UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM FITOTECNIA

GENETIC ANALYSIS AND PHYSICAL MAPPING OF OAT CROWN RUST RESISTANCE IN *Avena sativa L.*

Jéssica Argenta
Mestre em Fitotecnia /UFRGS

Tese apresentada como um dos requisitos
à obtenção do Grau de Doutor em Fitotecnia
Área de Concentração em Recursos Genéticos, Biotecnologia e Melhoramento Vegetal

Porto Alegre (RS), Brasil
Julho de 2023

CIP - Catalogação na Publicação

Argenta, Jéssica
GENETIC ANALYSIS AND PHYSICAL MAPPING OF OAT CROWN RUST RESISTANCE IN Avena sativa L. / Jéssica Argenta. -- 2023.
122 f.
Orientador: Marcelo Teixeira Pacheco.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Agronomia, Programa de Pós-Graduação em Fitotecnia, Porto Alegre, BR-RS, 2023.

1. Genetics. 2. Plant Breeding. 3. Crown Rust. 4. Disease Resistance. 5. Oats. I. Teixeira Pacheco, Marcelo, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

JÉSSICA ARGENTA
Engenheira Agrônoma- IFRS
Mestre em Fitotecnia - UFRGS

TESE

Submetida como parte dos requisitos
para obtenção do Grau de

DOUTOR(A) EM FITOTECNIA

Programa de Pós-Graduação em Fitotecnia
Faculdade de Agronomia
Universidade Federal do Rio Grande do Sul
Porto Alegre (RS), Brasil

Aprovado em: 14.08.2023
Pela Banca Examinadora

MARCELO TEIXEIRA PACHECO
Orientador(a)
UFRGS

SIMONE MUNDSTOCK JAHNKE
Coordenadora do Programa de Pós-Graduação em Fitotecnia

CARLA ANDRÉA DELATORRE
UFRGS

CRISTIANO MATHIAS ZIMMER
CORTEVA

JOSÉ ANTÔNIO MARTINELLI
UFRGS

CARLOS ALBERTO BISSANI
Diretor da Faculdade de Agronomia

LUIZ CARLOS FEDERIZZI
UFRGS

ACKNOWLEDGMENTS

I am grateful to God to have survived and completed my thesis during the three years of the pandemic, despite the challenges, where many others couldn't do the same. To the Federal University of Rio Grande do Sul (UFRGS) and to the Graduate Program in Plant Science for the opportunity to undertake the doctorate degree.

To the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting a scholarship for a Doctorate in Brazil and in Australia.

To Professor Marcelo Teixeira Pacheco, Luiz Carlos Federizzi, and José Antônio Martinelli, for their example and for their exceptional guidance, unwavering support, and friendship throughout my educational journey. Their influence and encouragement have played a significant role in shaping who I am today. It is thanks to them that I am where I am now. I am deeply grateful for this opportunity.

To the Plant Breeding Institute (PBI) for providing me with the opportunity to be a part of this project and gain invaluable international experience. I extend my special thanks to Professor Robert Park, Professor Peng Zhang, Dr. Laura Ziems, Dr. Mumta Chetri, Dr. Davinder Singh, and Technicians Matthew Williams, Jeremy Roake, Margarita Pietilainen, and Dorian Friendship for their immense support, guidance, and friendship.

To all the professors at the Federal University of Rio Grande do Sul who have played a role in my personal and professional growth. I am especially grateful to Carla Delatorre for her contributions and friendship throughout this journey. I also want to thank Itamar Cristiano Nava and Renata Pereira da Cruz for their valuable lessons and friendship.

To the DEPLAV technicians, Fabio Berndt and Gustavo Maia Leao, and special thanks to the UFRGS Agronomic Experimental Station assistants, Miguel Lenzi and Paulo Vinícius da Silva, for their help with field experiments.

To the people I met in Australia, special Moustafa El Sayyad, Jessica Shadbot, Disha Dhanda, and Ching-Yu Lu (Joey), for supporting me in the hard moments. Thank you for the amazing Aussie experience.

To the colleagues who became friends and companions along this journey and contributed directly or indirectly to my getting here: Jacqueline Flores Schmitz, Vanessa Duarte, Guilherme Oliveira, and Matheus Reinicke Wünsche.

To my parents, Ines Maria Rissardo and Augusto Argenta, and my family, for their support, encouragement, and understanding when I was absent.

To everyone who somehow contributed to my education.

GENETIC ANALYSIS AND PHYSICAL MAPPING OF OAT CROWN RUST RESISTANCE IN *Avena sativa L.*[1]

Author: Jéssica Argenta
Advisor: Marcelo Teixeira Pacheco

ABSTRACT

The most destructive foliar pathogen in oat is crown rust disease caused by the fungus *Puccinia coronata f. sp. avenae* (*Pca*). It can lead to significant yield losses in favorable epidemic conditions. The most effective method of control of crown rust is genetic resistance. Although in Brazil and Australia, this pathogen has asexual reproduction, it exhibits high variability in virulence phenotypes on oat varieties, and rapid emergence of new virulent pathotypes. Therefore, breeding for resistance is a continuous process of identifying and characterizing new and effective combinations of resistance genes to be incorporated into oat varieties. Almost a hundred genes have been reported in *A. sativa* to confer *Pca* resistance. Also, other *Avena* species, such as *A. strigosa* and *A. sterilis*, have been identified as valuable sources of resistance to introgression into cultivated oat. A great effort has been made to investigate the genetics of resistance to develop crown rust resistant oat varieties. However, the genetic and molecular mechanisms involved with the resistance in oats are poorly understood. Only a few resistance genes have been mapped to a known genomic location. The inheritance and identification of these genes, as well as their mapping, will assist the development of new oat varieties with higher genetic resistance. A population of *A. sativa* with introgressed resistance from *A. strigosa*, involving the resistant parent '07BT333', was characterized for the genetic inheritance of crown rust resistance. The results showed the presence of three loci providing resistance to crown rust and one resistance suppressor locus. Different combinations of alleles of these four loci exhibit four different resistance phenotypes, from immunity to complete susceptibility. Another set of 14 resistant *A. sativa* genotypes, carrying 13 characterized and one unknown resistance crown rust resistance genes, coming from *A. sterilis* and *A. sativa* were analysed. This set of parents (donors) was used to develop the first *A. sativa* nested association mapping (AsNAM) population. All 14 resistant parents were crossed to Swan, a crow rust susceptible oat cultivar. The derived subpopulations were phenotypically and genotypically assessed. Eight subpopulations segregated for one resistance genes, while the other six segregated for two to five resistance genes, all with complete dominance. A total of eight QTL were identified in eight different oat chromosomes (chr4A, chr1C, chr4C, chr1D, chr2D, chr3D, chr5D, and chr7D), using the IBD-methodology. Several resistance genes were linked or allelic. The chromosomic location was identified for ten resistance genes, which location was previously unknow for six of them. On the oat genome, at regions of the SNP markers associated with identified QTL, 31 candidate genes that may be involved with crown rust resistance were identified.

[1] Doctoral Thesis in Plant Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (122p.) August 2023.

ANÁLISE GENÉTICA E MAPEAMENTO FÍSICO DA RESISTÊNCIA À FERRUGEM DA FOLHA EM *Avena sativa* L.[1]

Autor: Jéssica Argenta
Orientador: Marcelo Teixeira Pacheco

RESUMO

A doença foliar em aveia mais destrutiva é a ferrugem da folha causada pelo fungo *Puccinia coronata f. sp. avenae* (*Pca*). Essa doença pode alcançar danos significativos sob condições favoráveis a epidemia. O melhor método de controle dessa doença e a resistência genética. Embora no Brasil e na Austrália esse patógeno tenha reprodução assexuada, apresenta alta variabilidade nos fenótipos de virulência em variedades de aveia e rápido surgimento de novos patótipos. Portanto, o melhoramento para resistência é um processo contínuo de identificação e caracterização de novas e efetivas combinações de genes de resistência a serem incorporados em variedades de aveia. Grandes esforços têm sido feitos a fim de investigar a genética da resistência e desenvolver cultivares resistentes a ferrugem da folha. No entanto o mecanismo genético e molecular envolvido na resistência em aveia ainda é pouco compreendido. Apenas alguns genes de resistência foram mapeados e possuem localização genômica conhecida. A herança e identificação desses genes, bem como seu mapeamento, auxiliarão no desenvolvimento de novas variedades de aveia com maior resistência genética. Uma população de *A. sativa* com resistência introgredida de *A. strigosa*, envolvendo o genitor resistente '07BT333', foi caracterizada para herança genética de resistência à ferrugem da folha. Os resultados demonstraram a presença de três loci conferindo a resistência a ferrugem da folha e um locus supressor da resistência. Diferentes combinações de alelos destes quatro locos exibem quatro diferentes fenótipos de resistência, de imunidade à susceptibilidade. Outro conjunto de 14 genótipos resistentes de *A. sativa*, carregando 13 genes caracterizados e um desconhecido de resistência à ferrugem da folha, provenientes de *A. sterilis* e *A. sativa* foram avaliados. Esse conjunto de parentais (doadores) foram usados para desenvolver a primeira população de mapeamento por associação aninhada de *A. sativa* (AsNAM). Todos os 14 parentais resistentes foram cruzados com Swan, uma cultivar suscetível a ferrugem da folha. As subpopulações derivadas de destes cruzamentos foram fenotipica e genotipicamente avaliadas. Oito subpopulações segregaram para um gene de resistência, enquanto as outras seis segregaram de dois a cinco genes de resistência, todos com dominância completa. Um total oito QTL foram identificados em oito cromossomos diferentes (cr4A, cr1C, cr4C, cr1D, cr2D, cr3D, cr5D e cr7D), usando a metodologia de IBD. Vários genes de resistência estavam ligados ou são alelos do mesmo gene. A localização cromossômica foi identificada para 10 genes, sendo que para seis destes, a localização era desconhecida anteriormente. No genoma da aveia, na região dos marcadores SNP associados com os QTL identificados, 31 genes candidatos foram encontrados e podem estar envolvidos com a resistência a ferrugem da folha.

[1] Tese de Doutorado em Fitotecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (122p.) agosto, 2023.

LIST OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LITERATURE REVIEW

ARTICLE 1

ARTICLE 2

1. INTRODUCTION

Oat (*Avena sativa* L.) is a globally significant cereal crop that is cropped for grain, human and animal consumption, and forage production. However, the growth and production of oats can be limited by crown rust, a disease caused by the fungus *Puccinia coronata f. sp*. Avenae (*Pca*). Crown rust is the primary disease affecting oats and can lead to significant yield losses in favorable epidemic conditions. This disease is a major concern in Brazil and Australia, where epidemics occur every year. In severe cases, the disease can cause yield losses of over 90 %.

The most effective method to control of crown rust is genetic resistance. Although in Brazil and Australia, this pathogen reproduces only asexually, it exhibits high variability in virulence phenotypes on oat varieties, and rapid emergence of new virulence races. Therefore, breeding for resistance is a continuous process of identifying and characterizing new and effective combinations of resistance genes to be incorporated into varieties. There are almost a hundred genes that confer complete resistance to crown rust, none of which are currently fully effective against all pathotypes of the pathogen. Some of them are still useful when combined with each other.

With advances in genetics and genomics, the sources of resistance can be better characterized and more efficiently used. These technologies can also enable the combination of resistance genes, within single genotypes (gene pyramiding), in oat breeding programs aiming to develop more durable resistance against oat crown rust. Combining multiple resistance loci makes it more challenging for the pathogen to overcome the resistance.

A great effort has been made to investigate the genetics of resistance to *Pca* in *Avena* species, more than any other pathogen of oats, aiming to developing crown rust resistant oat varieties. Considerable research has been dedicated to study the genetic factors that contribute to resistance to *Pca*, however only a few resistance genes have been mapped to a known chromosomic location.

Earlier research faced obstacles due to the absence of a reference genome for the hexaploid oat species, which delayed the identification of gene position, function, and sequence. It is now possible to identify genomic regions associated with desirable traits in oats, thanks to the availability of the oat genomes, being the genome of the of the oat genotype 'OT3098' the first publicly available. Therefore, new horizons are opened for oat genetic research and improvement of traits of interest, including development of molecular markers and mapping of crown rust resistance genes.

The genetic and molecular mechanisms involved with crown rust resistance in oats are poorly understood. The inheritance and identification of these genes, as well as the mapping of their location, will assist the development of new oat varieties with higher genetic resistance. Mapping tools have improved in the past few years, evolving from the traditional bi-parental population linkage mapping to association mapping, in genomic wide association studies (GWAS), using a diversity panel of genotypes. A mixed approach is the nested association mapping (NAM) population which combines the advantages of bi-parental populations and GWAS mapping. In this thesis a set of resistant oat genotypes were used as parental donors of crown rust resistance genes, all crosses to a common oat parent, universally susceptible to crown rust, to form the first NAM population of oats. Also, the phenotype can be accessed evaluating oat populations under field, with natural crown rust infection, or under controlled conditions, using selected of pathotypes. In this way, the main objectives of this study were:

I. To phenotypically characterize and determine the genetic inheritance of a crown rust resistance source, recently introgressed from *Avena strigosa* to *Avena sativa*, under field conditions*;*
II. to identify genomic regions controlling crown rust resistance in oats from fourteen crown rust resistance donors, using the nested association mapping design approach;
III. to identify linked SNPs to genomic regions associated with crown rust resistance with potential to use as molecular markers for marker assisted breeding;
IV. to detected candidate genes for controlling the crown rust resistance on the regions of the oat genome OT3098 v2 harbouring the significant SNPs.

2. LITERATURA REVIEW

2.1. Oat: from wild specie to important crop

Oat (*Avena sativa*) was domesticated later than the other cereals, although they all come from the Middle East. The cultivation of oat dates from 4,000 years ago (Zohary *et al*., 2000). The wild oat *Avena sterilis* were considered a weed in wheat and barley fields. However, the great adaptability of this cereal allowed early European farmers to domesticate this wild oat into heterogeneous and robust landraces. These landraces lost ancestral seed dormancy, retained their grain at maturity, and were adapted to long days in a cold and humid climate (Leggett and Thomas, 1995; Valentine *et al.*, 2016).

A. sativa is cultivated globally, including regions with mild climate, such as Europe (including Russia), Northern Asia, North and South America, Southern Africa, New Zealand, and Australia (Loskutov and Rines, 2011). It is the seventh most economically important cereal after corn, rice, wheat, barley, sorghum, and millet. The main producers are Russia, Canada, and Poland (FAOSTAT, 2022). It is estimated that oat yields have improved by 139% worldwide since the 1960s (Strychar, 2011). As a result, the USDA projects that the total area of oat harvested will remain stable at 11.3 million hectares until 2031 (USDA, 2022). Simultaneously, the average yield is predicted to increase, producing 2,430 million tons of grain (USDA-FAS, 2022).

Oat is a minor, versatile, whole-grain cereal with several uses (Zwer, 2017) as grain, pasture, forage, and cover crop, depending on the region. In addition, *A. sativa* is a low-input crop that positively contributes to soil health, particularly in crop rotation systems, by improving soil structure and reducing crop pests (Smulders, 2018). Traditionally, oat has served as feed for horse and cattle, providing a source of carbohydrates and bedding (Andersson and Börjesdotter, 2011; Marshall *et al*., 2013).

During the early 1900s, the industrial revolution led to replacing horsepower with diesel engines, thanks to the advancements in mechanization. Consequently, the agricultural industry shifted towards more profitable crops, such as winter wheat, which resulted in the

decline of traditional oat beers in favour of the more popular barley and wheat-based alternatives. These changes caused a reduction in the global cultivation of oats, leading to their classification as a 'neglected crop' (Smulders, 2018).

Despite this, the popularity of oats as human food is growing. Oat flour is recognized for its high nutritional value compared to other cereal grains. It comprises essential amino acids and generous amounts of soluble fibres, like beta-glycans, which can help reduce blood glycose, cholesterol, and obesity (Moore-Colyer, 1995). Currently, oat grain and its products are considered healthy and functional, and consumers view them as trendy. Numerous health benefits have been officially approved by the European Food Safety Authority (EFSA) and the United States Food & Drug Administration (FDA) (Marshall *et al*., 2013).

It is essential to acknowledge and appreciate the exceptional characteristics of oat as a food and feed crop, along with its favourable agronomic traits. This recognition and value should be extended worldwide to ensure that oat becomes a commercially competitive crop once again, as highlighted by Smulders in 2018.

2.2. Oat genome

The *genus Avena* L. belongs to the *Poacea*e family. Most species in this genus are annual and self-pollinated. The genus *Avena* contains up to 30 taxonomical species recognized, with several of them, within ploid level, being fully interfertile (Ladizinsky, 2012), including three levels of natural ploid: diploid (2n = 2x = 14), tetraploid (2n = 4x = 28), and hexaploid (2n = 6x = 42). The basic number of chromosomes is n = 7 (LEGGETT and THOMAS, 1995). Species of the genus show wide variation in genome size from 4.1 to 12.8 gigabase pairs (Gbp), which is determined by both the level of ploid and by variation in the types and quantity of repetitive DNA between the species (Fu, 2018).

|Hexaploid cultivated oat (*Avena sativa*) is an allopolyploid specie that has evolved through interspecific hybridization and polyploidization cycles, combining three distinct diploid genomes. The three genomes that make up this hexaploid oat received nominal genome designations of A, C, and D (Loskutov and Rines, 2011).

Studies on the origin of cultivated oats and their phylogenetic ancestors were carried out in organelles (chloroplasts and mitochondria) in the 1970s (Steer *et al.,* 1970). It is known that the *A. sativa* evolved from wild hexaploid species (*A. sterilis* and *A. fatua*) having the same genomic structure. It has been proposed that the hexaploid oat originated from hybridization between two diploid species, ‘A’ and ‘C,’ resulting in a tetraploid ‘AC’ genome, followed by another hybridization with a donor species of the diploid ‘D’ genome.

The genome evolved through duplication events to stabilize the chromosomal pairing (Fu, 2018). The origin of the D genome remains uncertain. It is believed that the D genome progenitor of hexaploid oat is closely related to the A-genome rather than the C-genome, and may be extinct (Peng *et al.*, 2022).

2.3. Crown rust: a global problem in oat cultivation

The crown rust fungus is the most destructive foliar pathogen of oat. This pathogen is widely distributed and observed in all regions where this cereal is cultivated (SIMONS, 1979) (Figure 1). Moreover, populations of this pathogen exhibit high variability in virulence phenotypes on oat varieties and rapid emergence of new virulence races (Park, 2008; Nazareno, 2018).

Crown rust epidemics have occurs annually in Brazil and Uruguay (Leonard and Martinelli, 2005), with reports also indicating a similar incidence of disease in South America (Gassner, 1916), Portugal (D'oliveira, 1942), Australia (Waterhouse, 1952), Israel (Wahl and Schreiter, 1953), south-eastern Europe (Kostic, 1959), the USA (Sherf, 1954), Tunisia (Hammami *et al*., 2010) and Canada (Chong *et al*., 2008).

Historical reports of damage to oat caused by *P. coronata* first appeared in the late 1800s (Nazareno *et al.*, 2018). In years when environmental conditions favour pathogen development, disease severity can peak at over 90 %. This pathogen affects grain quality and yields, particularly in susceptible genotypes (Martinelli *et al.*, 1994). For example, in cultivation conditions in southern Brazil, Chaves *et al.* (2002) observed reductions from 4 to 79.5 % in grain yield and up to 50 % in grain weight. In the USA, severe losses occurred in 2014, when equivalent to 18.7 % of the country's oat production was lost due to breakdown resistance. During this epidemic, yield losses were up 50 % (USDA-ARS CDL 2014; Nazareno *et al.*, 2018). According to Martinelli *et al.* (1994), the damage caused by the crown rust pathogen can reach 90 to 9 kg/ha for every 1 % of severity, according to the growth stage, from early tillering to grain dough, respectively.

