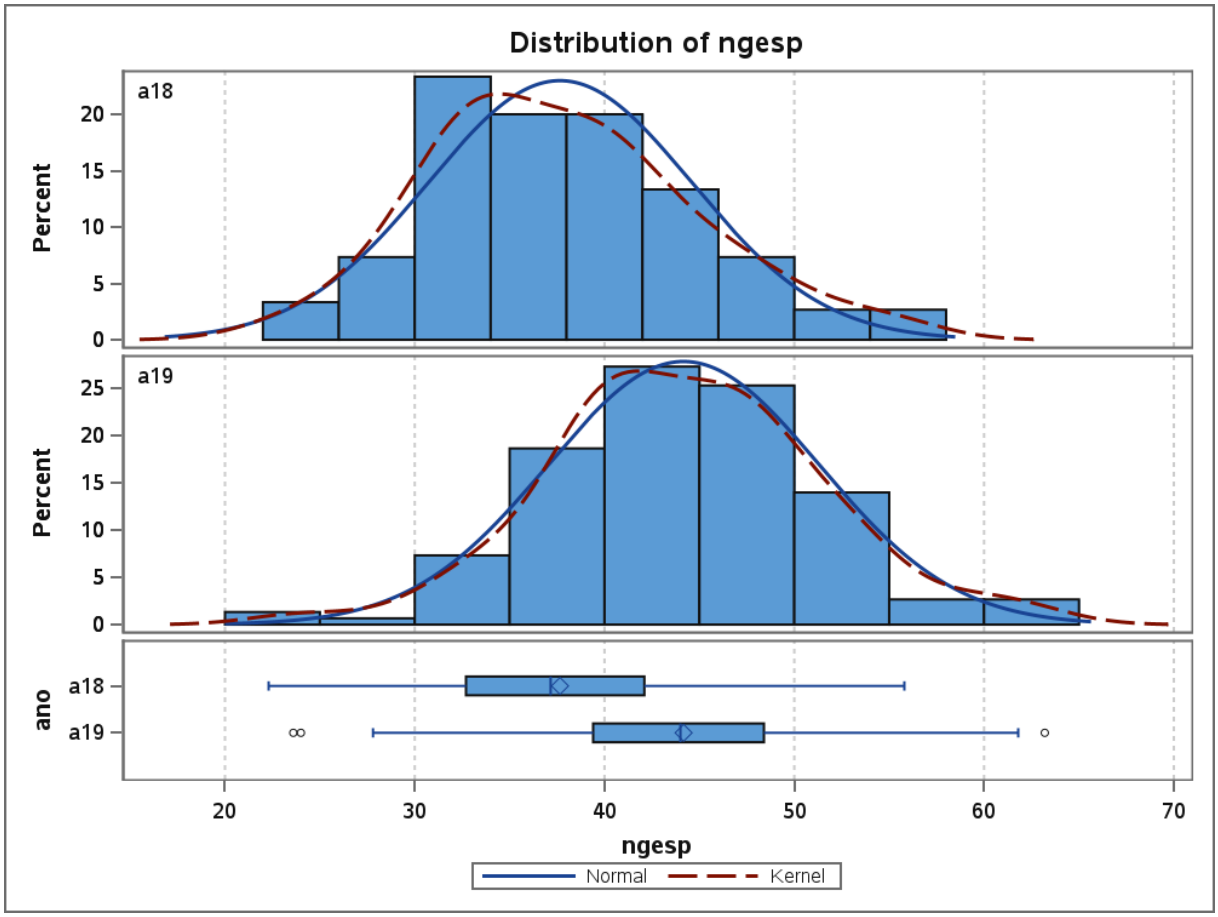

Figure 34. NGS (number of grains per spike) distribution for 150 RILs in two years.

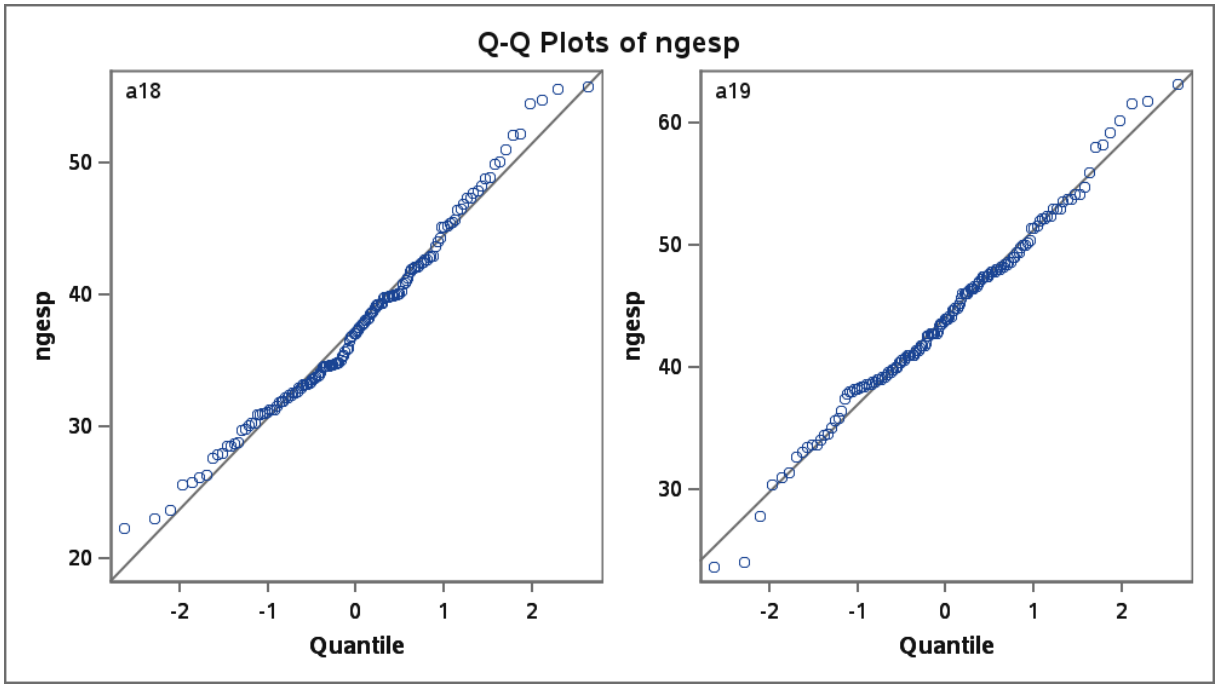

Figure 35. Q-Q Plots for the NGS (number of grains per spike) of 150 RILs in two years.

For GVS (germination visual score) (Table 17), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 1.0 to 3.0, and a mean equal to 1.41. In 2018, the 150 RILs showed an amplitude ranging from 1.0 to 3.0, with a mean of 1.89.

Table 17. Values of mean, amplitude and standard deviation of RILs for GVS.