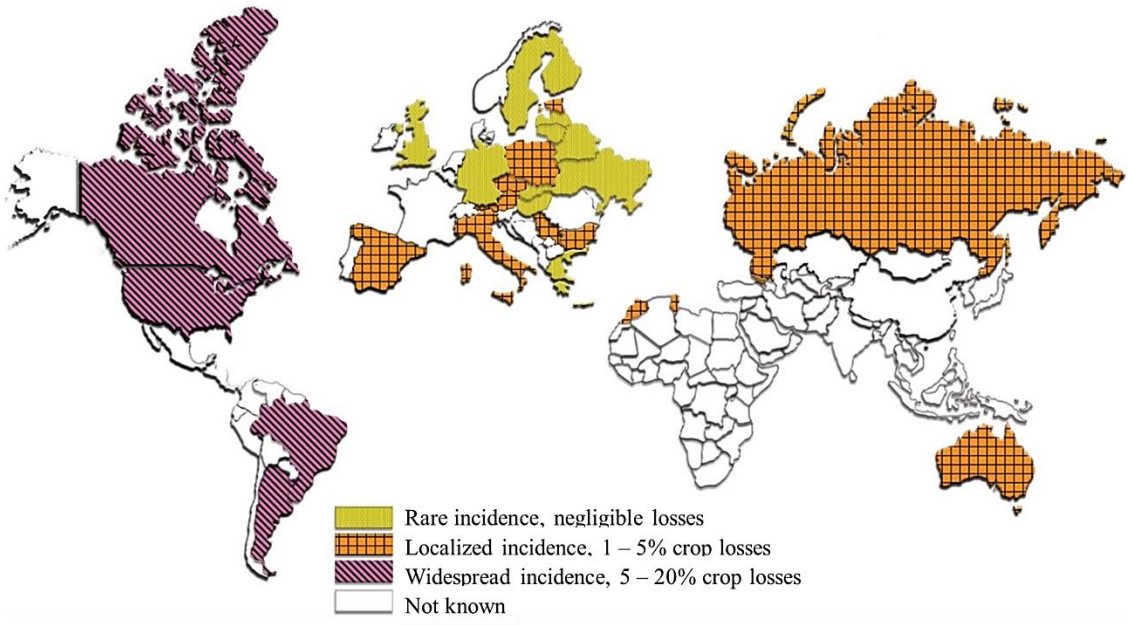


FIGURE 1. Global spread and economic impact of oat crown rust (*Puccinia coronate* f. sp. *avenae*) (Adapted from Gorash, 2017)

2.4. Pathogen and stages of the infectious process

Puccinia coronata f. sp. avenae is a biotrophic fungus belonging to the *Pucciniaceae* family, *Uredinales* order, and *Basidiomycotina* class (Forcelini and Reis, 1997; Simons, 1985). The pathogen acts as an obligatory parasite in a specific host-pathogen interaction at the species level (*formae specialis*) and group of cultivars (*physiological races*). Thus, crown rust can differ in virulence and present a high physiological specialization (Agrios, 2005).

This fungus is heteroecious (requires two host species to complete its life cycle), heterothallic (produces male and female gametes in different individuals), polycyclic (several secondary cycles throughout the infectious process) and completes each cycle in seven to ten days. The heteroecious condition occurs in the Northern Hemisphere, where plants of the *genus Rhamnus* acts as the alternative host (Nazareno *et al.*, 2018). *Rhamnus cathartica* is a native species to Europe and eastern Asia, which became invasive in North America after its introduction in the 1800s (Klenová-Jiráková *et al.*, 2010).

Oat crown rust has five infectious stages associated with the sexual and asexual stages of the fungus: uredial, telial, basidial, pycnial, and aecial. The stages vary concerning the fungal ploidy level, phenotypic characterization, pathogenicity, and associated virulence (Nazareno *et al.*, 2018). They also differ regarding the analogy of the structures formed and the penetration mechanism by which the pathogen accesses host tissues (Heath, 1997). The Life cycle of *P. coronata* is showed on Figure 2.

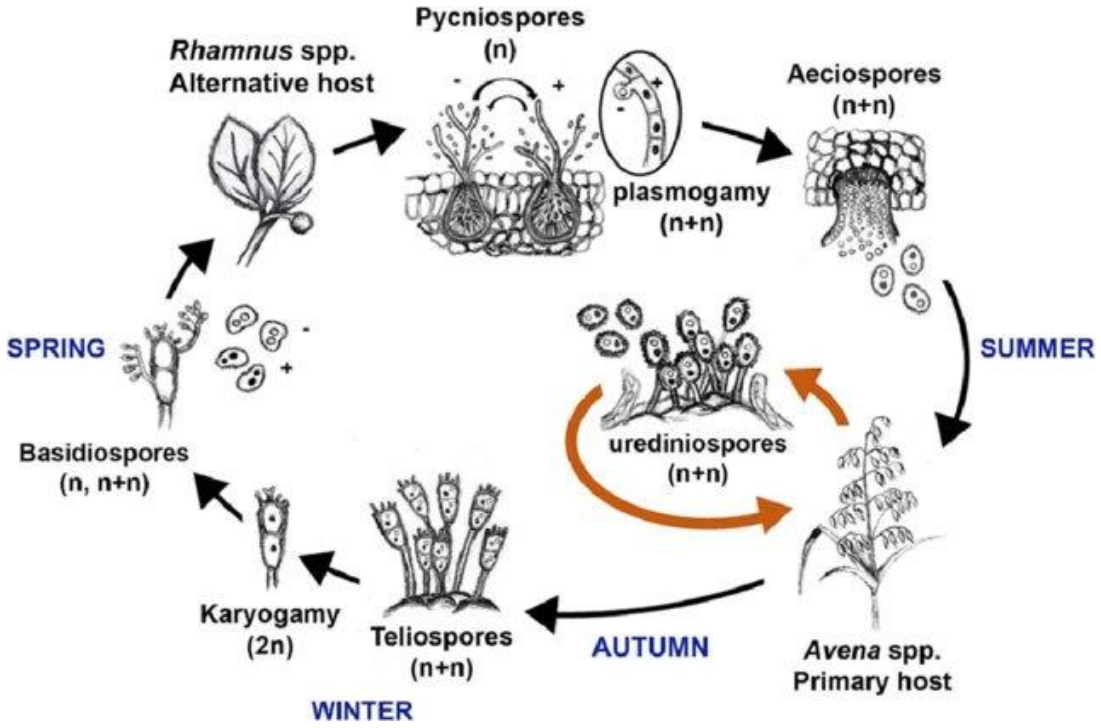


FIGURE 2. Life cycle of *Puccinia coronata* f. sp. *avenae*. (Nazareno *et al*., 2017).

After the deposition of uredospores on the leaf surface of oat plants and their germination, the disease develops on the host by initiating the called uredial phase. When the germ tube recognizes the stomata, penetrates through it, and is in the substomatal chamber, the formation of the substomatal vesicle occurs, which gives rise to the infective hyphae. When infectious hyphae encounter the cell wall of mesophyll cells, they differentiate into haustorium mother cells. The haustorium stem cells form a narrow clamp, which penetrates the host cell wall, probably by enzymatic dissolution or pressure (Heath, 1997; Hu and Rijkenberg, 1998). The development of haustoria takes place inside the lumen, but they remain on the outside of the plasma membrane of the host cells (Heath, 1997). The ramification of intercellular hyphae and haustoria formation continues until a fungal colony develops within the host tissue (Harder and Haber, 1992).

As infected plants mature, urediniospores formation ends, and teliospores production begins in the same or new fruiting structures, initiating the telial phase. Teliospores are sessile and serve as a hibernation structure for the fungus (Simons, 1985) and represent the only true diploid state of the fungus (Harder and Haber, 1992). Germination of teliospores and subsequent meiosis in the basidium results in haploid spores, called basidiospores, in the basidial phase. These infect young leaves of susceptible species of *Rhamnus* (Simons, 1985). The basidiospores germinate and penetrate directly through the epidermis during the sexual phase forming a haploid colony within the host (Harder and Haber, 1992).

The next phase of the crown rust life cycle is the pycnial phase, constituted by the production of pycnia, which are fruiting bodies usually formed on the adaxial face of *Rhamnus* leaves. The pycniospores are ejected into the air from the pycnia's interior, so insects or physical forces transport them between pycnia of different types (Harder and Haber, 1992). Pycniospores act as gametes, and their hyphae fuse with receptive hyphae when they encounter them, re-establishing the dikaryotic phase of the fungus. After plasmogamy, the formation of aecia occurs, initiating the aecial phase (Malvick, 1989). Aecia are cylindrical structures that produce aeciospores transported by air currents and reinfect oats. After infection by aeciospores, seven to 10 days later, there is the formation of uredia, which produce urediniospores, thus starting the uredial phase again, reinfecting the oat plant and completing the pathogen's life cycle (Harder and Haber, 1992).

The uredial and telial phases occur in different species of oats and other grasses, such as barley (*Hordeum vulgare* L.), ryegrass (*Lollium spp*), and bromus (*Bromus inermis*), among others. In addition, the production of spermatogonia and aecium occurs in species of the *genus Rhammus*. In North America, the fungus has a highly varied virulence phenotype due to the prevalence of the fungus's sexual phase (Chong *et al*., 2000).

In East Africa, South America, Australia, and New Zealand, the pathogen has no sexual phase due to a lack of alternative hosts. Instead, crown rust develops in a single host during its life cycle. Uredospores produced on oat plants that survive the summer or on green plants grown in different regions can be spread over long distances by wind and reinfect oat plants (Chaves and Martinelli, 2005). Therefore, in Brazil and Australia, *P. coronata* is limited to a repetitive asexual stage.

2.5. Disease symptoms

Crown rust is characterized by the occurrence of oval, yellow-orange uredia on the abaxial and adaxial surfaces of the leaf (Figure 3). However, uredia can be observed in all green parts of the plant, such as stems, panicles, and awns, depending on the severity of the disease (Simons, 1985). Symptoms in infected plants appear, on average, seven days after infection. The formation of pustules is observed when the spores break the cuticle, and the mass of urediniospores, present in the centre of the fruiting bodies, gives the pustules the yellow-orange colour, typical of the disease. When the pustules are ruptured, they release large amounts of spores. The wind carries these spores over distances of up to 2,000 km and repeats the infection cycle (Harder and Haber, 1992). After pathogen sporulation,

approximately 11 days after infection, green islands are formed in plant regions associated with the fungus's mycelial development (Scholes and Rolfe, 1996).

Different cultivars with varying degrees and types of resistance may exhibit varying responses to crown rust infection. These responses can range from tiny, pale flecks to medium-sized pustules typically surrounded by chlorotic or necrotic regions (Simons, 1985). After a few weeks, the edges of the uredopustules may turn black with the formation of teliospores. When infected plants reach maturity, the production of urediniospores stops, and only teliospores are produced (Figure 3A) (Simons, 1985; Martinelli, 2004).

During the infection's development, the disease harms green leaves, leading to alterations in photosynthetic rates and the distribution of photoassimilates between the leaves (source) and developing grains (drain organs). This results in withered grains with low nutritional value (Simons, 1985). In addition, the damage caused to the leaves reduces gas exchange. As the infection progresses, the affected areas expand, and the disease symptoms appear. The yellow spots on the leaves correlate with the fungus’s mycelium inside the tissues (Chaves and Martinelli, 2005). As the days pass, this photosynthetic decline causes the leaves to turn yellow, entering the senescence process.

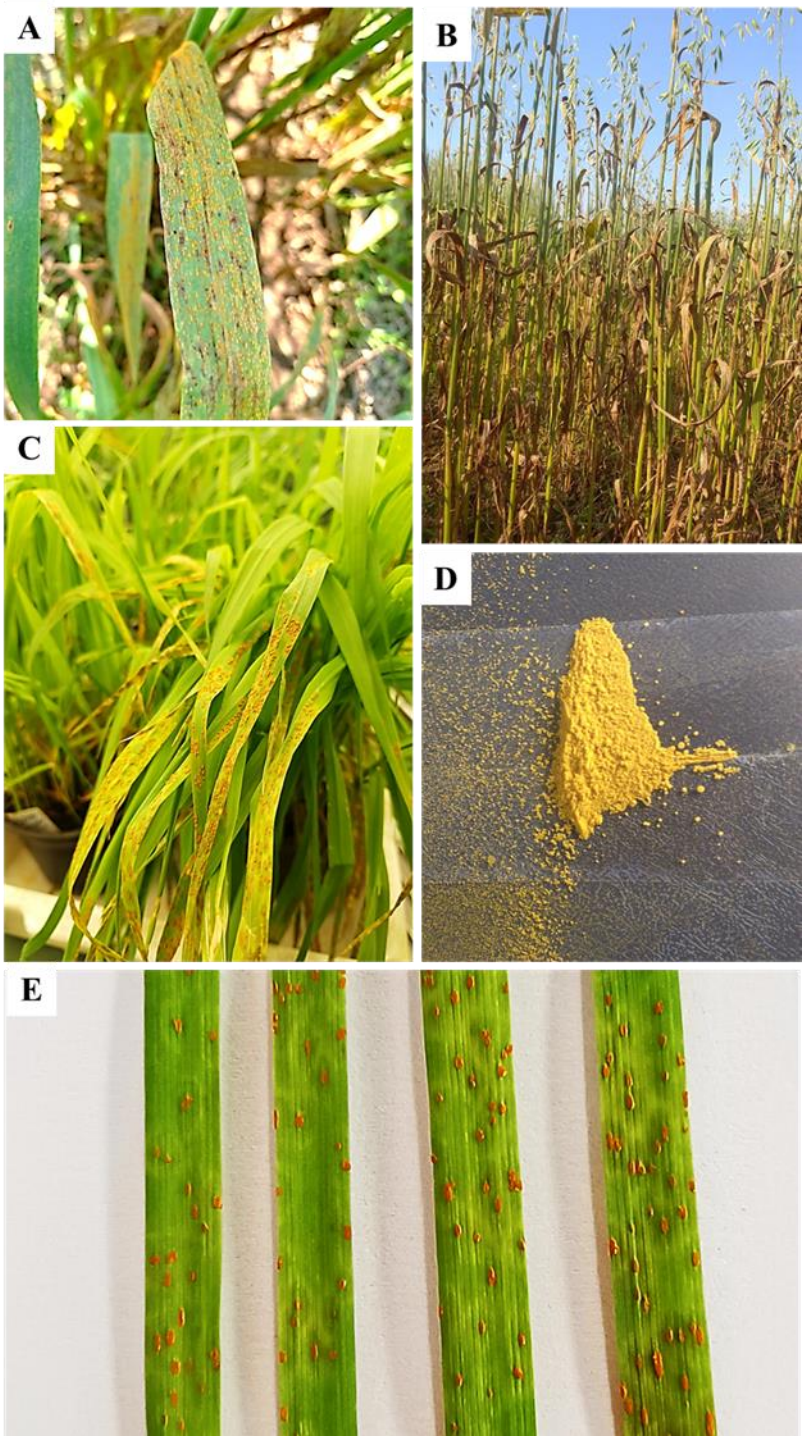


FIGURE 3. Symptoms of oat crown rust infection. (A, B) The disease of oat by *Puccinia coronate* f. sp. *avena* in the field. (C, D, E) The disease of oat by *P. coronata* in a controlled environment. (A) The leaves display a dense concentration of pustules (clusters of urediniospores). (B) Early senescence of the leaves due to the crown rust disease. (D) Mass of spores of *P. coronata*. (E) Susceptible genotype showing high infection type.

2.6. Oat crown rust control

The losses caused by the pathogen can be mitigated by applying fungicides and developing genetic resistance. Additionally, the literature suggests eradicating alternative hosts from regions close to the oat fields or implementing multilines (Pink, 2002; Mccartney *et al.*, 2011), which are not commonly utilized.

Investing in chemical products disease control can raise production costs. For example, although fungicides are known to limit yield losses in oat during rust outbreaks, they do not guarantee the complete protection of the crop. They are less effective than host resistance in reducing rust population size. Furthermore, they may have adverse environmental impacts due to soil residues or runoff into groundwater. Harder and Haber (1992) have also highlighted the challenges of obtaining fungicide registration for minor crops like oat.

Genetic resistance is more efficient and cost-effective than fungicide application for controlling crown rust in oats (Sanz *et al*., 2013). This approach reduces the number of necessary fungicide applications and increases oat yield. Therefore, it is highly favoured by farmers as it is highly economical, effective, and the safest approach for disease control (Gnanesh *et al*., 2014; Vieira *et al*., 2006; Simons, 1985; Nazareno *et al*., 2017).

2.7. Breeding to disease resistance

The concept of genetic resistance in plant breeding was explored in the re-discovery of Mendel's laws in 1900. During this period, in England, it was discovered that *Puccinia striiformi f.sp. tritici* resistance to wheat yellow rust was caused by a single recessive gene. This discovery allowed several breeding programs to initiate selection for resistance in the early 20th century. However, this same discovery created the expectation that developing new resistant varieties could provide permanent control of plant diseases (Pink, 2002). At this time, many genes that confer resistance to pathogens have been identified and introduced into cultivated species via crosses (Kover and Caicedo, 2001).

In 1919, McRostie identified the first gene for resistance to anthracnose (a foliar disease in the common bean) and demonstrated that different genes were responsible for resistance to different pathogenic variants, known as pathotypes. However, it was only when Flor (1956) presented the gene-by-gene theory for the flax and flax rust pathosystem (the relationship between a host and a parasitic species) that the relationships between pathogens and hosts began to be clarified. According to the gene–by–gene theory, resistance occurs

when, for each gene that conditions a resistance reaction in the host, there is a complementary gene in the pathogen that conditions virulence (Flor, 1956).

Breeding efforts to develop cultivars with genetic resistance to *P. coronata* created two significant cultivars: ‘Victoria’ and ‘Bond’. These cultivars were the only source of resistance to *Pca* used in Australia earlier to 1960 (Upadhyaya and Baker 1960). However, the development of virulence for both cultivars, which caused substantial losses in the southern USA, led to the search for new resistance to *P. coronata* (Coffman *et al.*, 1961).

Crown rust is common in the natural populations of *A. sterilis*, an ancestor of *A. sativa*. As a result, resistance genes in *A. sterilis* populations were incorporated into *A. sativa* and widely used in oat breeding (Leonard, 2002). Sources of resistance are also found in diploid species of the genus, such as *A. strigosa*, *A. bruhnsiana*, *A. ventricosa*, *A. longiglumis*, and *A. hirtula*, in tetraploid species, such as *A. magna*, *A. insularis*, *A. murphy*, and *A. macrostachya*, and in hexaploid species, such as *A. occidentalis* and *A. ludoviciana* (Loskutov and Rines, 2011).

According to Park *et al.* (2022), the majority of the 92 designated loci that provide resistance to *P. coronata* are attributed to alien species. Only 25 of these loci originate from hexaploid species *A. sativa* and *A. byzantina*, indicating the significance of other *Avena* species as potential sources of resistance (Supplementary Table 1). *A. sterilis* has been identified as a valuable source of resistance, with around 44 resistance loci already catalogued. One reason for this could be due to its sexual compatibility as a hexaploid, making it easier to transfer this resistance to cultivated oat (Park *et al*., 2022). In the 1980s, 'Fidler' became the first oat cultivar with the resistance gene *Pc*39 derived from *A. sterilis*. Some of these genes are still being utilized alongside other genes (Park *et al.*, 2022) or in regions where virulence towards these genes has not been identified (Sowa and Paczos-Grzęda, 2020).

Oats have two main types of resistance: all stage resistance (ASR) and adult plant resistance (APR). ASR, also called race-specific resistance, can be easily selected due to its high heritability and strong phenotypic effects, resulting in widespread use in breeding programs (Heath, 2000). However, it is not considered durable due to the increased diversity and genetic complexity of the *P. coronata* population (Leonard and Martinelli, 2005), which results in substantial selection pressure exerted on the pathogen population (Wairich, 2016). ASR resistance to oat crown rust can be overcome after a variety have been released or even during breeding. The resistance typically remains effective for only two to four years on average, which requires the continuous release of new oat cultivars.

From another side, APR is characterized by an efficient response to multiple pathogen races (non-race-specific resistance) without the differential host-pathogen interaction typical of ASR and without intense selection pressure on the pathogen. Thus, non-specific race resistance tends to be expressed, generating few symptoms throughout the crop cycle (Marshall and Shaner, 1992). The selection of genotypes with APR to oat crown rust involves the quantification of resistance component traits as lower frequency of infection, longer latency period, and smaller size of uredia (Díaz-Lago *et al.*, 2003), reduced spore production, early appearance of telia, a decreased ability to produce lesions, and incidence of necrosis or chlorosis surrounding the pustules (Chaves *et al*., 2004).