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		150	1.4067	0.5194	0.0424	1.0000	3.0000
a18		150	1.8933	0.5452	0.0445	1.0000	3.0000
Diff (1-2)	**Pooled**		-0.4867	0.5325	0.0615		
Diff (1-2)	**Satterthwaite**		-0.4867		0.0615		

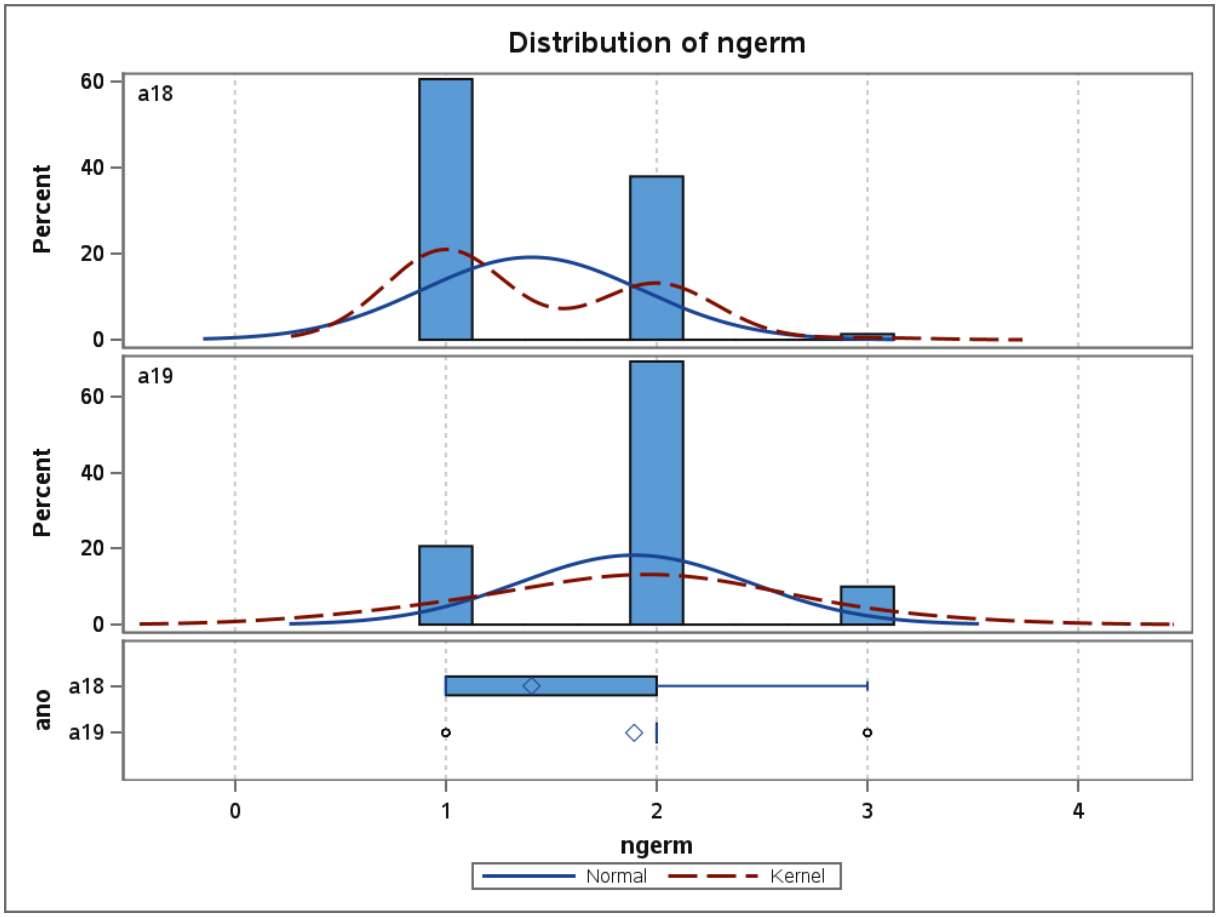

Figure 36. GVS (germination visual score) distribution for 150 RILs in two years.

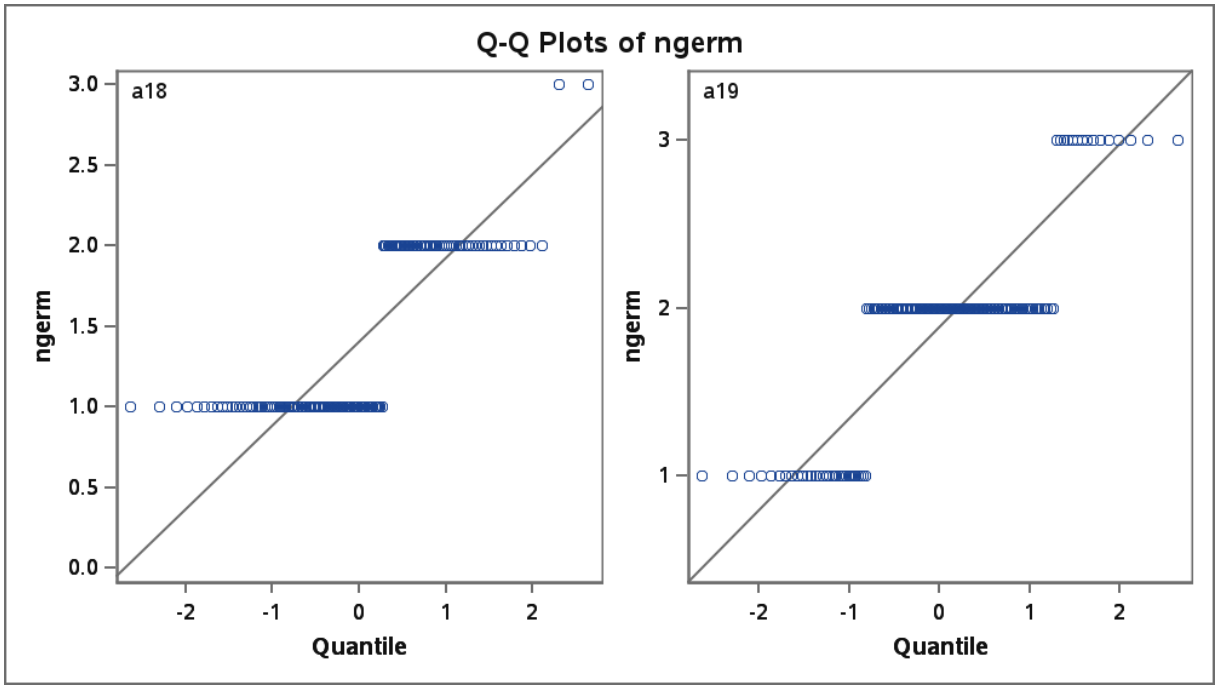

Figure 37. Q-Q Plots for the GVS (germination visual score) of 150 RILs in two years.

For GP (germination percent) (Table 12), in the year 2018, the results showed that the RILs displayed an amplitude ranging from 2.21 to 62.48, and a mean equal to 25.97. In 2019, the 150 RILs showed an amplitude ranging from 5.23 to 83.4, with a mean of 40.90.