2.8. Genetic resistance

Success in developing a resistant crop to a particular pathogen depends on knowledge of the genetics of host resistance (Kebede *et al*., 2019). On one hand, when one or more large effect genes can be easily identified through their phenotypic effect, they are called major genes. On the other hand, smaller genes individually have only a small impact on the expression of resistance creating a phenotypic variation (Russell, 2013). The greater the distribution of variation between genotypes, the greater the number of genes involved in the control of resistance (Camargo, 1995).

ASR is usually controlled by a single gene that encodes nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins (Dodds *et al*., 2006). Most of this gene confers a hypersensitive reaction phenotype (Klos *et al*., 2017) upon infection by specific races of the pathogen, resulting from the recognition of the pathogenic avirulence protein (Avr) by the corresponding plant-encoded R protein (Dodds and Rathjen, 2010). It is represented by a qualitative scale in infection types (IT), ranging from 0-4, according to Murphy (1961) (See Figure 4).

APR is considered a general form of resistance (Parlevliet, 1981) and in general is multigenic (Klos *et al*., 2017) and thus better represented on a quantitative scale, although a handful of major loci may control it (Fu *et al.*, 2009; Krattinger *et al*., 2009; Lin *et al.*, 2014; Moore *et al*., 2015). Some examples of APR genes in wheat include *Lr34,* which simultaneously provides resistance to leaf rust, stripe rust, and powdery mildew (Krattinger *et al.,* 2009); *Yr36,* which confers resistance to stripe rust (Fu *et al.*, 2009; Gou *et al.*, 2015); and *Lr67* gene that causes leaf tip necrosis and limits colonization of biotrophic pathogens (Moore *et al.*, 2015).

2.8.1. The *Pc* genes

Almost a hundred genes confer complete resistance to crown rust (Supplementary Table 1). The genes for resistance to *P. coronata* in *Avena* are designated by '*Pc*,' indicated by a number (*Pc1* to *Pc96* and *PcX*), with each number followed in most cases by a unique locus denoted by a lowercase letter. Many of these genes originated in other species of the genus, such as *A. sterilis* (*Pc38, Pc39, Pc48,* and *Pc68*), *A. strigosa* (*Pc94*), and *A. magna* (*Pc91*) (Gnanesh *et al.,* 2015).

Six loci have been identified as providing APR (*Pc27, Pc28, Pc69, Pc72*, *Pc73*, *Pc74*), while the remaining 86 loci provide ASR. Additionally, five loci have been found to have multiple alleles: *Pc2* (*Pc2*, *Pc2b*), *Pc3* (*Pc3*, *Pc3c*), *Pc4* (*Pc4*, *Pc4c*), *Pc6* (*Pc6*, *Pc6c*, *Pc6d*), and *Pc9* (*Pc9* and *Pc9c*) (Park *et al.,* 2022). Also was identified four pairs of complementary genes (when both are required for resistance expression): *Pc3 + Pc4*, *Pc3c + Pc4c*, *Pc7 + Pc8*, and *Pc24 + Pc25*.

Most alleles of *Pc* loci exhibit dominance, but some have been observed to be partially dominant, partially recessive, or recessive (Marshall and Shaner, 1992). An example of dominance is the gene *Pc68* (Wong *et al.,* 1983). Incomplete dominance is demonstrated by the genes *Pc56, Pc64, Pc65,* and *Pc66,* while *Pc54* and *Pc55* are inherited in a recessive manner (Park *et al.,* 2022). The action of some genes may be influenced by genetic background as *Pc45* (Harder *et al*., 1980). Resistance due to complementary gene pairs were identified in cultivars 'Bond' (*Pc3* and *Pc*4) (Baker and Upadhyaya, 1966), 'Santa Fe' (*Pc7* and *Pc8),* (Osler and Hayes, 1953), 'Garry' (*Pc*24 and *Pc*25) (Upadhyaya and Baker, 1960) and 'Ukraine' (*Pc3c* and *Pc4c*) (Upadhyaya and Baker, 1960).

To identify and effectively utilize cataloged *Pc* resistance genes, it is crucial to have a differential collection consisting of single gene reference stocks for every locus and pure isolate of *P. coronata* (Supplementar Table 2). In the differential collection each oat genotype contains one or more *Pc* genes in different genotypic backgrounds and is used to determine crown rust isolates pathogenicity based on compatible (virulent) or incompatible (avirulent) interactions. The response of each differential genotype to an isolate is recorded using a 0–4 infection type (IT) scale (Figure 4). The number of *Pc* genes in the differential sets used to identify pathotypes (races) in surveys varies among countries. It has been updated over time in response to identifying newly acquired pathogen virulence (Chong *et al.,* 2000).

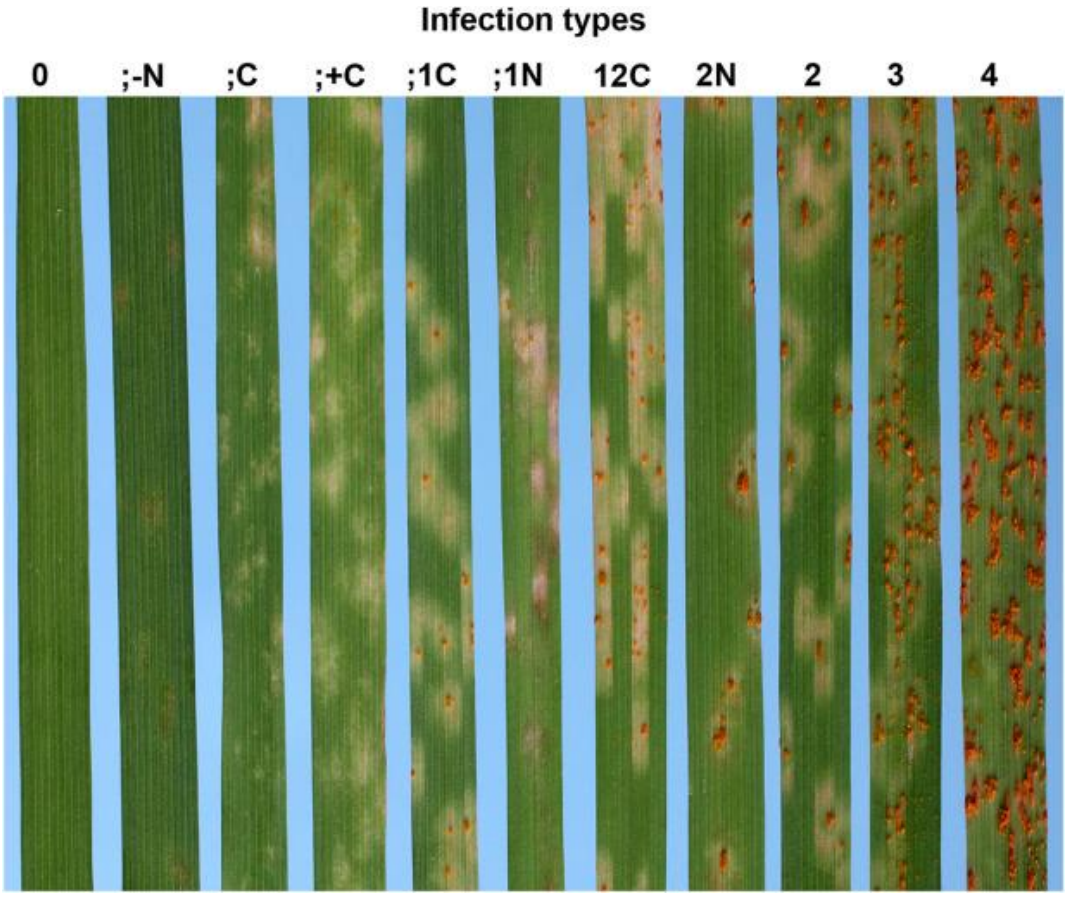


FIGURE 4. Examples of infection types (ITs) used to score ASR and to conduct physiological race assignments of *Puccinia coronate* f. sp. *Avenae*. Incompatible reactions include the following: 0, no urediniospores; fleck (;), presence of flecks; 1, few small pustules; 2, small pustules, presence of green islands; all of these may be accompanied by necrosis (N) and/or chlorosis (C). Compatible reactions include the following: 3, large pustules surrounded by chlorotic halos; 4, large pustules, often coalescing (CHONG *et al.*, 2000).

2.8.2. Crown rust resistance QTLs

A handful of quantitative trait loci (QTL) defined as genomic regions containing one or more genes presenting a quantitative variation characteristic, were identified, and validated for crown rust resistance. Acevedo *et al.* (2010) found that crown rust resistance in the 'MN841801-1' genotype is attributed to eight QTLs, being four of them consistent in all test environments (*Prq1a*, *Prq2*, *Prq7*, and *Prq8*) in the F_6 'MN841801-1' x 'Noble-2' population. This oat inbred line has been widely studied as it has shown durable resistance for over 35 years (Lin *et al.* 2014). In a study of partial resistance of this same oat line, Chong *et al*. (2000) concluded that it carries two adult plant resistance genes (APR) with additive effects (Lin *et al*. 2014). In another study, Lin *et al.* (2014) detected a QTL (Q*Pc*.crc-14D) highly associated with partial resistance to crown rust in oat chromosome 14D. This QTL has consistent effects in different environments (Lin *et al*., 2014).

In a population from the cross UFRGS 7 (susceptible) x UFRGS 910906 (partially resistant), Barbosa (2002) identified another eight QTLs in the F_2 generation and three QTLs in the F_6. Aside from those previously reported, four QTLs were mapped to specific oat chromosomes, with three QTLs (QCr.cdl9-12D, QCr.cdl9-19A, and QCr.cdl9lsu9-19A) coming from the oat cultivar CDC Boyer and one (QCr.cdl11 -13A) from the 94197A1-9-2 2-2-5 oat line (Babiker *et al*., 2015). In addition, these authors validated that the QTL of the studied line 94197A1-9-2-2-2-5 is significant in two different genetic backgrounds (Babiker *et al*., 2015).

Some detected QTLs are in genomic regions corresponding to areas known to contain resistance genes. For example, the QTLs identified by KLOS *et al.* (2017) are in regions linked to the ASR resistance genes *Pc48, Pc58a, Pc68, Pc71, Pc91,* and *PcKM*. In addition to this, a QTL was observed in the Mrg03, Mrg08, and Mrg23 linkage groups that had not previously been associated with crown rust resistance. More recent studies, such as that conducted by Sunstrum *et al.* (2019), found four QTLs. Two of these QTLs were attributed to known locations, while the remaining two were detected in new locations and thus may represent new crown rust resistance genes.

In a comparative analysis using SNPs linked to Q*Pc*.crc-14D, LIN *et al.* (2014) identified synergy between the chromosomes of wheat group 2, presenting ortholog to the *Yr16* rust resistance gene APR. The authors concluded that this QTL might function similarly to known leaf rust resistance genes from durable adult plants in wheat, such as *Lr3*4, *Lr46,* and *Lr67* (Hiebert *et al.*, 2010; Lin *et al.*, 2014).

2.9. Deployment of resistance genes

Although the use of resistant varieties is the most sustainable disease management to control plant disease, the genetic resistance to *Pc*a is deployed by this pathogen's rapid evolution. The need of deployment of new resistance genes occurs due to the great ability of the pathogen to genetically adapt to the host, which results in the evolution of new virulent races (Pink, 2002; Chaves and Martinelli, 2005). Nearly 400 physiological races of *Pc*a have already been identified worldwide (Harder and Haber, 1992), demonstrating the pathogenic variability of the pathogen.

The resistance of cultivars to crown rust has generally been shown to be temporary. Carson (2011) states that resistance to a newly released resistance gene in improved genotypes is limited to two to five years after release, which is a very short time compared to the development time of a cultivar by traditional methods. Kulcheski (2010) concluded

that under conditions in southern Brazil, two consecutive years are sufficient for an increase in the population of virulent races of *P. coronata* and the consequent overcome of the recently deployed resistance. This occurs due to the great diversity of the *P. coronata* population, high virulence complexity of its single races, and the intense selection pressure exerted on this pathogen population (Leonard and Martinelli, 2005).

In the 1980s, Fidler was the first oat cultivar developed with the resistance gene *Pc39*, derived from *A. sterilis*. Since then, several other genes have been used. However, a study conducted by Klos *et al.* (2017) found that 19 *Pc* genes are no longer effective in North America. This number can be greater, unfortunately, no studies are available about this kind of data for most counties. Among the genes that were overcome by virulent pathogen races in the USA are: *Pc14*, *Pc35*, *Pc36*, *Pc38*, *Pc39*, *Pc40*, *Pc51*, *Pc55*, *Pc57*, *Pc59*, *Pc60*, *Pc61*, *Pc70* and *Pc71*. The presence of virulent races was also observed only in specific oat varieties growing in the northern (*Pc46*, *Pc48*, *Pc52*, and *Pc63*) and the southern regions (*Pc67*) of the USA (Klos *et al*., 2017).

The ability of *P. coronata* to generate new races challenges breeders to find new and effective resistance genes that remain effective for a long period. Johnson (1984) defined durable resistance as 'resistance that has remained effective in a cultivar during its widespread cultivation for a long sequence of generations or a period, in an environment favorable to the disease'. This is the case of the MN 841801-1 oat line, which showed durable resistance for over 35 years (Lin *et al.,* 2014). In addition, MN841801, developed by the University of Minnesota, has exhibited a resistant phenotype over 30 years of testing under high disease pressure (Leonard, 2002). Acevedo *et al.* (2010) found that crown rust resistance in the 'MN841801-1' genotype is attributed to in the F_6 'MN841801-1' x 'Noble-2' population. The resistance in MN841801 is controlled by eight QTLs (Acevedo *et al.*, 2010). Recently, two lines derived from diploid *A. strigosa*, MNBT1020-1, and MNBT1021-1, were identified as valuable sources of APR to crown rust after over eight years of testing (Rines *et al.*, 2018).

While ASR has limited success, there have been cases where a single gene, such as the Lr34 gene in wheat for leaf rust resistance and the *mlo* mildew resistance gene in barley, that conditioned durable resistance. These genes have been effective for over 30 years in North America, South America, and Australia since the late 1970s.

One effective strategy for prolonging the effectiveness of genetic resistance against crown rust is to utilize quantitative resistance or a combination of ASR and APR in the same

genotype. Incorporating multiple loci that provide resistance can make it more challenging for the pathogen to overcome the resistance, thus increasing its durability.

2.10. Advances in oat genomics

A. sativa has a large genome (12.5 Gb) comprising 21 chromosome pairs and is a hexaploid species (genome AACCDD) (see section 2.2). This genome consists of several gene duplications and several translocation events, with high repetitive DNA sequences (Peng *et al*., 2022).

Homoeologous regions among oat chromosomes and matches with orthologous regions in the rice (*Oryza sativa*) genome have revealed that the hexaploid oat genome is highly rearranged compared to rice genome, a diploid species closer to the ancestral species of all grass species. The oat genome suffered frequent translocation events among chromosomes and other subgenome rearrangements (Chaffin *et al.* 2016). Thus, the high complexity of the oat genome has limited studies making difficult the development of linkage maps and the assignment of loci to chromosomes, as well as its sequencing, until very recently.

2.10.1. Genotyping

Since most plant and animal genomes are too large and economically impracticable, methods have been developed to select and sequence small reproducible fractions of genomic DNA (Bekele *et al.,* 2020). The advent of next-generation sequencing (NGS) has greatly facilitated the development of several methods that enable the simultaneous discovery and genotyping of thousands of single nucleotide polymorphism (SNP) markers, even in species for which little or no genetic information available (Torkamaneh *et al.*, 2016).

The most common methods for genotyping plant species are based on restriction enzymes cuts at specific genome sites. These tools include ‘restriction site associated DNA, ‘genotyping-by-sequencing – GBS,’ ‘sequence-based genotyping – SGB,’ and ‘diversity arrays technology sequencing - DArT-seq’ (Bekele *et al.*, 2020).

From all available technologies, GBS is the most widely employed. However, despite its effectiveness, GBS encounters challenges with missing data due to shallow and/or non-uniform sequence depth of fragments in the reduced representation. This issue can be addressed partially by utilizing data imputation, deeper sequencing, or restriction enzymes

that cut less frequently the genome. However, these solutions can often be costly or insufficient. (Bekele *et al*., 2020).

DArTseq is a platform that combines the next-generation sequencing (NGS) method with the existing DArT marker platform, producing a rapid SNP discovery method (Kilian *et al.,* 2012). The DArTseq platform, developed by Diversity Arrays Technology (https://www.diversityarrays.com), is a variant of genotyping-by-sequencing (GBS) that implements complexity reduction methods that effectively target low-copy sequences of the genome (Melville *et al.,* 2017). Furthermore, this process is optimized for each organism and application by testing different combinations of restriction enzymes (REs) and selecting the combination that is most effective in reducing genome complexity (Nguyen *et al*., 2018). This platform has traditionally been utilised in a lot of species, such as rice (*Oryza sativa*) (Courtois *et al*., 2013), barley (*Hordeum vulgare*) (Dracatos *et al*,. 2014) and maize (*Zea mays*) (Dos Santos *et al.,* 2016).

2.10.2. Genetic linkage mapping

Understanding the genetic background of phenotypic traits and switching selection from phenotype to genotype can be achieved with the help of genetic maps. Moreover, these maps enable identifying and cloning specific gene responsible for a target trait. The initial molecular maps of oat were obtained at the beginning of the 1990s. They were constructed in crosses among closely related diploid species, *A. atlantica* × *A. hirtula* (O'donoughue *et al.,* 1995) and *A. strigosa* × *A. wiestii* (Rayapati *et al.,* 1994). Instead of using cultivated hexaploid oat, genetic maps were constructed using wild diploid species. This approach simplified the identification of alleles of homologous loci and allowed for the analysis of a lower number of linkage groups. Both maps were based on RFLP markers. In the 2000s, two maps based on an *A. strigosa* × *A. wiestii* cross were constructed (Kremer *et al.,* 2001; Yu and Wise, 2000).

In the meantime, the first map of hexaploid oats was constructed based on a population of recombinant inbred lines (RILS) of two genetically very distant cultivars (*A. byzantina* cv. Kanota × *A. sativa* cv. Ogle) (O'donoughue *et al.,* 1995). This map originally consisted of 561 loci primarily based on RFLPs but has since been enriched with other marker types. Over time, numerous genetic maps of oat have been developed, but the Kanota × Ogle map remains the higher-density molecular map of oats, making it a popular choice. Several genes have been effectively located on this map (Rines *et al.,* 2006).

Despite these advances, the first physically anchored consensus map of hexaploid oat was developed only in 2013 by Oliver *et al.* (2013). It was based on 985 SNPs assayed in 390 recombinant inbred lines (RILs) derived from six hexaploid oat biparental populations, and determination of consensus linkage groups was possible through SNP deletion analysis in a set of monosomic hybrid stocks, representing 18 of the 21 oat chromosomes (Oliver *et al.*, 2013). This map was a substantial improvement over previous maps because of its low error rates. Further improvements were made by integrating high-density SNPs discovered via genotype-by-sequencing (GBS) (Tinker *et al*., 2014).

To create a comprehensive linkage map that accurately reflects most oat cultivars, Chaffin *et al.* (2016) saturated the map with additional populations and genetic markers, correcting errors in the previous map. Further revisions were performed by Yan *et al.* (2016) with high-density markers. Further genome analysis of 27 oat species identified the ancestral origins of 21 mapped chromosomes in hexaploid oat and thus deduced the likely pathway from which hexaploid oat originated by sequential polyploidization events (Yan *et al*., 2016).

Additionally, in *de novo* GBS analysis of 4,657 accessions of cultivated oat, Bekele *et al.* (2018) discovered 164,741 tag-level genetic variants comprising 241,224 SNPs that were used to expand the marker density of the existing oat consensus map by the addition of more than 70,000 GBS loci (Tinker *et al.,* 2016). Latta *et al.* (2019) utilized the high-density marker system to create the initial oat haplotype map through GBS marker analysis. This system enhanced the diploid maps as the 'A' genome (*A. strigosa* x *A. wiestii*, 2n = 14) and the tetraploid 'AB' genome (*A. barbata* 2n = 28).

These maps were utilized due the absence of a reference genome for the hexaploid oat species. This led to obstacles to assembly the position genes on a chromosome. Recently with the release of the first oat genome sequence opened new horizons to search and map crown rust resistance genes (see sections below).

2.10.3. Mapping genes

About 98 alleles located at 92 loci provide resistance to *P. coronata* in *A. sativa*, (Supplementary Table 1). However, only a few of these 92 loci have been mapped to a chromosome, and the allelic relationships of many remain unknown. Some studies that identified these regions could only assign the genes to linkage groups that did not indicate their physical location in the oat genome (Gnanesh *et al.,* 2015). So far, researchers have successfully mapped and designated 78 distinct loci that provide resistance to *P. triticina* in

wheat, highlighting the importance of conducting further mapping studies in oats (Park *et al*., 2022).

Among the genes already described are *Pc*38 (Wight *et al.*, 2005), *Pc39* (Zhao *et al.*, 2020; Sowa and Paczos-Grzeda, 2020; Mcnish *et al.*, 2020), *PcKM* / *Pc45* (Gnanesh *et al.*, 2015), *Pc48* (Wight *et al.*, 2005; Klos *et al.*, 2017; Mcnish *et al.*, 2020), *Pc53* (Admassu-Yimer *et al.*, 2018), *Pc54* (Admassu-Yimer *et al.*, 2022), *Pc58* (Hoffman *et al*., 2006; Klos *et al.*, 2017), *Pc68* (Chen *et al*., 2006; Klos *et al.*, 2017), *Pc71* (Bush and Wise, 1998; Klos *et al.*, 2017), *Pc91* (Gnanesh *et al.*, 2015; Klos *et al.*, 2017), *Pc92* (Rooney *et al.*, 1994), *Pc94* (Chong *et al.*, 2004; Chen *et al*., 2007), and *Pc98* (Zhao *et al.*, 2020).

The presence of linked genes or multiallelism has already been described for several the *Pc* genes in oat, reinforcing the importance of studying their physical location. Given the overlap in map locations and variation in resistance assessment methods, some QTLs may be the same or alleles of the same *Pc* genes (KLOS *et al.*, 2017). In an analysis by Gnanesh *et al.* (2015), the *PcKM* gene was identified. More recent evidence, in the form of resistance reactions to pure isolates of *P. coronata* characterized in a set of differential lineages, in addition to molecular marker data (Kebede *et al.*, 2019), pointed out that *PcKM* is the same gene as *Pc45* (Admassu-Yimer *et al.*, 2018). An example of two *Pc* genes being identified as located in a single or two separately loci, depending on the genetic background, are *Pc*54 and *Pc*35. A study by Martens *et al.* (1981) suggested that *Pc*54 and *Pc*35 could be alleles of the same gene or be tightly linked. However , Leonard *et al*. (2005) identified *Pc35* and *Pc54* in different linkage positions, possibly due to translocations, not uncommon in oat. Satheeskumar *et al.* (2011) described that the *Pc68* gene was linked to other crown and stem rust resistance genes, such as the *Pg3*, *Pg9*, *Pc44*, *Pc46*, *Pc50*, *Pc95*, and *PcX* genes. The *Pc53* gene is in the same linkage group as *Pc*45 (Gnanesh *et al.*, 2015; Klos *et al.*, 2017), forming a complex of genes in this region (Admassu-Yimer *et al.*, 2018). Moreover, Yu *et al.* (2001) reported the presence of a locus controlled by the genes *Pc81, Pc82, Pc83, Pc84*, and *Pc*85, forming a linkage block. Other studies have identified QTLs in linkage groups (Montilla-Bascón *et al*. 2015; Winkler *et al*., 2016).

Over time, as various methods of mapping populations were developed. The first-generation mapping population is created by crossing two contrasting parents and includes F_2, $F_{2:3}$, backcross populations (BC_1, BC_2), advanced backcross populations, NILs (Near Isogenic Lines), RILs (Recombinant Inbred Lines), DH population (Double Haploids), and CSSLs (Chromosome Segment Substitution Lines). These populations have been widely used for identifying and mapping qualitative traits, such as disease resistance (Gireesh *et al.*,

2021). Another strategy is BSA (Bulk segregation analysis) which uses a strategy of pooling individuals with extreme phenotypes (tails) to conduct economical and rapidly linked marker screening or quantitative trait locus (QTL) mapping (Li and Xu, 2022).

Thus, to overcome the shortfalls of first-generation mapping populations, researchers utilized natural populations and mutant populations as second-generation mapping populations for mapping complex traits. Association mapping, which utilizes panels containing a diverse set of genotypes with phenotypic and genetic diversity for target traits, are adopted for establishing marker-trait association using high-density genotyping and multi-environment phenotyping data (GWAS). It takes advantage of historical recombination that occurred during evolution, enabling higher precision and resolution for mapping complex traits (Mackay and Powell, 2007).

The third generation of mapping is based in multiparent populations as four-way crosses, diallel cross populations, multiparent advanced generation inter-cross (MAGIC) populations, and nested association mapping (NAM) populations (Gireesh *et al*., 2021). Between these strategies, NAM approach has been used to map important QTLs in various plant species such as maize (McMullen *et al*., 2009), *Arabidopsis thaliana* (Buckler *et al*., 2007), wheat (Bajgain *et al*., 2016), soybean (Song *et al*., 2017), and sorghum (Bouchet *et al*., 2017).

NAM combines the strengths of linkage mapping (biparental mapping) and linkage disequilibrium mapping (association mapping), resulting in improved accuracy and resolution of the mapped loci. It is a mating design that uses a shared parent, referred to as the "founder", across multiple crosses. The shared parent ensures a standardized genetic background, facilitating the identification of segregating alleles in different populations based on the presence of parent-specific alleles (Blanc *et al*., 2006; Yu *et al*., 2008). The NAM is primarily designed to address some of the inherent limitations of linkage analysis and GWAS. It has demonstrated its ability to detect quantitative trait loci (QTLs) of different sizes, including rare alleles, higher power of QTL detection than GWAS under high and low heritability, small population size and low effects of QTLs. due to its higher statistical power (Guo *et al*., 2010).

The scarcity of data regarding the chromosomal positioning of *Pc* genes and molecular markers associated with them has hindered the use of genomic techniques in oat breeding for crown rust. Therefore, the mapping of oat crown rust resistance genes will enhance our knowledge of oat genomics and enable scientists to utilize molecular tools in the creation of new oat cultivars with crown rust resistance (Admassu-Yimer *et al.,* 2018).

2.10.4. Genome sequencing

The availability of the genome database of rice and other species of *Poaceae* allowed several studies based on comparing the homology of reference genomes with the oat genome. A study carried out by Oliver *et al.* (2013), revealed 15 candidate genes for crown rust resistance based on the homology between rice chromosome 12D and rice chromosome 4 and between oat chromosome 13A and rice chromosome 6. These genes belong to the NBS-LRR, RPP, and RPM1 classes (Oliver *et al.*, 2013).

In partnership with several public and private research organizations, PepsiCo recently publicized the first *A. sativa* genome assembly v1 for use in open-source applications. An advanced version (v2) of the OT3098 assembly has also been released (https:// wheat. pw. usda.gov/GG3/graingenes_downloads/oat-ot3098-PepsiCo), incorporating gap filling as well as reorientation/flipping of specific chromosomes. When the entire chromosome is inverted or flipped, the positions of some of the chromosomes changed (Park *et al.*, 2022).

Earlier research faced obstacles due to the absence of a reference genome for the hexaploid oat species, which delayed the identification of gene position, function and sequence. It is now possible to determine the sequences of genomic regions associated with desirable traits on oats, thanks to the availability of the oat genome. It will open new horizons in prompting genetic improvement of traits of interest on oats, including high throughput mapping and cloning of crown rust resistance genes.

2.11. Phenotyping

While on controlled environment and on ASR resistance normally the scale is qualitative (Figure 4), the APR is evaluated on the field in a quantitative scale. The evolution of crown rust on the leaf surface changes each day and is destroyed at the end of the season (Figure 3B). In addition, the visual evaluation process is susceptible to human error and subjectivity. It is challenging for a plant breeder to maintain focus while working long hours in the field. The limitations of traditional phenotyping methods have made the development of new phenotyping technologies a research objective for many plant diseases. A common strategy is to collect photos of the plant tissues with the disease and measure the signs and symptoms using a methodology similar to human observation (Nilsson 1995). Using photo estimates by drones and with use of artificial intelligence have been applied to measure diseases (Mcnish, amd Smith, 2022; *Heineck et al.*, 2019). According to Mcnish, and Smith, (2022)

the image method to measure Crown rust in oats was greatest for adult plants before senescing. Its system UAV phenotyping methods used in their study, showed possibility of minimizing the effect of variable field conditions and multiple raters on measurement accuracy.

2.12. References

ACEVEDO, M. *et al.* Identification and validation of quantitative trait loci for partial resistance to crown rust in oat. **Phytopathology,** St. Paul, MN, v. 100, n. 5, p. 511–521, 2010.

ADMASSU-YIMER, B. *et al.* Mapping of crown rust *(Puccinia coronata* f. sp. *avenae*) resistance Gene *Pc54* and a novel quantitative trait locus effective against powdery mildew (*Blumeria graminis* f. sp. *avenae*) in the Oat (*Avena sativa*) Line Pc54. **Phytopathology**, St. Paul, MN, v. 25, PHYTO10210445R, 2022.

ADMASSU-YIMER, B; BONMAN, J. M.; KLOS, K. E. Mapping of crown rust resistance gene *Pc53* in oat (*Avena sativa*). **PLoS ONE**, San Francisco, v. 13, n. 12, 2018.

AGRIOS, G. N. Plant diseases caused by fungi. *In:* AGRIOS, G. N. **Plant Pathology**. 5. ed. San Diego: Elsevier, 2005. p. 385–614.

ANDERSSON, A.; BÖRJESDOTTER, D. Effects of environment and variety on content and molecular weight of β-glucan in oats. **Journal of Cereal Science**, Manitoba, v. 54, n. 1, p. 122-128, 2011.

AVENA SATIVA – OT3098 v2, PepsiCo. [Banco de dados] [2022]. Disponível em: https:// wheat. pw. usda.gov/jb?data=/ggds/oat-ot3098v2-pepsico. Acesso em: 20 sep. 2022.

BABIKER, E. M. Quantitative trait loci from two genotypes of oat (*Avena sativa*) conditioning resistance to *Puccinia coronata*. **Phytopathology**, St. Paul, MN, v. 105, n. 2, p. 239-245, 2015.

BAJGAIN P. *et al*. Nested association mapping of stem rust resistance in wheat using genotyping by sequencing. **PLoS ONE**, San Francisco, v.11, n.5, e0155760, 2016.

BAKER E.P.; UPADHYAYA, Y.M. Studies on the inheritance of rust resistance in oats. IV. Expression of allele morphism and gene interaction for crown rust resistance in crosses involving the oat variety Bond. **Proceedings of the Linnean Society of New South Wales**, Sydney, v. 91, p. 155–175, 1966.

BEKELE, W. A. Haplotype-based genotyping-by-sequencing in oat genome research. **Plant Biotechnology Journal**, Lancaster, v. 16, n.8, p. 1452–1463, 2018.

BEKELE, W.A. *et al*. A targeted genotyping-by-sequencing tool (rapture) for genomics-assisted breeding in oat. **Theoretical and Applied Genetics**, New York, v. 133, n. 2, p. 653-664, 2020.

BOUCHET, S. *et al*. Increased power to dissect adaptive traits in global sorghum diversity using a nested association mapping population. **Genetics,** Oxford, v.206, n.2, p. 573–585, 2017.

BUCKLER, E. *et al*. An Arabidopsis haplotype map takes root. **Nature Genetics**, London, v. 39, n. 9, p. 1056-1057, 2007.

BUSH, A. L.; WISE, R. P. High-resolution mapping adjacent to the *Pc71* crown-rust resistance locus in hexaploid oat. **Molecular Breeding**, New York, v. 4, n. 1, p. 13–21, 1998.

CAMARGO, L. E. A. Análise genética da resistência e da patogenicidade. *In:* BERGAMIN-FILHO, A.; KIMATI, H.; AMORIN, L. (ed.). **Manual de Fitopatologia**. 3 ed. São Paulo: Agronômica Ceres, 1995. v.1, p. 470-492.

CARSON, M. L. Virulence in oat crown rust (*Puccinia coronata* f. sp. *avenae*) in the United States from 2006 through 2009. **Plant Disease,** St. Paul, v. 95, n. 12, p. 1528–1534, 2011.

CDL - CEREAL DISEASE LABORATORY. **Resistance Genes:** Oat. *Pc* (Crown Rust). [2022]. Disponível em: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/resistance-genes/resistance-genes/. Acesso em: 16 set. 2022.

CHAFFIN, A. S. *et al.* A consensus map in cultivated hexaploid oat reveals conserved grass synteny with substantial subgenome rearrangement. **The Plant Genome**, Madison, v. 9, n. 2, plantgenome2015.10.0102, 2016.

CHAVES M S; MARTINELLI, J.A. Ferrugem da folha da aveia: aspectos epidemiológicos e perspectivas de controle através da resistência genética na região sul do Brasil. **Revista Brasileira de Agrociência**, Pelotas, v. 11, n. 4, p.397-403, 2005.

CHAVES, M. S. *et al.* Efeito da ferrugem da folha sobre o rendimento e qualidade de grãos em genótipos elite de aveia. *In*: REUNIÃO DA COMISSÃO BRASILEIRA DE PESQUISA DE AVEIA, 22., 2002, Passo Fundo. **Resultados Experimentais...** Passo Fundo: UPF, 2002. p. 463-470.

CHAVES, M. S.; MARTINELLI, J. A.; FEDERIZZI, L. C. Resistência quantitativa à ferrugem da folha em genótipos de aveia branca: I - Caracterização da reação em condições de campo. **Fitopatologia Brasileira**, Brasília, v. 29, n. 1, p. 35–42, 2004.

CHEN, G. *et al.* Identification of single-nucleotide polymorphisms linked to resistance gene *Pc68* to crown rust in cultivated oat. **Canadian Journal of Plant Pathology**, Ottawa, v. 28, n. 2, p. 214–222, 2006.

CHONG J, *et. al.* Development of sequence-characterized amplified region (SCAR) markers for resistance gene *Pc94* to crown rust in oat. **Canadian Journal of Plant Pathology**, Ottawa, v. 26, p. 89–96, 2004.

CHONG, J., *et al.* Virulence of oat crown rust [*Puccinia coronata* f. sp. *avenae*] in Canada during 2002–2006. **Canadian Journal of Plant Pathology**, Ottawa, v. 30, p. 115– 123, 2008.

CHONG, J.; LEONARD, K. J.; SALMERON, J. J. A north American system of nomenclature for *Puccinia coronata* f. sp. *avenae*. **Plant Disease**, St. Paul, v. 84, n. 5, p. 580–585, 2000.

COFFMAN, F.A; MURPHY, H.C.; CHAPMAN, W.H. Oat breeding. *In*: COFFMAN, F. A. (ed.). **Oat and oat improvement**. Madison, Wisconsin USA: The American Society of Agronomy, 1961. p. 263–329. (Agronomy Monographs)

COURTOIS, B. *et al*. Genome-wide association mapping of root traits in a japonica rice panel. **PloS One**, San Francisco, v. 8, n. 11, p. e78037, 2013.

DÍAZ-LAGO, J. E.; STUTHMAN, D. D.; LEONARD, K. J. Evaluation of components of partial resistance to oat crown rust using digital image analysis. **Plant Disease**, St. Paul, v. 87, n. 6, p. 667–674, 2003.

DODDS, P. N. *et al*. Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. **Proceedings of the National Academy of Sciences**, Washington, v. 103, n. 23, p. 8888-8893, 2006.

DODDS, P. N.; RATHJEN, J.P. Plant immunity: towards an integrated view of plant–pathogen interactions. **Nature Genetics**, London, v. 11, p. 539-548, 2010.

D'OLIVEIRA, B. A estação agronômica e os problemas nacionais de fitopatologia. **Revista Agronomia**, São Paulo, v. 30, p. 414–438, 1942.

DOS SANTOS, J. P. R. *et al*. Genomic selection to resistance to *Stenocarpella maydis* in maize lines using DArTseq markers. **BMC genetics**, London, v. 17, n. 1, p. 1-10, 2016.

DRACATOS, P. M. *et al*. Genetic mapping of a new race specific resistance allele effective to Puccinia hordei at the Rph9/Rph12locus on chromosome 5HL in barley. **BMC plant biology**, London, v. 14, n. 1, p. 1-12, 2014.

FAOSTAT. **The FAO's Crops and Livestock Products**. Rome, Italy, 2022. Disponível em: http://www.fao.org/faostat/en/#data/QCL>. Acesso em: 6 set. 2022.

FLOR, H. H. The complementary genic systems in flax and flax rust. **Advances in Genetics**, New York, v. 8, p. 29-54, 1956.

FORCELINI, C. A.; REIS, E. M. Doenças da aveia. *In*: KIMATI, H. *et al*. (Ed.). **Manual de fitopatologia**. 3ª ed. São Paulo: Agrônomica Ceres, 1997. p. 105-111.

FU, D. *et al*. A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. **Science**, Washington, v. 323, p. 1357–1360, 2009.

FU, Y. B. Oat evolution revealed in the maternal lineages of 25 *Avena* species. **Scientific Reports**, London, v. 8, n. 1, p. 1–12, 2018.

GIREESH, C. Nested Association Mapping (NAM) Populations: Present status and future prospects in the genomics era. **Critical Reviews in Plant Sciences**, London, v. 40, n.1, p. 49-67, 2021.

GNANESH, B. N. *et al*. Genetic analysis and molecular mapping of a seedling crown rust resistance gene in oat. **Theoretical and Applied Genetics**, New York, v. 128, n. 2, p. 247–258, 2015.

GNANESH, B. N. *et al*. Oat. *In*: PRATAP, A.; KUMAR, J. (ed.). **Alien Gene Transfer in Crop Plants**. New York: Springer-Verlag, 2016. v. 2.

GORASH, A. *et al.* Aspects in oat breeding: nutrition quality, nakedness and disease resistance, challenges and perspectives. **Annals of Applied Biology**, London, v. 171, n. 3, p. 281-302, 2017.

GOU J.Y. *et al.* Wheat stripe rust resistance protein WKS1 reduces the ability of the thylakoid-associated ascorbate peroxidase to detoxify reactive oxygen species. **Plant Cell,** Oxford, v. 27, p. 1755–1770, 2015.

GUO, B. *et al*. Nested association mapping for identification of functional markers. **Genetics**, Oxford, v. 186, n. 1, p. 373-383, 2010.

HAMMAMI, I. *et al*. Natural population of oat crown rust in Tunisia. **Phytopathology Mediterranea**, Berlin, v.49, p. 35– 41, 2010.

HARDER, D. E.; HABER, S. Oat diseases and pathologic techniques. *In*: Marshall H. G.; Sorrells, M.E. (ed.) **Oat Science and Technology**. Madison: American Society of Agronomy and Crop Science, 1992. p. 307-425. (Monograph No. 33 Agronomy Series).

HEATH, M. C. Nonhost resistance and nonspecific plant defences. **Current Opinion in Plant Biology,** London, v. 3, n.4, p. 315–319, 2000.

HEATH, M. C. Signalling between pathogenic rust fungi and resistant or susceptible host plants. **Annals of Botany**, Oxford, v. 80, n.6, p. 713–720, 1997.

HEINECK, G. C., *et al.* 2019. Using R-based image analysis to quantify rusts on perennial ryegrass. **Plant Phenome**, Washington, DC, v.2, p. 1-10, 2019.