Table 18. Values of mean, amplitude and standard deviation of RILs for GP

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		149	25.9705	13.0450	1.0687	2.2100	62.4800
a18		150	40.9034	15.4910	1.2648	5.2300	83.4000
Diff (1-2)	**Pooled**		-14.9329	14.3244	1.6568		
Diff (1-2)	**Satterthwaite**		-14.9329		1.6559		

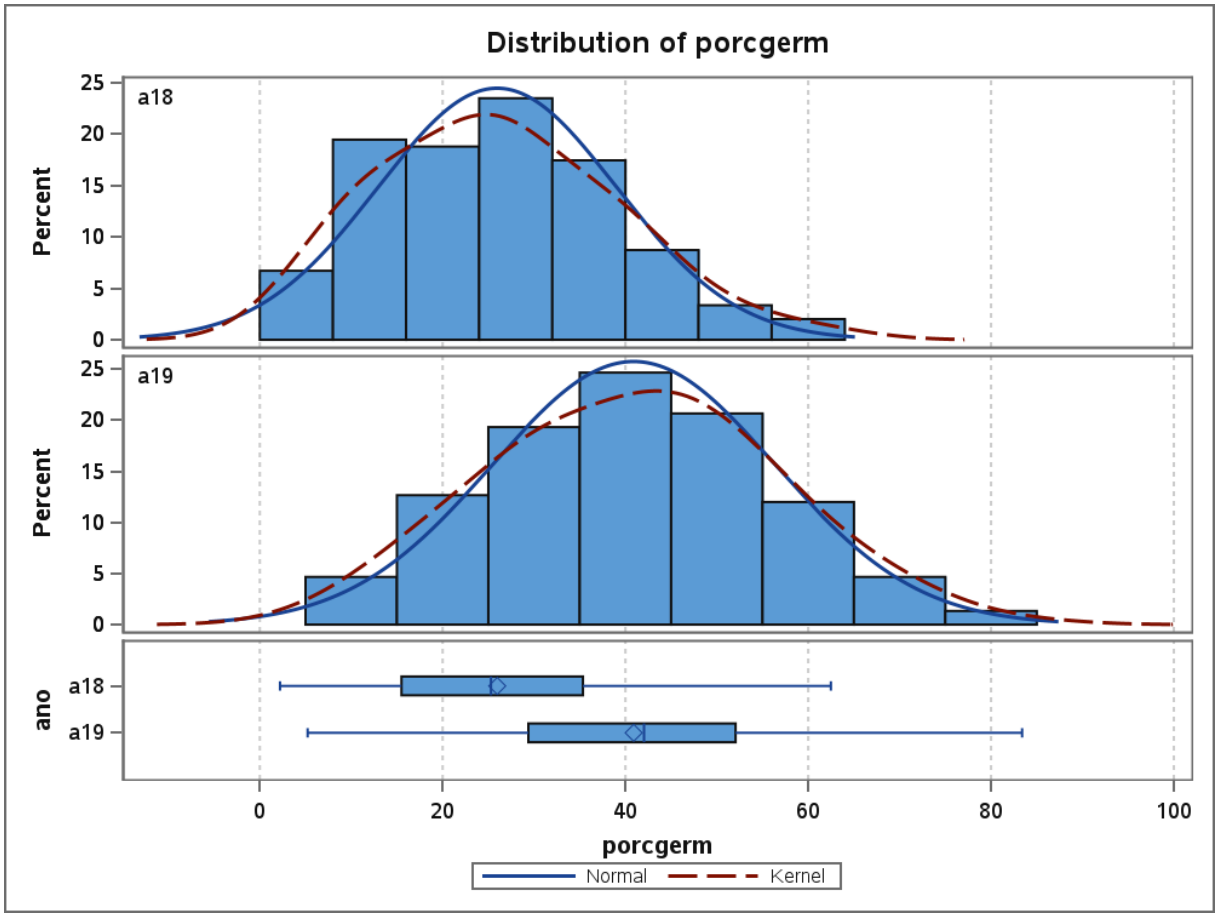

Figure 38. GP (germination percent) distribution for 150 RILs in two years.

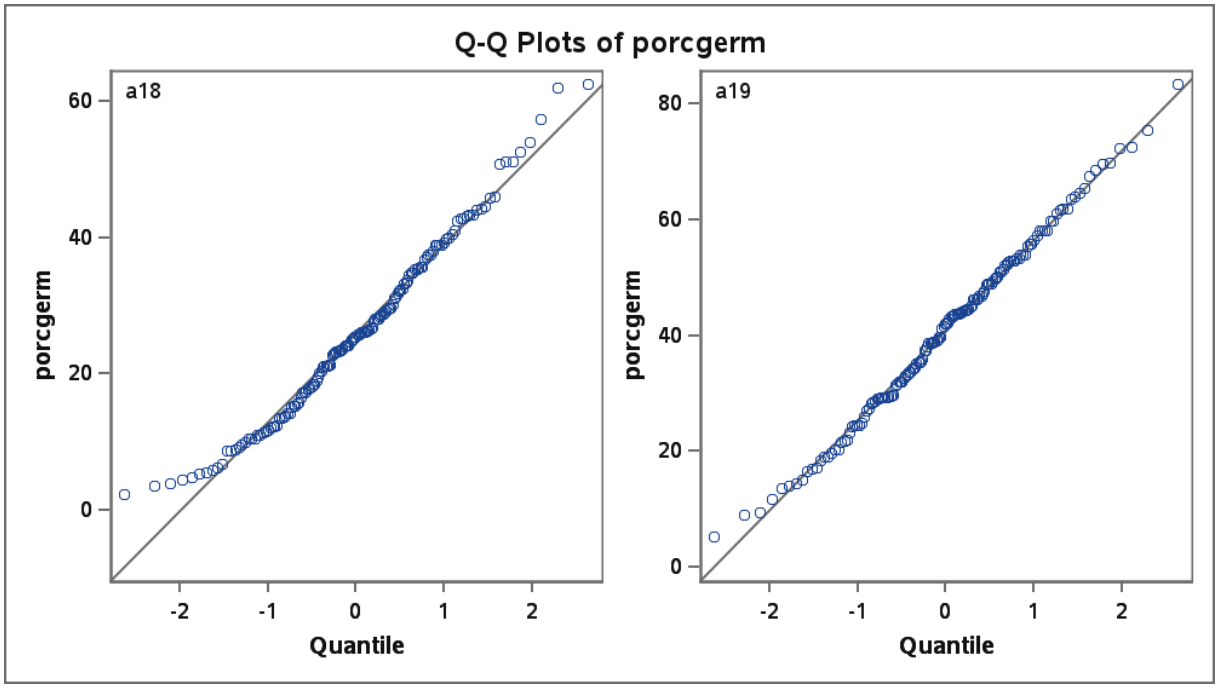

Figure 39. Q-Q Plots for the GP (germination percent) of 150 RILs in two years.

For FN (falling number) (Table 19), in the year 2017, the results showed that the RILs displayed an amplitude ranging from 71.0 to 498.0, and a mean equal to 287.0. In 2018, the 150 RILs showed an amplitude ranging from 242.5 to 501.0, with a mean of 377.0.