HIEBERT, C. W. *et al.* An introgression on wheat chromosome 4DL in RL6077 (Thatcher*6/PI 250413) confers adult plant resistance to stripe rust and leaf rust (*Lr67*). **Theoretical and Applied Genetics**, New York, v. 121, n. 6, p. 1083–1091, 2010.

HOFFMAN, D. L. *et al.* Characterization and mapping of a crown rust resistance gene complex (*Pc58*) in TAM O-301. **Crop Science**, Madison, v. 46, n. 6, p. 2630–2635, 2006.

HU, G.; RIJKENBERG, F. H. J. Scanning electron microscopy of early infection structure formation by *Puccinia recondita* f. sp. *tritici* on and in susceptible and resistant wheat lines. **Mycological Research**, Madison, v. 102, n. 4, p. 391–399, 1998.

JOHNSON, R. A Critical analysis of durable resistance. **Annual Review of Phytopathology**, Fort Collins, v. 22, n. 1, p. 309–330, 1984.

KEBEDE, A. Z. *et al.* Mapping oat crown rust resistance gene *Pc45* confirms association with *PcKM*. **G3: Genes, Genomes, Genetics**, Oxford, v. 9, n. 2, p. 505–511, 2019.

KILIAN, A. *et al*. Diversity arrays technology: a generic genome profiling technology on open platforms. *In*: POMPANON, F.; BONIN, A. (eds.) **Data Production and Analysis in Population Genomics**. Totowa, NJ.: Springer, 2012. p. 67–89. (Methods in (Molecular Biology, v. 888)

KLENOVÁ-JIRÁKOVÁ, H., *et al*. Diversity of oat crown rust (*Puccinia coronata f.sp. avenae*) isolates detected by virulence and AFLP analyses. **Plant Protection Science**, Praga, v. 46, n.3, p. 98–106, 2010.

KLOS, K. E. *et al.* Genome-wide association mapping of crown rust resistance in oat elite germplasm. **The Plant Genome**, Madison, v. 10, n. 2, p.1-13, 2017.

KOSTIC, B. The cereal rusts in the south-eastern part of Yugoslavia in 1958 and 1959. **Robigo**, Belgrado, v. 9, p. 8–12, 1959.

KOVER, P. X.; CAICEDO, A. L. The genetic architecture of disease resistance in plants and the maintenance of recombination by parasites. **Molecular Ecology**, London, v. 10, n. 1, p. 1-16, 2001.

KRATTINGER, S.G. *et al*. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. **Science,** Washington, v. 323, p. 1360–1363, 2009.

KREMER, C. A.; LEE, M.; HOLLAND, J. B. A restriction fragment length polymorphism-based linkage map of a diploid Avena recombinant inbred line population. **Genome**, Ottawa, v. 44, n. 2, p. 192-204, 2001.

KULCHELSKI, F. R. *et al.* Molecular mapping of *Pc68*, a crown rust resistance gene in *Avena sativa*. **Euphytica**, Dordrecht, v.175, n.3, p.423–432, 2010.

LEGGETT, J. M.; THOMAS, H. Oat evolution and cytogenetics. *In*: WELCH, R. W. **The oat crop:** Production and utilization. London: Chapman & Hall, 1995. p. 120-144.

LEONARD, K. J. Oat lines with effective adult plant resistance to crown rust. **Plant Disease**, St. Paul, v. 86, n. 6, p. 593–598, 2002.

LEONARD, K. J.; ANIKSTER, Y.; MANISTERSKI, J. Patterns of virulence in natural populations of *Puccinia coronata* on wild oat in Israel and in agricultural populations on cultivated oat in the United States. **Phytopathology,** St. Paul, MN, v. 94, n. 5, p. 505-514, 2004.

LEONARD, K. J.; MARTINELLI, J. A. Virulence of oat crown rust in Brazil and Uruguay. **Plant Disease**, St. Paul, MN, v. 89, n. 8, p. 802-808, 2005.

LEONARD, K.J.; ANIKSTER, Y.; MANISTERSKI, J. Virulence associations in oat crown rust. **Phytopathology,** St. Paul, MN, v.95, n.1, p. 53–61, 2005.

LI, ZHIQIANG; XU, YUHUI. Bulk segregation analysis in the NGS era: A review of its teenage years. **The Plant Journal**, London, UK, v. 109, n. 6, p. 1355-1374, 2022.

LIN, Y. *et al.* A major quantitative trait locus conferring adult plant partial resistance to crown rust in oat. **BMC Plant Biology**, London, v. 14, n. 1, p. 1–11, 2014.

LOSKUTOV, I. G.; RINES, H. W. Avena. *In*: CHITTARANJAN K. (Editor) **Wild crop relatives:** Genomic and breeding resources: Cereals. London: Springer, 2011. p. 109–183.

LYNGKJAER, M. F. *et al.* The barley *mlo*-gene: An important powdery mildew resistance source. **Agronomie**, London, v.20, p. 745-756, 2000.

MACKAY, I.; POWELL, W. Methods for crop linkage disequilibrium mapping. **Trends in Plant Science**, Amsterdam, v. 12, p.57–63, 2007.

MALVICK, D. K. **Crown rust of oats**. Springfield, [1989]. Disponível em: <http://www.ipm.illinois.edu/diseases/series100/rpd109/>. Acesso em: 15 jan 2020.

MARSHALL, A. *et al.* Crops that feed the world 9. Oats-a cereal crop for human and livestock feed with industrial applications. **Food security**, Amsterdam, v. 5, n. 1, p. 13-33, 2013.

MARSHALL, H. G.; SHANER, G. E. Genetics and inheritance in oat. *In*: MARSHALL, H.G.; SORRELLS, M.E. (Eds). **Oat Science and Technology**. Madison: American Society of Agronomy Inc., Crop Science Society of America Inc., 1992. p. 509-572.

MARTENS, J. W. *et al.* Evidence for complementary gene action conferring resistance to *Puccinia graminis avenae* in *Avena sativa*. **Canadian Journal of Genetics and Cytology**, Ottawa, v. 23, n. 4, p. 591–595, 1981.

MARTINELLI, J. A. Oat diseases and their control. *In:* SUTTIE, J. M.; REYNOLDS, S. G. (Ed.). **Fodder oats: A world review**. Roma: FAO, 2004. p. 197-214

MARTINELLI, J. A.; FEDERIZZI, L. C.; BENNEDETTI, A. C. Yield reductions of oat grains due to crown rust severity. **Summa Phytopathologica**, São Paulo, v. 20, n. 2, p. 116–118, 1994

MAUGHAN, P. J. Genomic insights from the first chromosome-scale assemblies of oat (*Avena* spp.) diploid species. **BMC Biology,** London, v. 17, [art.] 92, p. 1–19, 2019.

MCCARTNEY, C. A. *et al.* Mapping of the oat crown rust resistance gene *Pc91*. **Theoretical and Applied Genetics**, New York, v. 122, n. 2, p. 317–325, 2011.

MCMULLEN, M.D. *et al*. Genetic properties of the maize nested association mapping population. **Science**, Washington, v. 325, n. 5941, p. 737-740, 2009.

MCNISH, I. G, *et al*. Mapping crown rust resistance at multiple time points in elite oat germplasm. **The Plant Genome,** Madison, v. 13, n.1, e20007, 2020.

MCNISH, I. G.; SMITH, K. P. Oat crown rust disease severity estimated at many time points using multispectral aerial photos. **Phytopathology,** St. Paul, MN, v. 112, n. 3, p. 682-690, 2022.

MCROSTIE, G. P. Inheritance of anthracnose resistance as indicated by a cross between a resistant and a susceptible bean. **Phytopathology**, St. Paul, MN, v. 9, p. 141-148, 1919.

MELVILLE, J. *et al*. Identifying hybridization and admixture using SNPs: application of the DArTseq platform in phylogeographic research on vertebrates. **Royal Society open science**, [United Kingdom], v. 4, n. 7, p. 161061, 2017.

MONTILLA-BASCÓN, G. Genome-wide association study for crown rust (*Puccinia coronata f. sp. avenae*) and powdery mildew (*Blumeria graminis f. sp. avenae*) resistance in an oat (*Avena sativa*) collection of commercial varieties and landraces. **Frontiers in Plant Science**, Estocolmo, v. 6, p. 103, 2015.

MOORE J. *et al.* A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. **Nature Genetics**, London, v. 47, p. 1494–1498, 2015.

MOORE-COLYER, R. J. Oats and oat production in history and prehistory. *In*: WELCH, R. W. (Ed.) **The oat crop:** Production and utilization. Chapman & Hall, London, 1995. p. 1-33.

NAZARENO, E. S. *et al. Puccinia coronata* f. sp. *avenae*: a threat to global oat production. **Molecular Plant Pathology**, London, v. 19, n. 5, p. 1047-1060, 2018.

NGUYEN, N. H. *et al*. Genomic prediction using DArT-Seq technology for yellowtail kingfish Seriola lalandi. **BMC genomics**, London, v. 19, p. 1-9, 2018.

NILSSON, H. E. 1995. Remote sensing and image analysis in plant pathology. Annu. Rev. **Phytopathology,** St. Paul, MN, v.33, p. 489-528, 1995.

O'DONOUGHUE, L. S, *et al.* A molecular linkage map of cultivated oat. **Genome,** Ottawa, v.38, p. 368–380, 1995.

OLIVER, R. E. *et al.* SNP discovery and chromosome anchoring provide the first physically anchored hexaploid oat map and reveal synteny with model species. **PLoS ONE,** San Francisco, v. 8, n. 3, p. 1-12, 2013.

OSLER, R.D.; HAYES, H.K. Inheritance studies in oats with reference to the Santa Fe type of crown rust resistance. **Agronomy,** Madison, v. 45, n. 2, p. 49–53, 1953.

PARK, R. F. Breeding cereals for rust resistance in Australia. **Plant Pathology**, Seoul, v. 57, n. 4, p. 591-602, 2008.

PARK, R. F. *et al*. Breeding oat for resistance to the crown rust pathogen *Puccinia coronata* f. sp. *avenae*: achievements and prospects. **Theoretical and Applied Genetics**, New York, v.135, p. 3709-37-34, 2022.

PARK, R. F. *et al*. Rust resistance genotypes and expected rust responses of Australian common wheat, durum wheat and triticale varieties. **Cereal Rust Report**, Sydney, v. 17, 2020.

PARLEVLIET, J. E. Durability of resistance against fungal, bacterial, and viral pathogens; present situation. **Euphytica,** Dordrecht, v. 124, n. 2, p. 147–156, 2002.

PENG, Y. *et al*. Reference genome assemblies reveal the origin and evolution of allohexaploid oat. **Nature Genetics**, London, v. 54, n. 8, p. 1248-1258, 2022.

PINK, D. A. C. Strategies using genes for non-durable disease resistance. **Euphytica**, Dordrecht, v. 124, n. 2, p. 227–236, 2002.

PORTYANKO, V. A. *et al.* Quantitative trait loci for partial resistance to crown rust, *Puccinia coronata*, in cultivated oat, *Avena sativa* L. **Theoretical and Applied Genetics,** New York, v. 111, n. 2, p. 313–324, 2005.

RINES, H.W. Oat. *In*: KOLE, C. (ed.). **Genome Mapping and Molecular Breeding in Plants**. New York, NY: Springer. 2006. v. 1, p. 211-242.

ROONEY, W. L.; RINES, H. W.; PHILLIPS, R. L. Identification of RFLP markers linked to crown rust resistance genes *Pc 91* and *Pc 92* in oat. **Crop Science,** Madison, v. 34, n. 4, p. 940–944, 1994.

RUSSELL, G. E. **Plant breeding for pest and disease resistance:** studies in the agricultural and food sciences. London: Butterworth & Co Ltd., 2013. 485 p.

SANZ, M. J. *et al.* Identification of RFLP and NBS/PK profiling markers for disease resistance loci in genetic maps of oats. **Theoretical and Applied Genetics**, New York, v. 126, n. 1, p. 203–218, 2013.

SATHEESKUMAR, S. *et al.* Genetic association of crown rust resistance gene *Pc68*, storage protein loci, and resistance gene analogues in oats. **Genome**, Ottawa, v. 54, n. 6, p. 484–497, 2011.

SCHOLES, J. D.; ROLFE, S. A. Photosynthesis in localised regions of oat leaves infected with crown rust (*Puccinia coronata*): Quantitative imaging of chlorophyll fluorescence. **Planta,** Berlin, v. 199, n. 4, p. 573–582, 1996.

SHERF, A. F. The 1953 crown and stem rust epidemic of oats in Iowa. **The Proceedings of the Iowa Academy of Science,** Des Moines, v. 61, p. 161– 169, 1954.

SIMONS M.D. **Crown rust of oats and grasses**. Worcester Massachusetts USA: The Heffernan Press Inc., 1970. 47 p. (Monograph Number 5, The American Phytopathological Society)

SIMONS, M. D. Crown rust. *In:* BUSHNELL, W.R.; ROELFS, A.P. (Ed.). **The cereal rust: Volume II: Diseases, distribution, epidemiology, and control.** Orlando: Academic Press, 1985. p. 131-172.

SMULDERS, M. J. M, *et al*. Oats in healthy gluten-free and regular diets: A perspective. **Food Research International**, London, v. 110, p. 3-10, 2018.

SONG, Q. *et al*. Genetic characterization of the soybean nested association mapping population. **The Plant Genome**, Madison, v. 10, n.2, p. 1–14, 2017.

SOWA, S.; PACZOS-GRZEDA, E. Identification of molecular markers for the *Pc39* gene conferring resistance to crown rust in oat. **Theoretical and Applied Genetics,** New York, v. 133, n.4, p. 1081–1094, 2020.

STEER, M. W.; HOLDEN, J. H. W.; GUNNING, B. E. S. *Avena* chloroplasts: Species relationships and the occurrence of stromacentres. **Canadian Journal of Genetics and Cytology**, Ottawa, v. 12, n. 1, p. 21–27, 1970.

STRYCHAR, R.; WEBSTER, F. H.; WOOD, P. J. World oat production, trade, and usage. *In*: **Oats:** chemistry and technology. Cambridge: Academic Press, 2011. p. 77-94.

SUNSTRUM, F. G. *et al.* A genetic linkage map in southern-by-spring oat identifies multiple quantitative trait loci for adaptation and rust resistance. **Plant Breeding**, London, v. 138, n. 1, p. 82–94, 2019.

TINKER, N. A. *et al.* A SNP genotyping array for hexaploid oat. **The Plant Genome**, Madison, v. 7, n. 3, p. 1-8, 2014.

TORKAMANEH, D.; LAROCHE, J.; BELZILE, F. Genome-wide SNP calling from genotyping by sequencing (GBS) data: a comparison of seven pipelines and two sequencing technologies. **PloS one**, San Francisco, v. 11, n. 8, p. e0161333, 2016.

UPADHYAYA, Y. M.; BAKER E. P. Studies on the mode of inheritance of Hajira type stem rust resistance and Victoria type crown rust resistance as exhibited in crosses

involving the oat variety Garry. **Proceedings of the Linnean Society of New South Wales,** Sydney, v. 85, p.157–179, 1960.

USDA. **Agricultural Projections to 2031**. St. Paul, 2022. Disponível em: https://www.ers.usda.gov/webdocs/outlooks/103310/oce-2022-01.pdf?v=8139.5. Acesso em: 27 set. 2022.

USDA-ARS CDL. **Oat Crown Rust Resistance Genes**. St. Paul: USDA-ARS Cereal Disease Laboratory, 2022. Disponível em: http://www.ars.usda.gov/Main/docs.htm?docid=10342>. Acesso em: 28 set. 2022.

USDA-ARS CDL. **Oat Loss to Rust**. St. Paul: USDA-ARS Cereal Disease Laboratory, 2014. Disponível em: https://www.ars.usda.gov/ARSUserFiles/50620500/Smallgrainlossesduetorust/2014loss/2014oatloss.pdf. Acesso em: 28 set. 2022.

USDA-FAS. US Department of Agriculture, Foreign Agricultural Service. **World Agricultural Production.** Washington, DC: USDA Foreign Agricultural Service. Disponível em: https://apps.fas.usda.gov/psdonline/circulars/production.pdf. Acesso em: 28 Set. 2022.

VALENTINE, J.; COWAN, A. S. A.; MARSHALL, A. H. Genetic resources. *In*: **Oats:** chemistry and technology. Cambridge: Academic Press, 2016. p. 11.

VIEIRA, E.A. *et al.* Diversidade e estrutura genética para virulência de três populações sul brasileiras de *Puccinia coronata*. **Bragantia**, Campinas, v.65, n.1, p. 147-155, 2006.

WAHL, I; SCHREITER, S. A highly virulent physiological race of crown rust on oats in Israel. **Bulletin of the Research Council of Israel,** Jerusalem, v.3, p. 256– 257, 1953.

WAIRICH, A. **Caracterização fenotípica, identificação de QTLs da resistência parcial à ferrugem da folha e avaliação do perfil transcricional de genes candidatos Associados ao caráter em *Avena sativa* L**. 2016. Dissertação (Mestrado) - Programa de Pós-graduação em Fitotecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2016.

WATERHOUSE, P. M. Australian rust studies. IX. Physiologic rust determinations and surveys of cereal rusts. **Proceedings of the Linnean Society of New South Wales,** Sydney, v.77, p. 209–258, 1952.

WIGHT, C. P. *et al.* Discovery, localization, and sequence characterization of molecular markers for the crown rust resistance genes *Pc38*, *Pc39*, and *Pc48* in cultivated oat (*Avena sativa* L.). **Molecular Breeding**, New York, v. 14, n. 4, p. 349–361, 2005.

WINKLER, L. R. *et al*. Population structure and genotype-phenotype associations in a collection of oat landraces and historic cultivars. **Frontiers in Plant Science**, Estocolmo, v. 7, [art.] 1077, 2016.

WONG, L. S. L. The inheritance of resistance to *Puccinia coronata* and of floret characters in *Avena sterilis*. **Canadian Journal of Genetics and Cytology**, Ottawa, v. 25, p. 329–335, 1983.

YAN, H. Highdensity marker profiling confirms ancestral genomes of *Avena* species and identifies D-genome chromosomes of hexaploid oat. **Theoretical and Applied Genetics**, New York, v. 129, p. 2133–2149, 2016.

YU, G. X.; BRAUN, E.; WISE, R. P. *Rds* and *Rih* mediate hypersensitive cell death independent of gene-for-gene resistance to the oat crown rust pathogen *Puccinia coronata*

f. sp. *avenae*. **Molecular Plant-Microbe Interactions**, St. Paul, v. 14, n. 12, p. 1376–1383, 2001.

YU, G.X.; WISE R.P. An anchored AFLP- and retrotransposon-based map of diploid *Avena*. **Genome**, Ottawa, v. 43, p. 736–749, 2000.

YU, J. *et al*. Genetic design and statistical power of nested association mapping in maize. **Genetics**, Oxford, v. 178, n. 1, p. 539-551, 2008.

ZHAO J. *et al*. Mapping of the oat crown rust resistance gene *Pc39* relative to single nucleotide polymorphism markers. **Plant Disease**, St. Paul, v. 104 p. 1507–1513, 2020.

ZHAO, J. Chromosomal location of the oat crown rust resistance gene *Pc98* in cultivated oat (*Avena sativa* L.). **Theoretical and Applied Genetics**, New York, v. 133, p.1109–1122, 2022.

ZOHARY, D. *et al*. **Domestication of plants in the Old World:** The origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. 3. ed. Oxford: Oxford University Press, 2000. 316 p.

ZWER, P. Oats: grain-quality characteristics and management of quality requirements. *In*: **Cereal grains**. Swanston: Woodhead Publishing, 2017. p. 235-256.

3. ARTICLE 1

Enhancing crown rust resistance in *Avena Sativa* via *Avena Strigosa* introgression: phenotypic characterization and inheritance[1]

ABSTRACT

Crown rust is the primary disease affecting oats worldwide. *Avena strigosa* has been identified as valuable sources of ASR. This study aimed to characterize the phenotype and genetic inheritance of crown rust resistance from *Avena strigosa* introgressed in *Avena sativa* and evaluate its suitability as a disease resistance source in a breeding program. The resistance to crown rust segregated for four epistatic loci in the segregating F_2 population. Three of these loci, showing complete dominance, provided resistance to crown rust. Two loci promoting the resistance showed larger phenotypic effects and were called, in this study, as 'R1' and 'R2', while the third resistance locus, called 'R3', had a minor effect on the phenotype. The fourth locus, called 'S', behaved as a suppressor of the resistance, showing incomplete dominant gene action. Different combinations of alleles in loci 'R' and locus 'S' allowed the phenotype to show immunity, resistance, moderate susceptibility or complete susceptibility. The resistant parent '07BT333' has at least two loci ('R1' and 'R2') that significantly contribute to crown rust immunity.

KEY-WORDS: *Puccinia coronata*, immunity, suppressor, epistasis.

[1] Article formatted according to rules of Crop Breeding and Applied biotechnology rules. https://cbab.sbmp.org.br/

INTRODUCTION

Crown rust, which is caused by the fungus *Puccinia coronata f.sp. avenae* (Simons, 1979) is the most significant disease affecting oats worldwide. The existence of a common epidemiological unit among the countries in the Southern Cone, especially Brazil, Argentina, and Uruguay, with continuous and cyclic dissemination of uredospores in the region, makes epidemics almost annually (Leonard and Martinelli, 2005). However, using resistant cultivars to the pathogen can help reduce the losses caused by this disease.

All Stage Resistance (ASR) is easily selected due to its high heritability - controlled by few genes with greater effects on the phenotype - it is commonly used in breeding programs (Heath, 2000). Nevertheless, this type of resistance does not provide durable resistance due to the great diversity of the *P. coronata* population, the high complexity of the pathogen population (Leonard and Martinelli, 2005), and the intense selection pressure exerted on the pathogen population (Wairich, 2016) and the ability of *P. coronata* to generate new races. Consequently, ASR resistance to oat crown rust can be overcome even in the breeding process or after the release of cultivars, with two to four years of average effectiveness requiring the constant release of new oat cultivars. Breeders are being challenged to find new resistance sources, hence the need for new genes of *P. coronata* resistance for the introgression on cultivated oat germplasm. Over the past few decades, resistance in the hexaploid cultivated oat *Avena sativa* L. has depended on a series of introduced resistance genes (Nazareno *et al*. 2018).

Useful sources of ASR have been identified from wild species in Israel and other Mediterranean countries and introgressed in *Avena sativa* during the 1960s and the early 1970s (Leonard *et al.*, 2004). *A. sterilis* has proven a prosperous source of resistance, contributing to some 44 of the resistance loci catalogued until now (Leonard *et al.*, 2004). This may be partly because it is a sexually compatible hexaploid, making it far easier to transfer this resistance into cultivated oat (Park. *et al*., 2022). *A. strigosa* also is a source of resistance genes. Although their introduction into hexaploid *A. sativa* is much more difficult and demanding due to the lack of chromosome homology and the special technical requirements (Jellen and Leggett, 2006). However, many genes were successfully introgressed, including *Pc15, Pc16, Pc17, Pc19, Pc23, Pc30, Pc37, Pc81, Pc82, Pc83, Pc84, Pc85, Pc86, Pc87, Pc88, Pc89, Pc90,* and *Pc94* (CDL 2013). Some of these genes are still used today, in combination with other genes (Park *et al.*, 2022), or in production areas where virulence to those genes has not been reported (Sowa and Paczos-Grzęda, 2020). *Pc23* and *Pc94* have been incorporated into a stable *A. sativa* background and used in oat resistance breeding (Dyck and Zillinsky, 1963; Pretorius *et al.*, 2012). *Pc94* originating from *A.*

strigosa accession RL1697 is still in use in the modern varieties ‘Leggett’ and ‘Stride’ (Mitchell Fetch *et al.*, 2007; Fetch *et al.*, 2013). More recently, Rines *et al.* (2018) identified a new and highly effective source of adult plant resistance to oat crown rust in the diploid oat *A. strigosa* accession ‘PI 258731’ and introgressed into the hexaploid cultivated oat.

Hence, the objective of this work was to phenotypically characterize and determine the genetic inheritance of resistance to crown rust from *Avena strigosa* accession ‘PI 258731’, introgressed to *Avena sativa* (line '07BT333') in a segregating population of oats with adaptation to southern Brazil on field conditions. Furthermore, it is discussed the suitability of this resistance source to be incorporated in subtropical oat breeding programs.

MATERIALS AND METHODS

Plant material and population development

The population under investigation was obtained by crossing the *A. sativa* lines '07BT333', crown rust resistant parent, and 'UFRGS 970461', susceptible parent. The parent 'UFRGS 970461' was developed by the Oat Breeding Program of the Federal University of Rio Grande do Sul (UFRGS), which improves oat cultivars adapted to the subtropical environment of Southern Brazil. It exhibits early maturation, high-quality grains, and high grain yield. The line ‘07BT333’ was developed by the "USDA-ARS Plant Science Research Unit", at the University of Minnesota, USA.

The pedigree of ‘07BT333’ is “(*A. strigosa* CI6954SP/Black Mesdag) C2//Ogle-c*5”. Until 2021 ‘07BT333’ was immune to oat crown rust in Southern Brazil. It is derived from the *A. strigosa* accession 'CI6954SP'. The resistance was transferred to *Avena sativa* by direct cross of the diploid line 'CI6954SP' (2n=2x=14) with the hexaploid cultivar (*A. sativa*) 'Black Mesdag' (2n=6x=42). The chromosome number was then doubled using colchicine (2n=2x+6x=56) after embryo rescue of the haploid F_1 (2n=1x+3x=28). The octoploid hybrid was then crossed with the hexaploid cultivar 'Ogle' (2n=6x=42), followed by four backcrosses for 'Ogle' and self-fertilizations of the last backcross, with selection for resistance to crown rust and for chromosome number equal to 42 (Rines *et al.*, 2007). Despite its high level of crown rust resistance, the hexaploid inbred line '07BT333' is unsuitable for Brazilian environments due to its late cycle, poor grain quality, and low grain yield.

The oat line ‘UFRGS 970461’ pedigree is “UFRGS 15/UFRGS 14”. ‘UFRGS 14’ (80SA65 // Coronado^2/Cortez^3/Pendek/ME1563) was a cultivar, released in Brazil in 1993 and very popular among farmers, while ‘UFRGS 15’ (Coronado^2/Cortez^3/Pendek/ME1563 // C16CRcpx/C7512/SRcpx/74C8014) was released in 1994, and also a very successful oat

cultivar in Southern Brazil. The choice of ‘UFRGS 970461’ as a parent for the F_2 population was also based on the fact that this line is the basis of modern UFRGS germoplasm with UFRGS 14 and UFRGS 15 on the pedigree. Also, this line is the male parent of ‘URS Taura’, release in 2009, which is an early, short straw, very high yielding oat cultivar, with the largest cultivated area among all oat Brazilian cultivars, adding up all years of adoption.

The cross between the resistant parent '07BT333' and the susceptible inbred line 'UFRGS 970461' was conducted in 2015. F_1 seeds were sown in the field in 2016. At the maturation stage, all seeds produced by F_1 plants were harvested in bulk, originating the F_2 seeds, which were stored in a seed cold room.

Field experiments

In the oat growing season (winter/spring) of 2020, the F_2 seeds were conducted under field conditions, subjected to natural epidemic of crown rust. On June 29th, 400 F_2 seeds and the respective parental lines were sown as individual plants, spaced 30 cm apart within and between sowing rows. Ten seeds were sown at each 3 m long row. Sowing rows were distributed in four sowing blocks of 12 rows each (Figure 1 A to 1 C). The two parental lines were sown at the beginning of each block and after every two F_2 sowing rows, alternating the order of each parent. This arrangement allowed that each F_2 sowing row had a parental row at one side and a F_2 row at the other side. To ensure maximum pathogen infection and phenotyping accuracy, the experiment was surrounded by plants of the highly susceptible cultivar 'URS 22', which served as a inoculum spreader (Figure 1D). The ‘URS 22’ plants were arranged in four continuous sowing rows with a density of approximately 300 seeds per m^2 (Figure 1A to 1C).

Phenotypic assessment

The infection was monitored throughout the cycle of the plants, with four phenotypic classes being assigned to the F_2 plants and the parental lines. According to the level of the observed crown rust resistance, each plant was initially classified as : ‘immune’ (when there were no pustules); ‘resistant’ (presence of "flecks" or presence of small pustules, at low to moderate frequency, accompanied by chlorosis and/or necrosis); ‘moderately susceptible’ (moderate frequency of large pustules showing almost no chlorosis, or high frequency of small pustules with significant photosynthetic area remaining on leaf laminae); ‘susceptible’ (very high frequency of pustules, occupying practically the entire leaf area of each plant) (Figures 1E to 1H). For inheritance investigation reasons, initial phenotypic classes ‘highly

resistant' and 'moderately resistant' were grouped as the single phenotypic class 'resistant' (presence of "flecks" or rare to moderate frequency of small pustules, accompanied by chlorosis and/or necrosis).

Statistics Analysis

From the frequency of the phenotypic classes observed in the segregating population and among the parental genotypes, genetic models with one to four loci were proposed to estimate the number of genes, controlling the resistance to crown rust, in the segregating F_2 population, as well as their mode of action. The goodness-of-fit of each proposed genetic model was tested using the chi-square test ($\chi 2$), according to the equation presented by Steel *et al.* (1997), with n-1 degrees of freedom.:

$$x^2 = \sum \frac{(Obs - Exp)^2}{Exp}$$

Where: Obs = number of plants observed in each phenotypic class.

Exp = the number of plants expected in a particular phenotypic class, according to the proposed model.

n = number of phenotypic classes of the genetic model.

To calculate the number of expected genotypes in the F_2 generation for each proposed genetic model, the following formula was applied:

$$Gp = 3^n$$

Where: Gp = possible genotypes

n = number of loci involved in the trait expression.

RESULTS AND DISCUSSION

Due to the continuous evolution of the *P. coronata* pathogen, the effectiveness of the current crown rust resistance genes has rapidly declined. Thus, there is a need to search for new resistance genes. *A. strigosa*, which the diversity center is located in northern Portugal and north-western Spain, is valuable source of resistance due to its diversity (Loskutov 2008).

This study was conducted to determine the genetic inheritance of the crown rust resistance present in the oat line '07BT333'. The resistance was introgressed from the line of *Avena strigosa* 'CI6954SP' into the *Avena sativa* cultivar 'Ogle', which is adapted to temperate conditions. The phenotypic characterization of this source of resistance was carried out in the field, under conditions very favourable to crown rust development (Figure

1C and 1D), together with the evaluation of the segregating F_2 population derived from crossing between ‘07BT333’ and the oat line 'UFRGS 970461', adapted to subtropical conditions.

The line ‘07BT333’ exhibited high resistance level under the Southern Brazilian environment, where crown rust epidemics occur every year, reaching high levels of infection in susceptible oat cultivars, indicating its suitability for use in oat Brazilian breeding programs. Most of the ‘07BT333’ plants showed an immune phenotype, however two out 80 plants presented very low levels of crown rust infection, showing very few pustules accompanied by chlorosis, with low apparent sporulation (Table 1). Besides the pathogen field population being a mixed of several pathotypes, the presence of the parental ‘07BT333’ resistant of all the races on the pathogen population allow us to make the genetic analysis. However, despite its high level of crown rust resistance, the oat line ‘07BT333’ is not adapted to Southern Brazilian environments, showing very late cycle, very low physical grain quality, and very low grain yield. This line have been crossed with several oat genotypes developed in Brazil, showing high grain quality and yield. From these crosses, the cross with the oat line ‘UFRGS 970461’ was chosen to investigate the inheritance of this crown rust resistance source in a Brazilian oat genotype background.

The presence of the highly susceptible oat cultivar 'URS 22', as a crown rust spreader, around the experiment guarantied very high levels of inoculum (Figure 1A to 1D), avoiding the occurrence of scapes, which are susceptible plants with low level of infection and being mistaken as resistant or moderately susceptible. Crown rust phenotypic evaluations started at the end of August. During the first two weeks, the spreader rows showed a low frequency of pustules. On September 15th, 2020, the first evaluation was conducted in the experiment, when several crown rust symptoms were already present in susceptible parent ‘UFRGS 970461’. Two more crown rust evaluations were performed on September 22nd and September 29th, allowing a very accurate visual evaluation of crown rust severity and infection reaction. On October 6th the high incidence of crown rust had caused premature senescence of leaves in the most susceptible genotypes (as shown in Figure 1D), making severity evaluation impossible.

The goodness-of-fit of the observed phenotypic data distribution was tested for different genetic models considering from one to four loci controlling the trait. We started from the simplest models to more complex ones. Genetic models with only one gene allow only two or three phenotypic classes in a F_2 population. Therefore, it was necessary to group different phenotypic classes. We tried all possibilities of grouping the initial five phenotypic

classes, in different ways, to fit the expected number of classes of each genetic model possible.

The best fit (p=0.93) for the observed phenotypic distribution was a genetic model considering four loci controlling the resistance of crown rust, with the presence of four phenotypic classes in F_2 population ('immune', 'resistant', 'moderately susceptible', and 'susceptible'). This model predicts the segregation of 81 possible genotypes, expecting that 14.06 % of the F_2 individuals fall in the 'immune class', 24.61 % to belong to the 'resistant' class, 7.03 % behave as 'moderately susceptible', and 54.30 % to be 'susceptible' (Table 1).

The best-fitted genetic model, with four loci controlling the resistance to crown rust, considers the presence of a suppressor locus for resistance, called here as locus '**S**', segregating in the F_2 population. The suppression of the expression of the resistance originally coming from the *Avena strigosa* 'CI6954SP' was reported by Rines *et al.* (2007), when describing the introgression of this resistance source to *Avena sativa*. These authors found suppression of the resistance in crosses involving the susceptible oat genotypes 'Pendek-*Pc38*' and 'Black Mesdag', but not involving 'Ogle'. Therefore, the presence of a suppressor locus depends on the genetic background of the susceptible parental genotype chosen. The susceptible male parent of the F_2 population ('UFRGS 970461') descends from the Dutch cultivar 'Pendek' from both parents. Thus, its genealogy may explain the presence of the resistance suppressor gene, but we cannot discard the hypothesis that this suppressor may be common in the oat germplasm, once it is expected the 'Pendek' has contributed to a very small portion of the 'UFRGS 970461' genome (see Material and methods).

The expected genotypes for each observed phenotype are presented in Table 1. The expression of the suppressor locus '**S**' showed incomplete dominance. Therefore, its heterozygous form ('**S**s') does not have the same capability of inhibiting the expression of resistance genes that control the genetic resistance to crown rust, as its homozygous form ('**SS**'). While the resistance is controlled by at least three genes, once we found that the F_2 population was segregating for three independent completely dominant loci (called '$\mathbf{R_1}$','$\mathbf{R_2}$', and '$\mathbf{R_3}$'), contributing in an epistatic manner to the resistance (Table 1).

According to the proposed genetic model, when the suppressor locus '**S**' has two functional alleles (**SS** homozygous), the phenotype will always be susceptible, independent of the combination of functional alleles at the '**R**' loci. When only of functional allele of the '**S**' locus is present (**S**s heterozygous), the phenotype can be from resistant to susceptible, depending on the combination of functional alleles at the '**R**' loci, but never immune (see

Table 1 for details). Immunity is only possible in the absence of any functional allele at the suppressor locus (ss homozygous) (Table 1).

In a previous study using the same oat line '07BT333', Roesler (2017) did not find a suppressor locus acting on the crown rust resistance in the segregating populations, but it was present in crosses involving the sister line '07BT306' and two different susceptible parents, one being a line also descending directly of the susceptible parent 'UFRGS 970461'. The crown rust suppression found by Roesler (2017) showed also an incomplete dominance mode of action. In our study, the heterozygous condition ('**S**s') the plant may exhibit some level of resistance, that can even be high, depending on the combination of resistance genes present in the genotype (Table 1).

It is not uncommon to find inhibitory genes in crow rust resistance. According to Wilson and McMullen's (1997), the Pc38 parent may possess a factor that prevents the expression of the resistant gene *Pc62*. Similarly, Rayapati *et al*. (1993) and Wise *et al*. (1996) identified an inhibitor in *A. wiestii* (line C.I. 1994) that inhibits the expression of a resistance gene found in *A. strigosa* (line C.I. 3815).

The '$\mathbf{R_1}$' locus is crucial for resistance but not sufficient for immunity on its own, while the '$\mathbf{R_2}$' locus is incapable of conferring resistance without a functional allele at '$\mathbf{R_1}$' locus. Thus, the immune genotypes must not have a functional allele at locus 'S' (ss homozygous) and carry at a functional allele from the locus '$\mathbf{R_1}$' ($\mathbf{R_1R_1}$ or $\mathbf{R_1}r_1$ genotypes) combined with a functional allele from the locus '$\mathbf{R_2}$' ($\mathbf{R_2R_2}$ or $\mathbf{R_2}r_1$ genotypes), independently of the alleles present in the locus '$\mathbf{R_3}$' (Table 1).

The '$\mathbf{R_3}$' locus do not contributes to immunity and functions as a modifier gene, a gene with small effect on the resistance phenotype, contributing to expression of a 'resistant' phenotype under two situations: i) in the absence of the any functional alleles at the suppressor locus '**S**' (ss homozygous) and at the resistance locus '$\mathbf{R_2}$' (r_2r_2 homozygous), combined with the presence of a functional allele at the '$\mathbf{R_3}$' ($\mathbf{R_3R_3}$ or $\mathbf{R_3}r_3$ genotypes) plus a functional allele at the locus '$\mathbf{R_1}$' ($\mathbf{R_1R_1}$ or $\mathbf{R_1}r_1$ genotypes); ii) in presence of single functional allele at suppressor locus '**S**' (**S**s heterozygous), combined with the presence of a functional allele in each 'R' loci ($\mathbf{R_1}$_+$\mathbf{R_2}$_+$\mathbf{R_3}$_+**S**s genotypes). The genotypes with the presence of a functional allele at the locus '$\mathbf{R_3}$' without a functional allele at locus '$\mathbf{R_1}$' can only express a moderately susceptible phenotype, and only in absence of a functional allele at the suppressor locus '**S**' (Table 1).

Different resistance classes, in a segregating population, may be due to susceptible parent contributing genes for resistance. This suggests that no longer effective resistance

genes may still interact with resistance genes from resistant parents, leading to epistatic contributions to resistance, as observed in this study and by Roesler (2017), who found that the populations with crown rust resistance from the accession 'CI6954SP' of *A. strigosa* were segregating for one or three resistance ('R') loci, depending on the resistant line and on the susceptible parent. Also, the pathogen population can influence the effective number of loci contributing to resistance to crown rust. In Southern Brazil, the crown population is highly complex, being formed by races carrying numerous virulence genes. Therefore, the races present in the field, in our investigation, must be more complexes than the ones used by Rines *et al.* (2007) in a previous study on same crown rust resistance from the *A. strigosa* accession 'CI6954SP', made in North America under controlled conditions. These authors reported that their segregant populations were segregating for only one resistance, at the time when the resistance from accession 'CI6954SP' had been recently introgressed into *A. sativa*.

According to Leonard and Martinelli (2005), the *P. coronata* population structure in southern Brazil is diverse and fundamentally constituted by super-races, which contained, at the time of their survey, on average around 12.5 virulence genes per race, ranging from 2 to 26. This complexity was reached besides the absence of sexual stages in the pathogen cycle in South America and despite the small size of the differential set of oat lines used, that was only 27, which underestimates the crown rust races complexity.

Our findings allow to conclude that the resistant line '07BT333' must carry, at least, two resistance genes, present in the two major loci, we called as '**R_1**' and '**R_2**', once this resistant line showed an immune phenotype under the experiment conditions, which is predicted to happen only if '**R_1**' and '**R_2**' contributes together with effective alleles to the genotype (Table 1). This was also the conclusion made Roesler (2017). Nevertheless, we cannot exclude the possibility of '07BT333' carry also a third resistance gene, present in lous we called '**R_3**'. It is because we cannot differentiate between genotypes carrying or not carrying this minor effect locus in the immune plants (Table 1).