Table 19. Values of mean, amplitude and standard deviation of RILs for FN

Year	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
a17		184	287.0	67.8260	5.0002	71.0000	498.0
a18		183	377.0	47.3329	3.4990	242.5	501.0
Diff (1-2)	**Pooled**		-90.0199	58.5117	6.1086		
Diff (1-2)	**Satterthwaite**		-90.0199		6.1028		

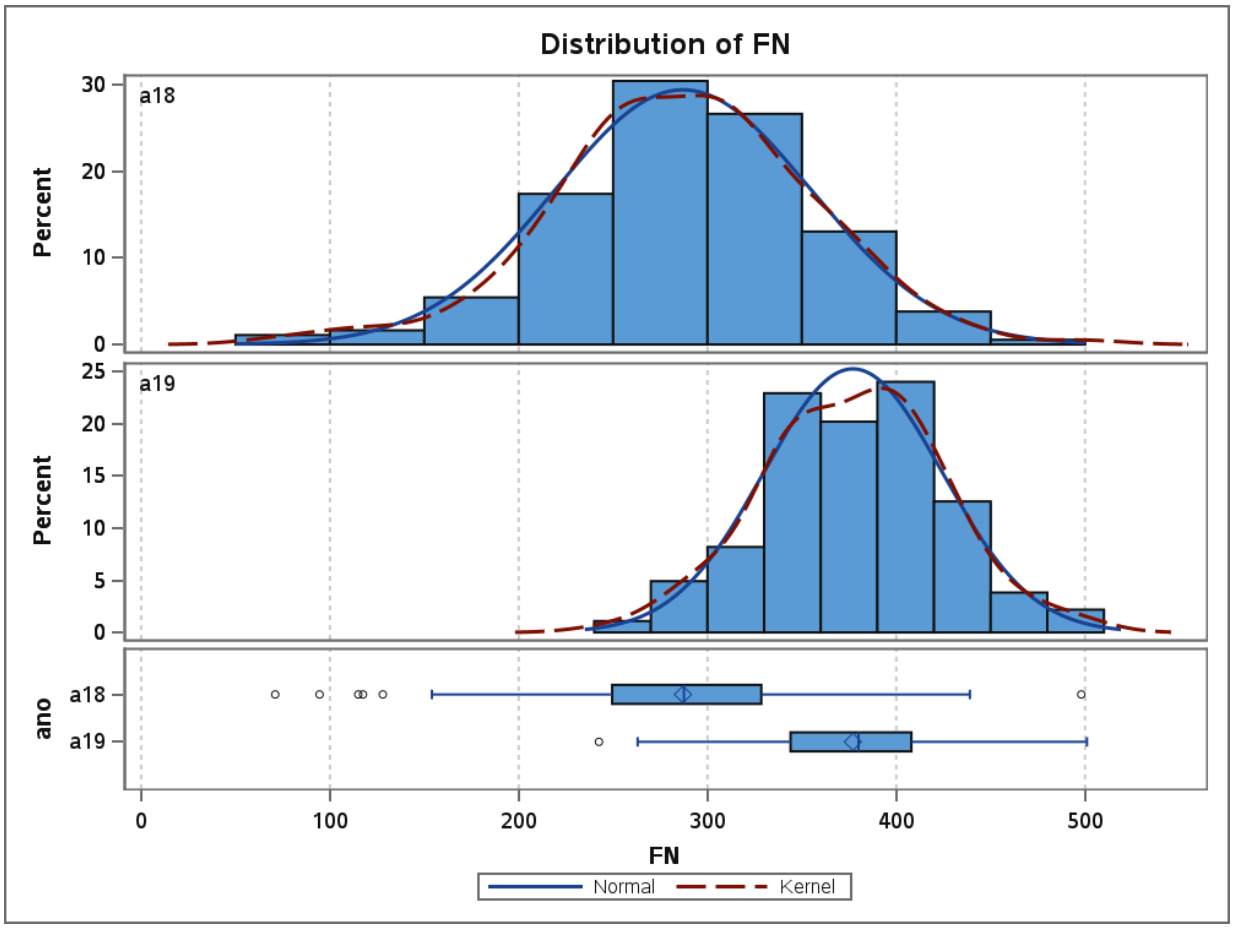

Figure 40. FN (Falling Number) distribution for 150 RILs in two years.

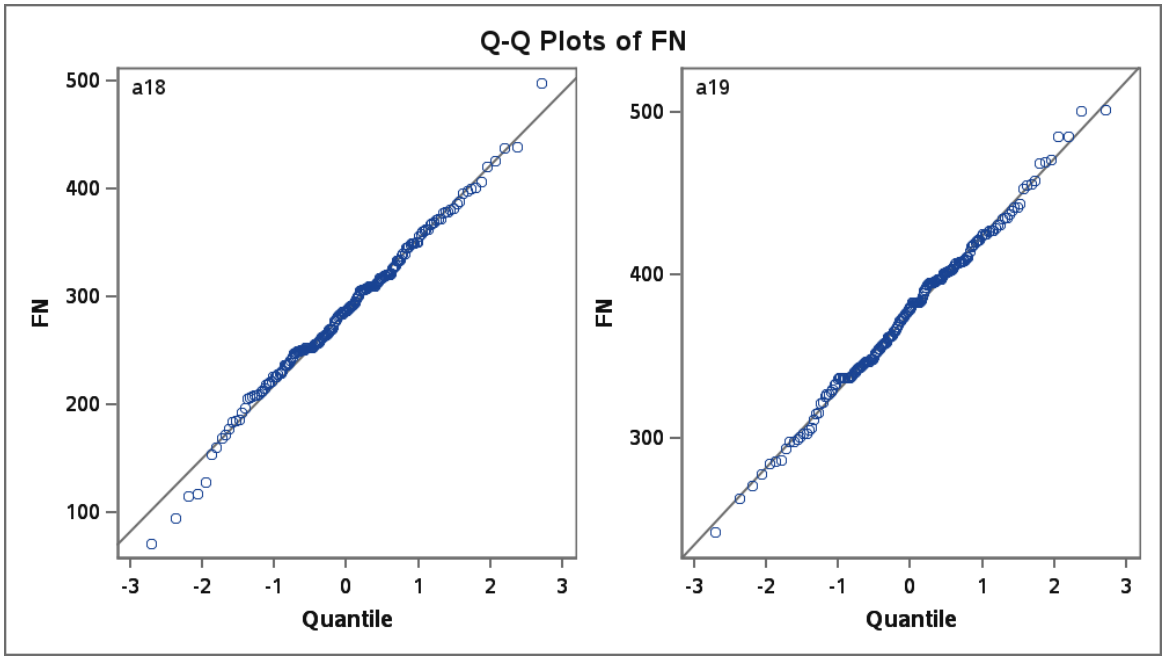

Figure 41. Q-Q Plots for the FN (Falling Number) of 150 RILs in two years.

3.4.2 Repeatability

The coefficient of repeatability is defined as the correlation between the measurements obtained for the same individual, repeated in time and space, being equivalent to the maximum value that broad sense heritability can achieve (Falconer, Mackay, 1996). Obtaining a high repeatability coefficient value for a given trait means that it is possible to predict the actual value aof the individual with few measurements. Therefore, increasing the number of evaluations does not mean that the accuracy to predict the phenotype b=value will be increased.

The values of r ranged from 0.23 (GVS) to 75.72 (DTH). Therefore, the traits showing higher heritability were DTH, DTM, PHT, TKW and NSS, while the traits showing lower heritabilities were GVS, GWS and NGS.