It is difficult to determine the origin of the '**R_3**' locus in the current study, as it only has a minor impact on the phenotype. It could have come from either the resistant parent '07BT333' or the susceptible parent 'UFRGS 970461', but it is more likely to have come from the latter. This is because the functional allele of this locus is not necessary for immunity, although this predicted condition may only be an effect of the crown rust population virulence composition at the time of the experiment. Our observations also suggest that the expressivity of the main locus '**R_1**' of '07BT333' might not be 100 %, as small pustules have been observed in plants of this parent (Table 1). This subtle change in phenotype observed

in the resistant parent, is also expect to happen in the segregating population, but would not alter the conclusions we made on the best-fitted genetic model, as it is expected that only one of the plants initially classified as 'highly resistant' would in fact carry an 'immune' genotype.

Transferring crown rust resistance from poorly adapted genotypes, as the oat line '07BT333' to elite oat lines adapted to the subtropical environment, can be a very challenging task, once the selection process involves not only selecting for effective alleles controlling the resistance, but also selecting for many other desirable agronomic traits, which favourable alleles must be distributed throughout the genome. The presence of a crown rust resistance suppressor gene, from certain genetic backgrounds, will add an extra challenge for the oat breeder, as the suppressor genes may entirely or partially inhibit some genes that confer resistance.

Sometimes, what appears to be a lack of effective alleles for crown rust resistance may be the result of the presence of a suppressor gene in the genotype. Even though, in the segregating populations, the oat breeder must eliminate the plants that show a susceptible phenotype, once there is no guarantee that they will carry the right combination of resistance alleles, at the different genes controlling the trait, and will produce resistant descents in the next generation. The breeder should work with large F_2 populations, ranging from one to two thousand plants, when working with crown rust resistance from the *A. strigosa* accession 'CI6954SP', allowing to select to other traits among the resistant oat plants. According to our genetic model almost 40 % of the F_2 will show an immune or resistant phenotype, but part of the resistant plants will never give immune progeny (those 'r_2r_2' homozygous), as well as part will segregate for susceptibility (those carrying the '**S**' functional allele). Therefore, the oat breeder should concentrate on selecting the immune plants, selecting a large set of them, since from the 14 % of expected immune plants, only around 10 % (¾ of 14 %) will carry a functional allele for the '$\mathbf{R_3}$', increasing the chance that the select plants may be homozygous for the three resistant loci ($\mathbf{R_1R_1}$+$\mathbf{R_2R_2}$+$\mathbf{R_3}$ $\mathbf{R_3}$+ss) as advanced inbred lines, improving the probability of the selected resistance to last longer than average, compared to those lines carrying effective alleles at only two loci ($\mathbf{R_1R_1}$+$\mathbf{R_2R_2}$+r_3r_3+ss). In North America crown rust resistance is usually defeated by pathogen in a very short time, from two to five years after cultivar release (Carson, 2011), and it is even worse in the subtropical South America.

CONCLUSIONS

Based on our results, the resistant parent '07BT333' must carry, at least, two main loci with large phenotypic effect on crown rust resistance. One of these loci ('**R_1**') is essential to the expression of the resistance, while the second major effect locus ('**R_2**') is necessary for the expression of the immune phenotype. A third resistance locus ('**R_3**'), with minor effect on the phenotype, was also segregating in the F_2 population and contributed to the resistance in certain genotypic arrays, but did not contribute to immunity. All three resistance loci showed a complete dominance mode of action. In addition, a suppressor locus ('**S**') of the crown rust resistance was also segregating in the population; this locus showed incomplete dominance and the presence of only one functional suppressor allele is sufficient to inhibit immunity, but not resistance, depending on the combination of resistance alleles at the three other loci. The resistance of the oat line '07BT333' do not show complete expressivity and it is possible to find plant with very low levels of infection, without meaning that the resistance has been overcome by the pathogen.

ACKNOWLEDGMENT

To the Higher Education Personnel Foundation (CAPES) for the Scholarship granted to the first author.

REFERENCES

CARSON ML (2011) Virulence in oat crown rust (*Puccinia coronata* f. sp. *avenae*) in the United States from 2006 through 2009. **Plant Disease 95**: 1528–1534.

CDL - Cereal Disease Laboratory**. Resistance Genes. Oat. *Pc*** (Crown Rust). Available online:<https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/resistance-genes/resistance-genes/>. Access on September 16th, 2022).

Chaves MS, Martinelli JA, Wesp CDL and Graichen FAS (2008). The cereal rusts: an overview. **Pest Technology 2**: 38-55

Dyck PL and Zillinsky FJ (1963). Inheritance of crown rust resistance transferred from diploid to hexaploid oats. **Canadian Journal of Genetics and Cytology 5**: 398-407.

Heath, M. C. (2000) Nonhost resistance and nonspecific plant defenses. **Current Opinion in Plant Biology 3**: 315-319.

Jellen EN and Leggett JM (2006). Cytogenetic manipulation in oat improvement. **Genetic resources, chromosome engineering, and crop improvement 2**: 199-232.

Leonard KJ, Anikster Y and Manisterski J (2004). Patterns of virulence in natural populations of Puccinia coronata on wild oat in Israel and in agricultural populations on cultivated oat in the United States. **Phytopathology 94**: 505-514.

Leonard, K. J. and Martinelli and J. A. (2005). Virulence of oat crown rust in Brazil and Uruguay. **Plant Disease 89**: 802-808.

Loskutov IG (2008). On evolutionary pathways of *Avena species*. **Genetic Resources and Crop Evolution 55**: 211–220

Martinelli JA, Federizzi LC and Bennedetti AC (1994). Yield reductions of oat grains due to leaf rust severity. **Summa Phytopathologica 20**: 116-118.

Mitchell Fetch JW, Duguid SD, Brown PD, Chong J, Fetch TG, Haber SM [...] and Stadnyk KD (2007). Leggett oat. **Canadian Journal of Plant Science 87**: 509-512.

Nazareno ES, Li F, Smith M, Park RF, Kianian SF and Figueroa M. (2018). *Puccinia coronata* f. sp. *avenae*: a threat to global oat production. **Molecular Plant Pathology 19**: p. 1047-1060.

Park RF, Boshoff WHP, Cabral AL, Chong J, Martinelli JA, McMullen MS, [...] and Singh, D. (2022). Breeding oat for resistance to the crown rust pathogen *Puccinia coronata* f. sp. *avenae*: achievements and prospects. **Theoretical and Applied Genetics 135**: 3709–3734 .

Pretorius ZA., Szabo LJ., Boshoff WHP, Herselman L and Visser B (2012). First report of a new TTKSF race of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) in South Africa and Zimbabwe. **Plant Disease 96**: 590-590.

Rayapati PJ, Gregory JW, Lee M and Wise RP (1994). A linkage map of diploid *Avena* based on RFLP loci and a locus conferring resistance to nine isolates of *Puccinia coronata* var. '*avenae*.' **Theoretical and Applied Genetics 89**: 831-837.

Rines HW., Porter HL, Carson ML and Ochocki GE (2007). Introgression of crown rust resistance from diploid oat *Avena strigosa* into hexaploid cultivated oat *A. sativa* by two methods: direct crosses and through an initial 2 x· 4 x synthetic hexaploid. **Euphytica 158**: 67-79.

Rines HW, Miller ME, Carson M, Chao S, Tiede T, Wiersma J, Kianian SF (2018). Identification, introgression, and molecular marker genetic analysis and selection of a highly effective novel oat crown rust resistance from diploid oat, *Avena strigosa*. **Theoretical and Applied Genetics 131**: 721–733.

Roesler EA (2017) **Herança da resistência qualitativa à ferrugem da folha em genótipos de aveia (*Avena sativa* L.)**. 167 f. Dissertação (Mestrado) – Programa de Pós-Graduação em Fitotecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2017.

Simons MD, Rothman PG and Michel LJ (1979). Pathogenicity of *Puccinia coronata* from buckthorn and oats adjacent to and distant from buckthorn. **Phytopathology 69**: 156.

Sowa S and Paczos-Grzęda E (2020). Identification of molecular markers for the *Pc39* gene conferring resistance to crown rust in oat. **Theoretical and Applied Genetics 133**: 1081-1094.

Steel RGD, Torrie, JH and Dickey DA (1997). **Principles and procedures of statistics: A biometrical approach**. 3rd ed., McGraw Hill, New York, 666p.

Wairich A (2016) **Caracterização fenotípica, identificação de QTLs da resistência parcial à ferrugem da folha e avaliação do perfil transcricional de genes candidatos associados ao caráter em *Avena sativa* L**. Dissertação de mestrado - Programa de pós graduação em Fitotecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, 2016.

Wilson WA. and McMullen MS (1997). Dosage-dependent genetic suppression of oat crown rust resistance gene *Pc-62*. **Crop Science 37**: 1699-1705.

Wise RP, Lee M and Rayapati PJ (1996). Recombination within a 5-centimorgan region in diploid *Avena* reveals multiple specificities conferring resistance to *Puccinia coronata*. **Phytopathology 86**: 340-346.

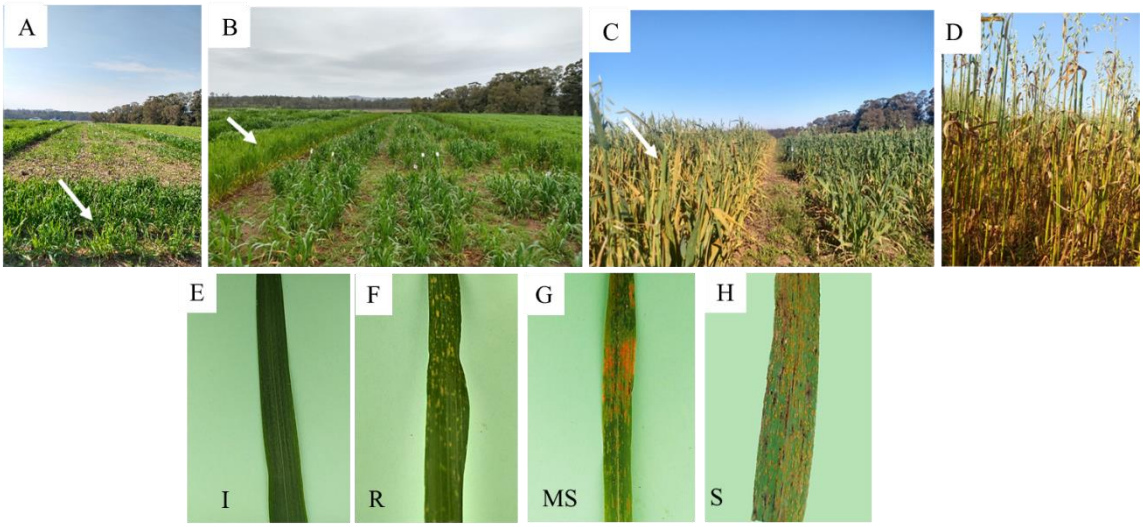


Figure 1. Crown rust phenotyping of the '07BT333 / UFRGS 970461' segregating F_2 population and parental lines. **A** and **B**: Overview of the field experiment. **C** and **D**: Details showing the high crown rust incidence on the high susceptible spreader oat cultivar 'URS 22' (white arrows). **E**, **F**, **G**, **H**, **I**: Leaves of oat genotypes with different classes of resistance and susceptibility. **E**: Immune (I). **F**: Highly resistant (HR). **G**: Moderately Resistant (MR). **H**: Moderately susceptible (MS). **I**: Susceptible (S). **F** and **G**: Resistant (HR+MR).

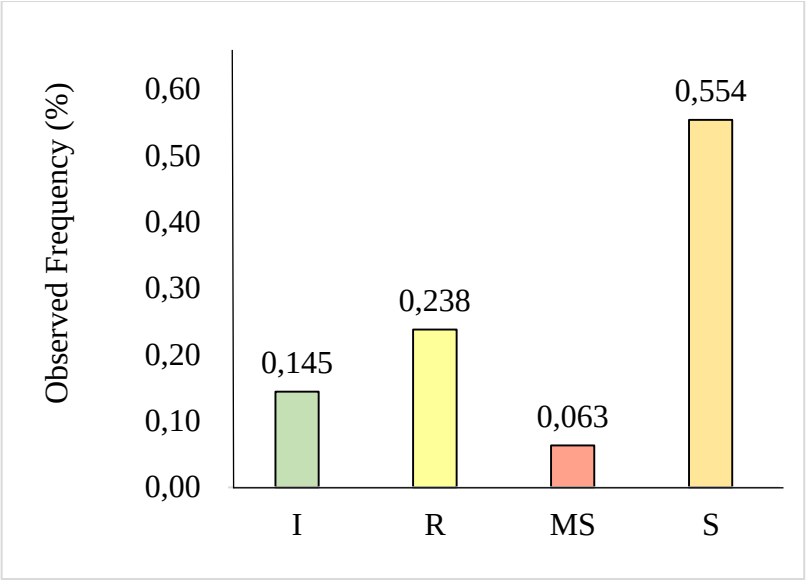


Figure 2. Frequency distribution of four phenotypic classes of crown rust resistance observed in the F_2 segregating oat population '07BT333 / UFRGS 970461', under field conditions. N = 332 plants. S: Susceptible; MS: Moderately susceptible; R: Resistant; I: Immune.

Table 1. Observed and expected phenotypic and genotypic frequencies of parental and F_2 genotypes of the '07BT333 / UFRGS 970461' oat segregating population, according to the genetic model of four epistatic loci controlling crown rust resistance.

Parents	F_2 segregating population					
	Phenotype	**Expected Genotypes**	**Nº obs plants**[§]	**O.F**[†]	**E.F**[‡]	χ^2
	Immune	**R_1** _ + **R_2** _ + ___ + ss	48	0.1446	0.1406	0.037
	Resistant	**R_1** _ + r_2r_2 + **R_3** _ + ss **R_1** _ + **R_2** _ + **R_3** _ + **S**s	79	0.2380	0.2461	0.089
	Moderately Susceptible	**R_1** _ + r_2r_2 + r_3r_3 + ss r_1r_1 + **R_2** _ + ___ + ss r_1r_1 + r_2r_2 + **R_3** _ + ss	21	0.0633	0.0703	0.235
	Susceptible	**R_1** _ + **R_2** _ + r_3r_3 + **S**s **R_1** _ + r_2r_2 +**R_3** _ + **S**s r_1r_1 + ___ + ___ + **S**s ___ + ___ + ___ + **SS** r_1r_1 + r_2r_2 + r_3r_3 + ss	184	0.5542	0.5430	0.077
		Total	**332**	**1.0000**	**1.0000**	**0.439**
		Probability				**0.932**
07BT333	Immune		78	0.9750	1.0000	
	Resistant		2*	0.2500		
UFRGS 970461	Susceptible		110	1.0000	1.0000	

[§]Number of observed plants. [†]Observed plant frequency. [‡]Expected plant frequency.
χ^2: Chi-square goodness of fit test.
R_1, R_2, and R_3: Loci that confer resistance to crown rust, with complete dominance.
R_1 and R_2: Loci with large phenotypic effect on crown rust resistance.
R_1: Indispensable locus for expressing resistance to crown rust but is insufficient alone.
R_3: Locus with minor phenotypic effect (modifier) on crown rust resistance.
S: Crown rust resistance suppressor locus, with incomplete dominance.
* The two resistant plants of '07BT333' had very few small pustules, surrounded by chlorosis.

4. ARTICLE 2

FIRST NESTED ASSOCIATION MAPPING POPULATION IN OAT (*Avena sativa* L.) IDENTIFIES THE LOCATION OF MULTIPLE GENES CONFERRING RESISTANCE TO THE CROWN RUST PATHOGEN *Puccinia coronata* f. sp. *Avenae*[1]

ABSTRACT

Crown rust, caused by the fungus *Puccinia coronata* f. sp. *avenae* (*Pca*), is the most destructive foliar pathogen of oat. Almost one hundred genes conferring resistance to crown rust have been catalogued in *Avena*. However, few of these genes have been mapped, and the chromosomal location of most remains to be determined. The goal of the present study was to use nested association mapping in *A. sativa* L. (AsNAM) to detect the chromosomal locations of 13 catalogued *Pc* genes and an oat genotype with uncharacterized but highly effective crown rust resistance and to identify putative candidate genes underlying each. The population consisted of 14 biparental $F_{2:3}$ families, each derived from crosses between a donor carrying *Pca* resistance and the crown rust susceptible founder variety 'Swan'. A total of 2,356 $F_{2:3}$ lines were phenotyped for seedling response to defined pathotypes of *Pca*, from which the AsNAM population of 707 individuals was built. AsNAM analysis based on DArT-Seq genotype data identified 15,940 high-quality single nucleotide polymorphisms (SNPs). Using the IBD method, were identified eight QTL associated with seedling resistance to *Pca* with varying effects on phenotypic variance. Five new genes had their location on oat chromosomes (chr.) described for the first time: *Pc13* (chr3D), *Pc36* (chr1C), *Pc60* (chr1C), *Pc64* (chr7D),

[1] Article formatted according to Theoretical and Applied Genetics rules.
https://www.springer.com/journal/122

and *Pc70* (chr5D). Also, previously mapped genes had their position confirmed: *Pc38* (chr7D), *Pc45* (chr2D), *Pc46* (chr3D), and *Pc5*0 (chr3D). Results showed some populations had a single dominant gene controlling resistance, while others had more complex resistance. Several genes were linked or allelic (*Pc13*, *Pc46* and *Pc50*; *Pc36* and *Pc60*; *Pc38* and Pc64). Flanking SNPs were reported for each QTL. Location analysis of the significant SNPs revealed annotation 31 putative genes, belonging to eight protein families to encode proteins related to molecular functions and biological processes with disease resistance.

Keywords: Resistance; loci; chromosome; all stage resistance; QTL

INTRODUCTION

The crown rust fungus *Puccinia coronate* f. sp. *avenae* is the most destructive foliar pathogen of oat (*Avena sativa* L.). This pathogen is widely distributed, being present in all regions where this cereal is cultivated (Simons 1970). Populations of *Pca* exhibit high variability in virulence phenotypes, with rapid emergence of new virulent races both in the presence and absence of sexual recombination (Park 2008; Park *et al.,* 2022; Nazareno, 2018). In years when then environmental conditions favor pathogen development, disease severity can peak at over 90 % in susceptible genotypes (Martinelli *et al*., 1994). The use of genetic resistance is considered more efficient in controlling crown rust in oats from an economic and environmental point of view (Sanz *et al*., 2013). There are two main types of rust resistance characterized in oats: all stage resistance (ASR) and adult plant resistance (APR). ASR can be selected easily due to high heritability and strong phenotypic effects that are typically immune or hypersensitive responses at all plant stages. The ability to select for ASR at seedling growth stages has resulted in its widespread use in breeding programs (Heath 2000). ASR genes fit the gene–for–gene model, whereby for each gene controlling a resistance reaction in the host, there is a corresponding gene in the pathogen controlling pathogenicity (Flor 1956). Genetic loci that have been characterised as conferring resistance in oat to *Pca* are given the designation "*Pc*". To identify and effectively utilize catalogued *Pc* resistance genes it is crucial to have a differential collection consisting of single gene reference stocks for every resistance locus and pure isolates of *Pca* of well-defined pathogenicity (pathotypes)

for each. This collection contains one or more *Pc* genes in different genotypic backgrounds and is used to determine pathogenicity based on compatible (virulent) or incompatible (avirulent) interactions (Chong *et al.,* 2000).

Ninety-eight alleles at 92 loci conferring resistance to *Pca* have been designated in the genus *Avena* (Park *et al*., 2022). Few of these loci have been mapped to chromosomes, and linkage relationships between them remain largely unknown. Past *Pc* gene characterization studies could only associate these genes with groups of linked loci without indicating their position within the oat genome (Gnanesh *et al*., 2015). The lack of information on the chromosomal location of *Pc* genes in oat and their corresponding molecular markers has hindered the utilization of genomic methods in oat breeding for crown rust resistance (Admassu-Yimer *et al.,* 2018). This highlights the importance of conducting further mapping studies in oats to facilitate gene interaction studies and to better understand genetic resistance to the pathogen.