Table 20. Repeatability analysis

	SL	DTH	DTM	PHT	SW	GWS
r	66.94	75.72	72.70	60.25	29.10	14.22
cv%	6.58	2.40	1.78	4.80	16.66	19.49
cvg%	6.62	3.00	2.05	4.18	7.55	5.61

	NSS	GVS	NGS	FN	TKW	GP	YLD
r	50.32	0.23	16.59	64.25	56.53	38.56	55.15
cv%	7.07	32.23	15.88	12.51	7.34	34.26	22.86
cvg%	5.04	1.10	5.01	11.90	5.92	19.19	17.93

r = repeatability, equivalent to broad sense heritability. cv% = coefficient of variation of the experiment. cvg% = genetic coefficient of variation.

3.4.3 Correlation

Table 21. Pearson's correlation matrix among the characters evaluated for 150 RILs in two years (2017 and 2019) (CGF/FAEM/UFPel, 2025).

	DTH	DTM	PHT	YLD	TKW	SL	NSS	SW	GSW	NGS	GVS	GP	FN
DTH	1.00	0.84 ***	0.32 ***	-0.47 ***	-0.45 ***	-0.17 ***	-0.14 *	-0.46 ***	-0.47 ***	-0.30 ***	-0.43 ***	-0.41 ***	-0.61 ***
DTM		1.00	0.30 ***	-0.25 ***	-0.23 ***	0.00 ns	-0.01 ns	-0.23 ***	-0.25 ***	-0.13 *	-0.40 ***	-0.38 ***	-0.59 ***
PHT			1.00	0.07 ns	-0.12 *	0.16 ***	0.09 ns	-0.11 *	-0.12 *	-0.02 ns	-0.21 ***	-0.28 ***	-0.29 ***
YLD				1.00	0.44 ***	0.42 ***	0.41 ***	0.60 ***	0.62 ***	0.59 ***	0.27 ***	0.19 ***	0.32 ***
TKW					1.00	0.27 ***	0.29 ***	0.57 ***	0.56 ***	0.35 ***	0.30 ***	0.38 ***	0.17 ***
SL						1.00	0.65 ***	0.64 ***	0.58 ***	0.60 ***	0.09 ns	0.03 ns	0.06 ns
NSS							1.00	0.66 ***	0.62 ***	0.71 ***	0.19 ***	0.16 ***	0.06 ns
SW								1.00	0.98 ***	0.87 ***	0.32 ***	0.35 ***	0.20 ***
GSW									1.00	0.88 ***	0.33 ***	0.36 ***	0.22 ***
NGS										1.00	0.27 ***	0.24 ***	0.13 *
GVS											1.00	0.62 ***	0.15 ***
GP												1.00	0.10 ns
FN													1.00

*** = 0.0001 ** = 0.001 *= 0.05 ns = not significative

The Pearson's correlation obtained for the evaluated characters indicated significant correlations for many characters. The higher significant correlations were detected between DTH and DTM (0.84), GSW and SW (0.98), NGS and SW (0.87), NGS and GSW (0.88). The highest negative significant correlation was obtained

between DTH and FN (-0.61). Other correlations were either low or intermediate and many were not significant.

3.4.3 Cluster Analysis

The Cluster analysis of the 2018 grouped the 150 RILs and the 2 parental genotypes into 5 groups (Figure 42). Group 1 consisted of 23 genotypes, group 2 with 121 genotypes, group 3 with 6 genotypes, group 4 with one genotype (line 219) and group 5 with one genotype (line 77).

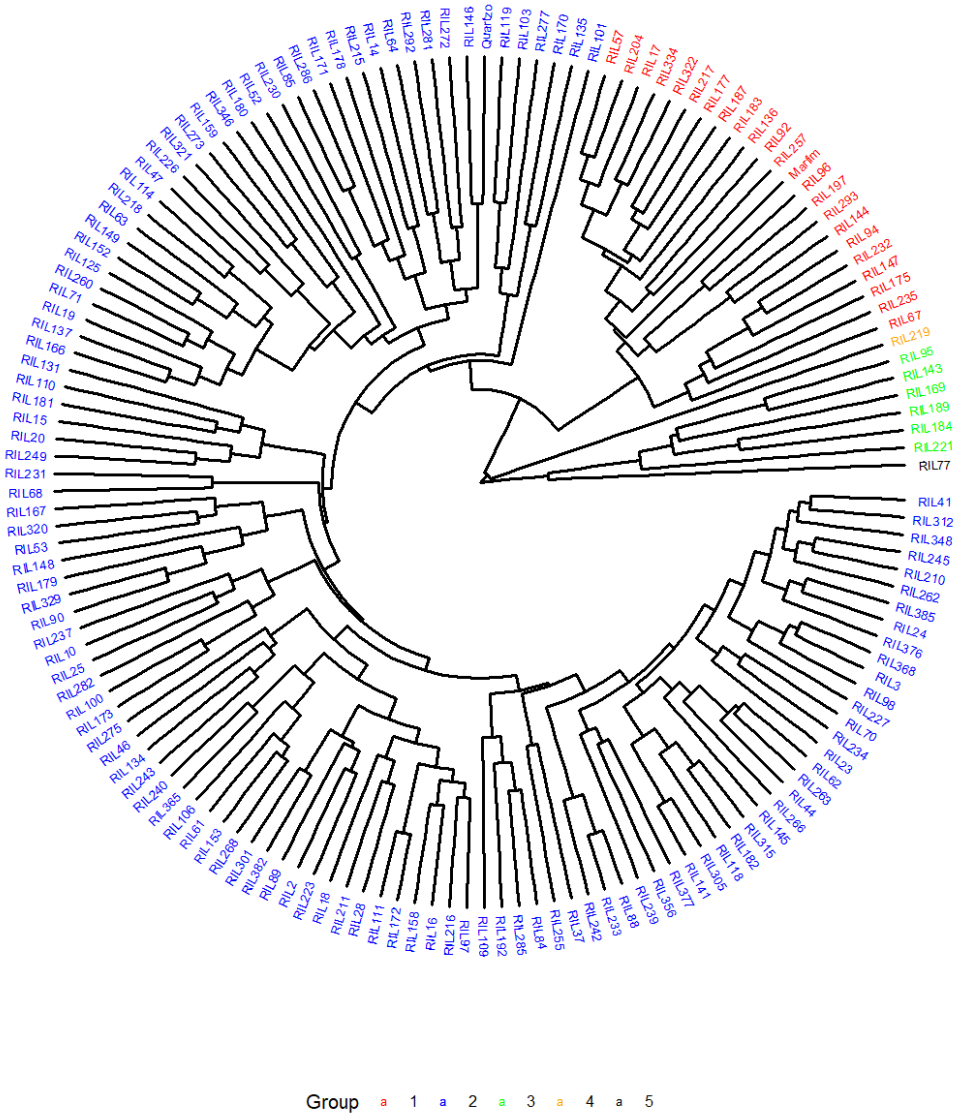

Figure 42. Cluster analysis of characters evaluated for 150 wheat RILs and their parents, in 2018.

In the summary table (Table 22), one can see the heritability values for the two environments (2017 and 2018).

Table 22. Heritability values, considering the BLUE values for each variable in the analysis of 150 RILs and their parents.

Location: 1

Trait	Role	Genotypic Variance	Heritability	p Value
DAE	Gen	8.5385	0.5453	
DAE	Check	5.2367	0.5969	
DAM	Gen	10.7040	0.5573	
DAM	Check	14.0794	0.6151	
EST	Gen	3.7233	0.5274	
EST	Check	9.8672	0.6089	
REN	Gen	0.1206	0.5130	
REN	Check	0.0847	0.5398	
PMG	Gen	4.9728	0.5470	
PMG	Check	0.6455	0.5542	
CE	Gen	0.3729	0.5602	
CE	Check	0.5442	0.6171	
NET	Gen	0.1877	0.5102	
NET	Check	0.0000	0.0000	
ME	Gen	0.0184	0.5139	
ME	Check	0.0074	0.5348	
MGE	Gen	0.0163	0.5171	
MGE	Check	6e-04.0000	0.0000	
NGE	Gen	15.5057	0.5342	
NGE	Check	0.0000	0.0000	
GEV	Gen	0.0434	0.5159	
GEV	Check	0.0981	0.5973	
GEQ	Gen	96.0553	0.5423	
GEQ	Check	28.3187	0.5536	
FN	Gen	0.0000	0.0000	
FN	Check	442.8440	0.5489	

Location: 2

Trait	Role	Genotypic Variance	Heritability	p Value
DAE	Gen	2.2496	0.5347	
DAE	Check	9.1626	0.6167	
DAM	Gen	1.8058	0.5261	
DAM	Check	7.0163	0.6139	
EST	Gen	6.3845	0.5422	
EST	Check	3.8634	0.5922	
REN	Gen	0.2931	0.5386	
REN	Check	0.0147	0.5278	
PMG	Gen	8.6073	0.5812	
PMG	Check	0.0401	0.5138	
CE	Gen	0.3387	0.5715	
CE	Check	0.2035	0.6102	
NET	Gen	0.5356	0.5279	
NET	Check	0.1029	0.5475	
ME	Gen	0.0469	0.5474	
ME	Check	0.0434	0.5983	
MGE	Gen	0.0343	0.5526	
MGE	Check	0.0301	0.6075	
NGE	Gen	29.0645	0.5646	
NGE	Check	25.8651	0.6135	
GEV	Gen	0.0000	0.0000	
GEV	Check	0.0000	0.0000	
GEQ	Gen	91.4086	0.5502	
GEQ	Check	0.0000	0.0000	
FN		NA NA	NA	Convergence Failed in BLUE model
FN		NA NA	NA	Convergence Failed in BLUE Genotype Check model

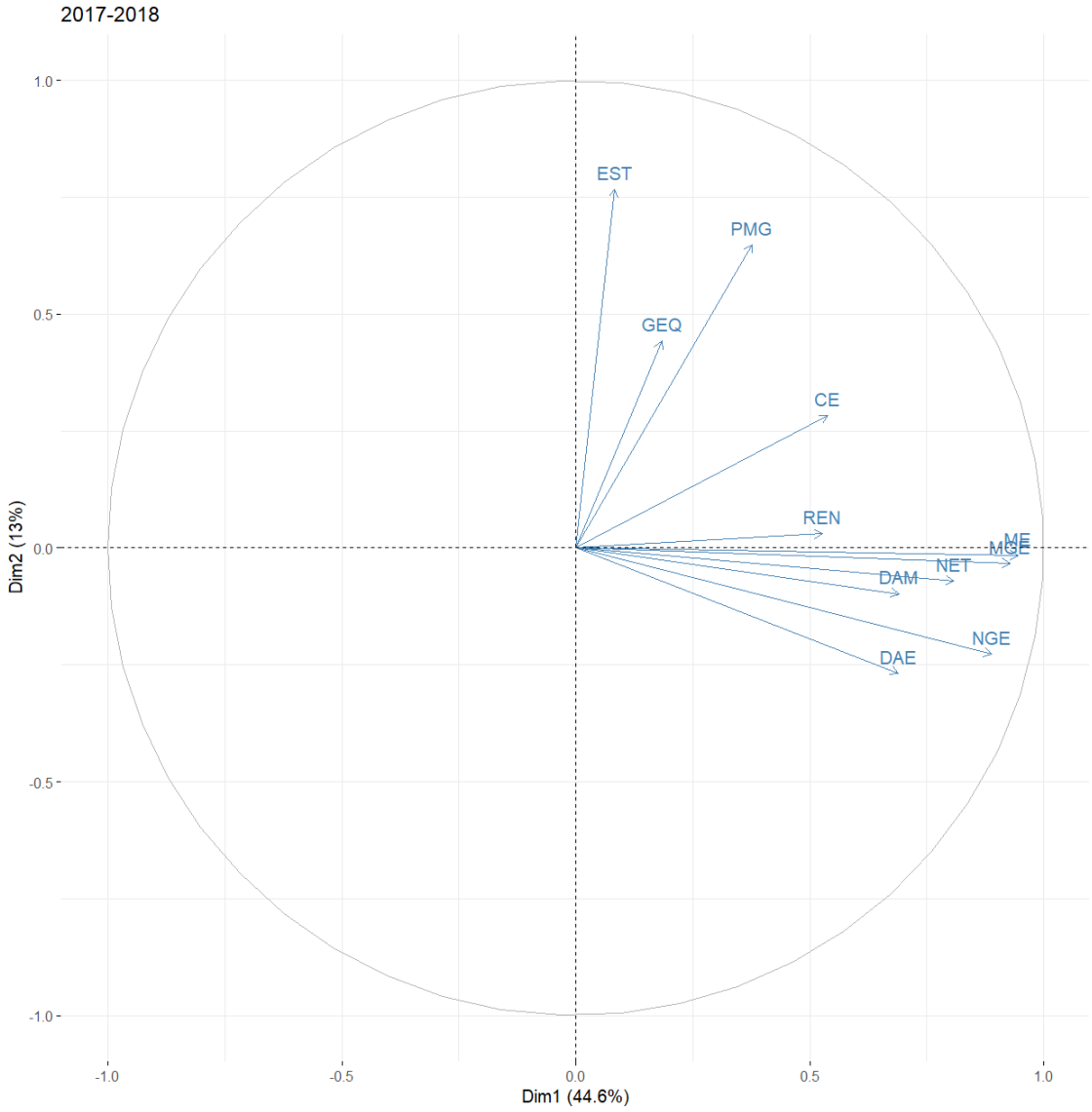

Figure 43. Diagram showing the contribution of variables (characters) to each dimension in the Principal Components (PC) analysis.

In the PC analyses, the joint analysis (2017 + 2018) displayed 44.6% of the variation in the first PC and 13% in the second PC, totalling 57.6%. In Figure 43, one can observe that the variables DTH, DTM, NSS, NGS, WS, GWS and YLD, all contributed to the PC1, that explained 44.6% of variation. The variables PHT, GVS and PG, contributed to PC2, that explained 13% of the variation.

In Figure 44, one can see the individuals spread across the axes according to the contribution of their character values. For example, lines (RILs) 20, 145 and 131 appear close together in the right upper corner. Since PMG vector is right nearby, we can infer that these lines are similar for PMG and different from the average. The PMG values are 38.12, 37.10 and 37.76 for RILs 20, 145 and 131, indicating this to be the case (data not shown).

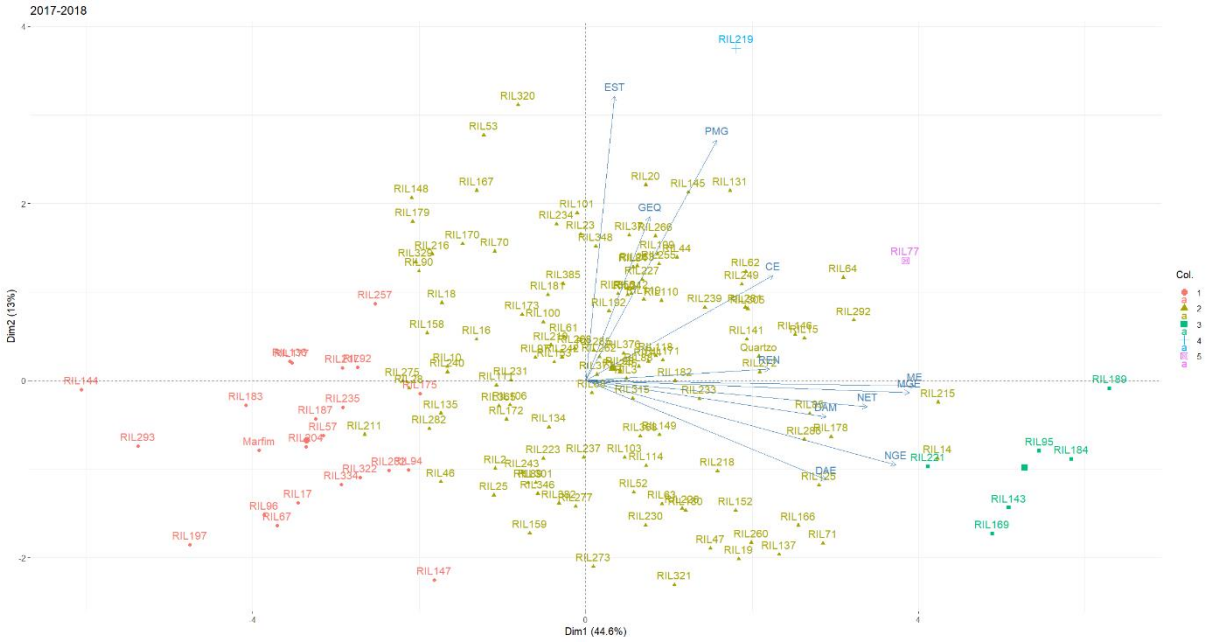

Figure 44. Diagram showing the contribution of variables (characters) to each dimension in the Principal Components (PC) analysis and distribution of individual genotypes.

3.5 DISCUSSION

This work aimed to characterize 150 RILs from the wheat cross ORS Quartzo x ORS Marfim. The distribution of the individuals for the different characters evaluated was examined using a ttest for each distribution of the character in each year. As can be seen by the analysis of variance equalities, some variances were not equal, probably due to the effect of environmental differences between years.

The repeatability coefficient was used to calculate a broad sense heritability for the group of individuals. Therefore, the traits showing higher heritability were DTH, DTM, PHT, TKW and NSS, while the traits showing lower heritability values were GVS, GWS and NGS. DTH and DTM are traits related to cycle, although known to be influenced

by year, i.e., number of days with higher radiation as opposed to cloudy days can influence the cycle. Also, other environmental factors can accelerate or delay cycle, such as temperature and water and nutrient availability for example. PHT is also a trait that can be influenced by particular conditions hapenning in a given year. TKW and NSS can also be influenced differentially in different years, since relate to grain filling and flower development, respectively. However, these traits showed higher heritability, indicating a higher stability between years. On the other hand, GVS, GWS and NGS, suffered with the higher effects from the environment, therefore displaying lower heritability values.

The analysis of correlation between traits indicated particular associations that are important to this study. The positive correlations detected between DTH and DTM (0.84), GSW and SW (0.98), NGS and SW (0.87), NGS and GSW (0.88), all suggest that most of the lines with longer time to heading, also had a longer time to maturation. There are some studies that suggest that for cereals, it could be advantageous to have a shorter vegetative cycle and a longer reproductive cycle in order to better fill the grains. However, this was not observed in the present work for most of the lines in this cross. The highest negative significant correlation was obtained between DTH and FN (-0.61). This negative association suggests that lines with longer DTH, tend to present lower falling number probably due to lower PHS resistance. Also, a negative correlation between DTH and GP (-0.41) was observed, however this negative association can not be explained by PHS resistance. Since no correlation between GP and FN was obtained, this linear correlation between DTH and GP may be a result of secondary correlations affecting this association.

The Cluster analysis using BLUP values of the year 2018 grouped the 150 RILs and the 2 parental genotypes into 5 groups. Group 1 consisted of 23 genotypes, group 2 with 121 genotypes, group 3 with 6 genotypes, group 4 with one genotype (line 219) and group 5 with one genotype (line 77). Most of the lines (120) grouped with the ORS Quartzo, indicating that the lines resemble this parent best. This is interesting, because this parent was a very successful wheat cultivar in Brazil, occupying a large area and being used in crosses to generate more recent cultivars. The remaining 23 RILs grouped with ORS Marfim and the other 3 groups showed fewer genotypes, perhaps with some transgressive traits, that could be further investigated.

Considering BLUP values for each year, the higher values of heritability found were for DTM and SL, with 0.62 for both in 2017. In 2018, the higher values of heritability

found were 0.62 for DTH, DTM, SL and NGS. The higher heritability values present in both years were DTM and SL.

The PC analysis, although did not explain a significant share of the variation (ca. 57%), could be used to infer some interesting commonalities between the genotypes. The PC1 was influenced by most variables (DTH, DTM, NSS, NGS, WS WGS and YLD), while PC2 was influenced by PHT, GP and TKW. The estimatives of heritability using repeatability coefficient and BLUP for GP showed 0.38 and 0.55, respectively, indicating that the BLUP analysis can better detect the genetic effects. For FN, only the repeatability values were obtained, reaching 0.64.

The study of RILs has the advantage of having a permanent population that can be replicated and to combine phenotyping in different years to the genotyping. Due to problems with the pandemics, this work could not be presented as originally planned. The phenotyping data for field and laboratory traits has been performed for 150 lines, and DARTSeq genotyping was also performed. The original 483 lines were phenotyped for three traits (DTH, DTM and YLD) in the two years, while a subset of 150 lines were phenotyped as described earlier. The results show interesting separation of the lines and further GWAS analysis may reveal sources of PHS resistance present in this progeny.

3.6 CONCLUSION

PHS is an important trait in many regions of the globe. The BLUP analyses showed a heritability value of 0.55 for GP, one of the two characters indicating PHS resistance. For FN, a heritability of 0.64 was obtained with the repeatability coefficient. The analysis of a RIL population from the cross ORS Quartzo x ORS Marfim revealed differences in the progeny that suggest transgressive segregants for many traits, forming 5 major groups. The higher significant correlations were detected between DTH and DTM (0.84), GSW and SW (0.98), NGS and SW (0.87), NGS and GSW (0.88). The highest negative significant correlation was obtained between DTH and FN (-0.61).

References

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Ali, A., Cao, J., Jiang, H., Chang, C., Zhang, H. P., Sheikh, S. W., Shah, L., & Ma, C. (2019). Unraveling Molecular and Genetic Studies of Wheat (Triticum aestivum L.) Resistance against Factors Causing Pre-Harvest Sprouting. Agronomy 2019, Vol. 9, Page 117, 9(3), 117. https://doi.org/10.3390/AGRONOMY9030117

BIOTRIGO (2025). Catálogo de cultivares 2023–2024 https://biotrigo.com.br/cultivares/#portfolio-biotrigo-sul

Borém, A., Miranda, G. V., (2013). Melhoramento de plantas. 6 ed. Universidade Federal de Viçosa. 523 p.

Chang, C., Zhang, H., Lu, J., Si, H., & Ma, C. (2023). Genetic Improvement of Wheat with Pre-Harvest Sprouting Resistance in China. Genes, 14(4), 837. https://doi.org/10.3390/GENES14040837/S1

Cherepovitsyna, A., Ridoutt, B., Halecki, W., & Bedla, D. (2022). Global Wheat Production and Threats to Supply Chains in a Volatile Climate Change and Energy Crisis. Resources 2022, Vol. 11, Page 118, 11(12), 118. https://doi.org/10.3390/RESOURCES11120118

CIMMYT (2025) https://www.cimmyt.org/es

CONAB. (2025, February). Acompanhamento da safra brasilera de graõs 2024/2025. Monitoreamento Agrícola Safra 2025, 12, 99–101. https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos

Cunha, G. R., & Pires, J. L. F. (2004). Germinação pré-colheita em trigo. Embrapa.

De Castro, R. L., Chais, A., Guarienti, E. M., Pires, J., So e Silva, M., Caierão, E., & Scheeren, P. (2015). Germinação pré-colheita nas cultivares de trigo do Rio Grande do Sul, ano 2014.

EMBRAPA TRIGO (2017). BRS Parrudo. https://www.embrapa.br/busca-de-publicacoes

Falconer, D. S. (1996) Introduction to quantitative genetics. Pearson Education India. 4.ed. Londres: Longman, 479 p.

FAO. (2025). FAOSTAT. Crops and Livestock Products. https://www.fao.org/faostat/en/#data/QCL

Fernie, A. R., & Yan, J. (2019). De novo domestication: an alternative route toward new crops for the future. Molecular plant, 12(5), 615-631.

Guarienti, E. M., Leonardo, J., Pires, F., Lima De Castro, R., Rocca Da Cunha, G., Paula, A., Valentini, F., Cunha, A., Caierão, R. E., Melo, E., Francisco De Assis, R.,

Genei, F., Dalmago, A., Fuhrmann, J., Lauro, W., Okuyama, A., Lago, L., Luiz, M., De Mello, G., … Tonon, D. (2017). Método para Avaliar a Germinação Pré-Colheita em Genótipos de Trigo: Desenvolvimento Analítico e Aplicação. https://www.embrapa.br/fale-conosco

Halecki, W., & Bedla, D. (2022). Global wheat production and threats to supply chains in a volatile climate change and energy crisis. Resources, 11(12), 118.

Hill, W. G., Goddard, M. E., & Visscher, P. M. (2008). Data and theory point to mainly additive genetic variance for complex traits. PLoS genetics, 4(2), e1000008.

Ministerio de Agricultura e Pecuaria (2009). Regras para anlálises de sementes. https://www.gov.br/agricultura/pt-br

Narvariya, S. S., & Singh, A. K. (2019). Qualitative Traits, Genetic Variability and Character Association for the Potential Use in Breeding Programme of Mango (Mangifera indica L.). Int. J. Curr. Microbiol. App. Sci, 8(2),

Nörnberg, R., Silva, J. A. G. D., Luche, H. D. S., Tessmann, E. W., Kavalco, S. A. F., Zimmer, C. M., ... & Oliveira, A. C. D. (2015). Tolerance to preharvest sprouting and yield of wheat genotypes from different breeding programs. Pesquisa Agropecuária Brasileira, 50(08), 698-706.

Okuyama, L. A., Riede, C. R., & Kohli, M. M. (2020). Association between falling number and grain characteristics to evaluate preharvest sprouting in wheat. Journal of Experimental Biology and Agricultural Sciences, 8(6), 758–764. https://doi.org/10.18006/2020.8(6).758.764

OR SEMENTES (2025). Catálogo de cultivares https://www.orsementes.com.br/cultivares

Patwa, N., & Penning, B. W. (2020). Environmental Impact on Cereal Crop Grain Damage from Pre-harvest Sprouting and Late Maturity Alpha-Amylase. In Sustainable Agriculture in the Era of Climate Change (pp. 23–41). Springer International Publishing. https://doi.org/10.1007/978-3-030-45669-6_2

Pedrozo et al. Repeatability of fruits and seeds production and selection of Brazil nut genotypes in native populations in Roraima. **Revista Árvore**, Viçosa-MG, v.39, n.5, p.863-871, 2015.

Peske, S. T., Villela, F. A., & Meneghello, G. E. (2012). Sementes: fundamentos científicos e tecnológicos. Editora Universitária/UFPel, Pelotas.

REUNIÃO DA COMISSÃO BRASILEIRA DE PESQUISA DE TRIGO E TRITICALE RCBPTT. Informações técnicas para trigo e triticale, safra 2015. Brasília, DF: Embrapa, 2017, 2018.

Reynolds, M. P., & Braun, H. J. (2022). Wheat Improvement: Food Security in a Changing Climate. In Wheat Improvement: Food Security in a Changing Climate. Springer International Publishing. https://doi.org/10.1007/978-3-030-90673-3

SADE et al. Assessment of floral characteristics for hybrid wheat (Triticum aestivum L.) production in Texas. Agrosystems, Geosciences & Environment, Texas, USA, 2022

Schereen, P. L., & Caierão, E. (2015). Cultivares. In Trigo: do plantío à colheita (pp. 91–119). Universidade Federal de Viçosa.

T. Pearson. (2010). High-Speed Sorting of Grains by Color and Surface Texture. Applied Engineering in Agriculture, 26(3), 499–505. https://doi.org/10.13031/2013.29948

Tai, L., Wang, H. J., Xu, X. J., Sun, W. H., Ju, L., Liu, W. T., Li, W. Q., Sun, J., & Chen, K. M. (2021). Pre-harvest sprouting in cereals: genetic and biochemical mechanisms. Journal of Experimental Botany, 72(8), 2857–2876. https://doi.org/10.1093/JXB/ERAB024

Vetch, J. M., Stougaard, R. N., Martin, J. M., & Giroux, M. J. (2019). Review: Revealing the genetic mechanisms of pre-harvest sprouting in hexaploid wheat (Triticum aestivum L.). In Plant Science (Vol. 281, pp. 180–185). Elsevier Ireland Ltd. https://doi.org/10.1016/j.plantsci.2019.01.004

Wade Thomason et. al. (2024, August 7). Understanding Pre-harvest Sprouting of Wheat. Publications and Educational Resources. https://www.pubs.ext.vt.edu/424/424-060/424-060.html

Wheat.org. (2025). Wheat. https://wheat.org/