Earlier research faced obstacles due to the absence of a reference genome for hexaploid oat species, which delayed the identification of gene position, function, and sequence. The recent public release of chromosome-scale assemblies for the diploid species *A. atlantica* and *A. eriantha* (Maughan *et al.,* 2019) and the hexaploid *A. sativa* genotype OT3098 (https://wheat.pw.usda.gov/ GG3/grain genes_ down oats/oat- ot3098-pepsico) have provided invaluable genetic resources that will accelerate efforts to map *Pc* genes.

To comprehend the genetic architecture of complex traits, QTL analysis is usually conducted through linkage analysis in bi-parental mapping populations and GWAS in diversity panels (Li *et al*., 2016). The nested association mapping (NAM) approach combines these two methods, addresses some of the inherent limitations in each, and is considered third-generation genetic mapping (Gireesh *et al.,* 2021). It requires a mating design that involves a shared parent across multiple crosses. The common parent is referred to as the "founder," and other parents are referred to as “donors”. The method was first developed in maize (McMullen *et al*., 2009) and has been successfully adapted to map complex traits in model plants such as arabidopsis (Buckler *et al*., 2007), wheat (Bajgain *et al*., 2016), soybean (Song *et al*., 2017), and sorghum (Bouchet *et al*., 2017). The NAM strategy employed in this study combines the strengths of linkage mapping and linkage disequilibrium mapping, resulting in improved accuracy and resolution of the mapped loci due to a standardized genetic background, facilitating the identification of segregating alleles in different populations based on the presence of parent-specific alleles referred to as identity-by-descent (IBD) (Blanc *et al*., 2006; Yu *et al*., 2008). The

origins of offspring alleles from parental genotypes can be estimated through IBD probabilities. The IBD information forms the basis for creating design matrices, or genetic predictors, to which QTL allele effects can be estimated (Li *et al*., 2022).

This work describes the first *Avena sativa* NAM (AsNAM) population built for QTL Mapping of ASR to oat crown rust. The AsNAM population was based on 707 $F_{2:3}$ genotypes derived from crossing 14 diverse oat genotypes (*A. sativa*), carrying genes for crown rust resistance, with the Australian oat cultivar 'Swan', highly susceptible to this disease, and 15,940 single nucleotide polymorphisms (SNPs). The AsNAM population was established to fulfill the main objectives: i) to screen the population for resistance against *P. coronata*; ii) to find genomic regions associated with crown rust resistance (QTLs); iii) to identify the location of previously reported genes in the literature, and iv) to identify putative candidate genes underlying the identified resistance QTL.

MATERIAL AND METHODS

Plant material and population development

The AsNAM population was based on 707 $F_{2:3}$ lines belonging to 14 subpopulations originating from 14 selected oat genotypes (donors) crossed with oat cultivar 'Swan' (founder) (Fig. 1). The donor parents are represented in Table 1, 13 of which carry a catalogued *Pc* gene and Ensiler, a cultivar developed by the University of Wisconsin (Brinkman *et al*., 1990), which carries resistance that was effective against all Australian isolates of *Pca* subjected to race analysis from 1999 through 2017 (Park RF unpublished) (Table 1). The Australian cultivar Swan carries *Pc1* (Park RF unpublished) and is highly susceptible to all the pathotypes used in this study (Table S1). Each population was developed and advanced to the F_3 generation at the Plant Breeding Institute of the University of Sydney, Cobbitty, NSW, Australia.

Within each cross, each $F_{2:3}$ family was classified as "Non-segregating Resistant" (NSR), "Segregating" (Seg), or "Non-segregating Susceptible" (NSS) to crown rust. A total of 2,356 $F_{2:3}$ families were phenotyped for seedling response to crown rust, allowing the genetic analysis of the number resistance genes segregating in each of the 14 subpopulations (Table 2). After phenotyping for crown rust response, 50 lines (25 NSR and 25 NSS) were selected from each tail of the distribution, all descending from different F_2 plants, to represent resistant and susceptible phenotypic classes following the protocol

described in Ziems *et al*. (2017) (Fig. 1, Fig. S1). When there were fewer than 25 plants in any of the tails, plants from the segregating class were chosen according to their reaction, i.e., resistant plants were chosen to complete the NSR sample and susceptible plants to complete NSS sample (Fig. S1).

Evaluation of crown rust response

Thirty seeds of each $F_{2:3}$ family and parental lines were sown in 9 cm diameter pots filled with a mix of composted pine bark (8 parts) and coarse sand (2 parts). Pots were fertilized at sowing with a complete fertilizer (Aquasol®, 25 g/10 L water) and urea 3 days before inoculation. Seedlings at the 1-2 leaf stage were sprayed with urediniospores of *P. coronata* f. sp. *Avenae* suspended in light mineral oil (Pegasol®), using a motorized sprayer (GAST Manufacturing Inc., Benton Harbor, MI) and a small atomizer (G-R Manufacturing Manhattan, KS). The inoculated plants were incubated for 24h in a dew chamber at 18-23 °C in the dark. After the dew treatment, the infected seedlings were transferred to a greenhouse growth room at 23°C, under natural daylight.

Eleven pathotypes were selected based on virulence for Swan (*Pc1*) and avirulence on the resistance gene of interest (Table 1). All are maintained as pure isolates in liquid nitrogen storage at the Plant Breeding Institute, University of Sydney, NSW, Australia. Australian oat crown rust differential lines were included to confirm resistance response of the parents to those associated with known resistance genes (Table S1). Each subpopulation was phenotyped at the F_3 generation with specific pathotypes selected to illicit the desired crown rust gene resistance response (Table 2). The populations Swan/Ensiler, Swan/PC46, Swan/PC50, and Swan/PC68 were screened with a single pathotype, selected based on pathogen virulence, due to the low availability of seed for these subpopulations.

Seedling tests were conducted in greenhouse facilities at the University of Sydney Plant Breeding Institute in 2022 and 2023. Crown rust infection types (IT) were scored 10-12 days after inoculation on a “0”-“4” scale described by Nazareno *et al*. (2018). ITs of “0” to 3” were considered to indicate resistance, while those of “3+” to “4” indicated susceptibility.

Genetic inheritance analysis

To determine the nature of resistance in the subpopulations evaluated, genetic models were proposed to identify the number of genes and their gene action involved in controlling resistance to crown rust, to fit the observed frequency of the phenotypic classes (NSR, Seg, and NSS). Goodness of fit of the expected Mendelian ratio of 1 Non Segregating Resistant : 2 Segregating : 1 Non Segregating Susceptible, for a single segregating dominant gene was tested at first. When this simple genetic model did not fit the data, more complex models were tested to determine the most suitable for the observed segregation (Table 2). The goodness-of-fit of each genetic model was tested through the Chi-squared test ($\chi 2$), based on the expected and observed frequencies of each phenotypic class, according to the equation presented by Steel *et al.* (1997):

$$\chi^2 = \Sigma\ [(Obs - Exp)^2 / Exp]; \text{ with n-1 degrees of freedom.}$$

Where: Obs = number of families observed in each phenotypic class; exp = number of families expected in a particular phenotypic class; and n = number of phenotypic classes of the genetic model.

DNA extraction

High-quality DNA was isolated from $F_{2:3}$ selected plants after scoring IT reaction, on each population and parents. The method used was an adaption of the CTAB Protocol according to Doyle and Doyle (1987) as follows. Approximately 0.2 g of plant material of each genotype was collected in an Eppendorf tube and kept over silica gel for 2–3 days for drying. The maceration of the plant tissue was performed with the aid of the Tissue Lyser II equipment (Qiagen). For each macerated tissue sample was added 800 µL of warmed CTAB buffer (containing 2 µL mL-1 of β-mercaptoethanol). The samples were shaken and incubated in a water bath at 65°C for 40 minutes. After incubation, 600 µL of Chloroform: phenol (24:1 v:v) was added. Each sample will be gently homogenized by tube inversion for two minutes until the two layers mix. The emulsion was centrifuged at 4000 rpm for 20 minutes. The aqueous phase was removed and transferred to a clean tube of 1.5 ml, add 2/3 to 1 volume of cold isopropanol and gently mixed to precipitate the nucleic acids. The samples were centrifuged at 10,000 rpm for 10 minutes, the supernatant discarded, and DNA dried until there was no isopropanol. 500 µl of washing buffer was

added to each tube and washed by gentle inversion. The sample was then centrifuged at 10,000 rpm for 10 minutes and left to stand for 2 minutes to dry the pellet. 100 µL TE (pH 8) with RNAase (1 µL per 100 µL TE) was added to each tube and kept in the oven at 37 °C for 1–2 h. The DNA samples were diluted to 50 ng/µL using double-distilled autoclaved water. DNA integrity and quality were evaluated by electrophoresis on 1.5% agarose gel. The DNA concentration was determined with a NanoDrop2000 spectrophotometer (Nanodrop® Technologies) and normalized to 80 ng/µl.

Genotyping

Resistant and susceptible tails of each $F_{2:3}$ subpopulation (Fig. S1) were genotyped by Diversity Arrays Technology genotyping-by-sequencing Inc., Canberra, ACT, Australia (www.diversityarrays.com). 728 DNA samples, including each parental twice (genotypic data pooled for maximum coverage), were genotyped with Oat DArTseq (1.0) - DArTseq High-density sequencing (2.5 milion reads), which returned 96,419 polymorphic DArT-Seq markers.

Markers sequences were aligned against the OT3098 oat reference genome (v2) (https://wheat.pw.usda.gov/GG3/graingenes-downloads/pepsico-oat-ot3098-v2-files-2021), by the same company that provided the markers, Diversity Arrays Technology.

Filtering for quality markers and genotypes

SNPs were filtered by lack of chromosome position, missing data, and minor allele frequency. Heterozygote calls were converted to missing allele calls to allow for the imputation algorithm to discern parental genotype. Poor quality markers were filtered and removed through the following data curation: minor allele frequencies (MAFs) < 0.05%; failed to provide information for >20 % of the lines without a mapped position on the Oat Genome OT3098 V2 were removed. After filtering markers, 15,940 SNPs were used for the combined AsNAM analysis. High-quality SNPs were successfully assigned to all 21 oat chromosomes. The number of markers per chromosome ranged from 378 (Chr1C) to 1,362 (Chr4D). SNP markers were located every 0.75Mbp on average. Most SNPs were assigned to the D genome, with 6,690 markers, followed by the C genome (4,654 makers) and the A genome (4,596 markers). Five individuals did not have genotypic information available, and three had > 20 % missing genotypic information. The total size of each

population is indicated in Fig. 1. The final AsNAM was performed with 707 genotypes, which included the 15 parental lines.

QTL analysis

Prior to analysis, IT ("0"–"4") data were converted to binary scores, where 0 = susceptible and 1 = resistant. Data analyses were performed using R Software (R Core Team 2021). Genetic relationships among the genotypes were investigated based on SNP markers using the "synbreed" package to produce a similarity matrix (Wimmer *et al.,* 2012). Principal component analysis (PCA) was also performed using R Software and the same package. The first three principal components were drawn as a biplot using "ggplot 2" (Wickham 2016) to visualize genetic variation between subpopulations.

Multiparent whole-genome QTL mapping was used to identify QTL in the AsNAM population with the "statgenMPP" R package, which utilizes an IBD-based mixed model approach for QTL mapping (Li *et al*., 2022). The design matrices contain the expected number of allele copies from a particular parent. Initially a mixed model for single-locus genome scanning was conducted incorporating a random cofactor with the highest −log10(P-value) of potential QTLs until no new QTL were discovered. Then IBD probabilities for AsNAM populations were calculated with the maximum distance between evaluation points set as 5 Mbp.

Markers with a minimum logarithm of odds (LOD) value of 3.0 were considered significant and designated as QTL and marker effects were calculated. The allele that confers resistance for each putative QTL was determined from marker effects. The percentage of phenotypic variance explained (R^2) and allelic effects were also estimated. The QTLs detected were mapped on the Oat OT3098 v2 genome and visualized with Software Mapchart 2.32 (Voorrips 2002).

Marker position data analysis

All significant SNP markers and belonging to the same haplotypes (window of significant SNP markers) (Table S3) were analyzed for biological understanding, data availability, and genomic complex of annotated genes on the Oat OT3098 v2 genome using 'EsemblPlants' database (https://plants.ensembl.org) (Yates *et al.,* 2022). This public platform includes annotation gene sets, variants, protein annotation, regulatory elements, repeat masking, and phylogenetic trees. A BLAST analysis with each desired marker sequence was run against the Oat OT3098 v2. It usually returned many hits, but just one

had the full length of the blasted sequence (64 bp) and was also located in the expected oat chromosome. The chromosome region of the correct hit, where the SNP sequence is located, is displayed as a detailed highlighted region. Next, we analyzed all the transcript ID genes on the marker's position and next to them. The transcript gene ID was identified and verified it might be involved in disease resistance, according to functions and family. Its sequence details and annotation were confirmed on the 'Grain Genes' database (Yao *et al.*, 2022). After, a wider region around the SNP position was inspected directly on the Oat OT3098 v2, at Grain Genes (https://wheat.pw.usda.gov/jb/?data=/ggds/oat-ot3098v2-pepsico) and other identified candidate genes were also investigated about function and family on the 'EsemblPlants' database. The candidate genes were then analyzed on the 'Gene Ontology' Enrichment Analysis (GO) (Thomas *et al.*, 2022) to assemble functions related to disease resistance. The GO provides the ontology of defined terms representing the gene product in three domains: cellular component, molecular function, and biological process.

RESULTS

Phenotypic analysis of resistance to crown rust

Pathotype tests were conducted to assess virulence and avirulence reactions in the parental lines (Table S1). All the susceptible plants showed IT 3+. The infection types of all resistant parental lines ranged from 0; to 12N. Swan was susceptible to all pathotypes used, showing compatible IT 3+ (Tables S2 and S3).

Genetic inheritance of the resistance to crown rust

The phenotypic distribution of each population is shown in Fig. S1. Genetic analysis was conducted for each subpopulation evaluated in the $F_{2:3}$ generation to determine the number of genes controlling resistance. The expected segregation of a single gene with complete dominance was observed in nine of the subpopulations, including Swan/Ensiler, Swan/PC36, Swan/ PC38, Swan/PC45, Swan/ PC46, Swan/ PC50, Swan/PC51 (Pathotype 500), Swan/ PC68, and Swan/ PC70.

When subpopulation SWAN/PC51 was challenged by pathotype 271 the best-fitted model was an epistatic one with three dominant genes segregating for resistance, but only two dominant genes, each carrying at least one dominant allele, were necessary at once to confer a resistant phenotype. Under this model, the expected segregating ratio is 10

NSR : 44 Seg : 10 NSS (Table 2). This same pattern of segregation was observed for the subpopulations SWAN/PC55, SWAN/PC58 and SWAN/PC60 (challenged by pathotype 501). The other subpopulation with the donor PC60, challenged by pathotype 271, segregated according to the model 12 NSR : 177 Seg : 67 NSS, where four genes were segregating for resistance, but only three of them, each carrying at least one dominant allele, were required at once to express the resistant phenotype.

Phenotypic evaluation of the SWAN /PC64 subpopulation showed 4 NSR, 36 Seg and 22 NSS families, this segregation fitted an epistatic genetic model of 1 NSR : 8 Seg : 7 NSS (Table 2), in which two dominant genes were segregating in the subpopulation and both of them, with at least one dominant allele each, were needed at once to exhibit a resistant phenotype.

The most complex segregation was observed in the subpopulation SWAN/PC13, challenged by two different pathotypes (135 and 179), showing the mendelian segregation ratio 16 NSR : 630 Seg : 378 NSS, in which there are five dominant genes segregating in the subpopulation and four of these genes, with at least one dominant allele each, have to be present at once in the genotype in order to be resistant to crown rust.

Population structure and IBD analysis

A weak to intermediate structuring among the lines were observed in the AsNAM population (Fig. 2; Fig. S3). Two main clusters were formed according to the genetic relationship among the oat lines, as revealed by the PCA analysis (Fig. S3). The first cluster comprised predominantly individuals crossed with donor parents carrying the resistance genes ***Pc38***, ***Pc45***, ***Pc46***, ***Pc50***, and ***Pc55***, which are all backcross derivatives of the variety ‘Pendek’, and, therefore, were expected to be similar. The remaining progenies formed a second cluster (Table 1 and Fig. S3).

SNP discovery and QTL mapping

The allele effects of each detected QTL were estimated by the IBD-based AsNAM analysis (Figure 4 and Table 3). The IBD-based mixed model approach for QTL mapping discovered eight QTL on eight different chromosomes (**chr1C**, **chr1D**, **chr2D**, **chr3D**, **chr4A**, **chr4C**, **chr5D**, **chr7D**), named **Qpc.NAM.01** to **Qpc.NAM.08** (Figs 3 and 4). Five new genes had their position reported for the first time in this study: ***Pc13*** (**chr3D**), ***Pc36*** (**chr1C**), ***Pc60*** (**chr1C**), ***Pc64*** (**chr7D**), and ***Pc70*** (**chr5D**). Four QTL associated

with the genes ***Pc38*** (**chr7D**), ***Pc45*** (**chr2D**), ***Pc46*** (**chr3D**), and ***Pc50*** (**chr3D**), previously mapped in the literature, had their location confirmed in this study (Table S2). The detected QTLs explained a wide range of phenotypic variance (R^2), with the lowest R^2 of 2 % and the highest of 21 %, explaining an average of 10 % of the phenotypic variance per subpopulations, even though in four of them, none of the phenotypic variance could be explained (Table 3). The eight QTL were:

- **Qpc.NAM.01**: **Chr1C**, explaining 10 % of the phenotypic variance. This QTL was associated with positive effects displayed by two parental lines, PC36 and PC60 (Table 3). Further analyses revealed that the marker SNP_5442448 was significantly associated with the QTL (Table S3).

- **Qpc.NAM. 03**: **Chr2D**. This QTL had its strongest association with the PC45 donor, with a LOD peak of 18.24 and explained 14 % of the phenotypic variance (Table 3). Three SNP markers, SNP_20626437, SNP_20625033_1, and SNP_5440447, flanked this QTL, but only SNP_20625033_1 was significantly associated to this QTL (Table S3).

- **QTL Qpc.NAM.04**: **Chr3D**. It was common between parents Ensiler, PC13, PC46, and PC50, peaking in the same chromosome location in all four subpopulations (Figure 5). It explained the highest phenotypic variance, 21 %, of all eight significant QTL, and a LOD score of 33.08 (Table 3). One significative SNP was reported on the QTL and another one was positioned next to the significant marker (SNP_10583358 and SNP_77981947 respectively).

- **Qpc.NAM.07**: **Chr5D**. This QTL was most strongly associated with resistance in the PC70 donor, with a strong effect (0.77) of this region on the phenotype (Table 3). One SNP marker was associated with this QTL (SNP_16624935; Table S3). This QTL explained 11 % of the variance with an LOD score of 8.68 (Table 3).

- **Qpc.NAM.08**: **Chr7D**. The resistance donors PC38 and PC64 showed a high contribution effect on this QTL, however the effect of PC38 (0.79) was more than twice that of PC64 (Table 3). Together, they accounted for 13 % of the variance, with the most significantly associated marker being SNP_3458380 (Table S3).

- **Qpc.NAM.02** (LOD 3.37), **Qpc.NAM.05** (LOD 4.05) and **Qpc.NAM.06** (LOD 3.0): **Chr1D**, **chr4A**, and **chr4C**, respectively. These QTL did not show strong effects from any of the parental lines (Table 3).

No relevant phenotypic effects were detected in the subpopulations Swan/PC51, Swan/PC55, Swan/PC58, and Swan/PC68 (Table 3).

Gene annotation

Ensembl Plants database was used to investigate the chromosomic regions identified as associated with crown rust resistance, revealing numerous genes of interest in the regions. The genes were selected according to function and type of coding protein. All significant QTL were associated with putative genes (Table 4).

The identified putative genes were predicted to encode proteins related to molecular functions and biological processes based on gene ontology analysis. A total of 31 genes were identified, belonging to eight protein families which are or might be involved with resistance (Table 4): WPP domain (tryptophan-proline-proline motif), Cystatin domain, NB-LRR (Nucleotide-Binding and Leucine-Rich Repeat domain), Protein Kinase domain, CYP (Cytochrome P450), Protein Kinase domain, ABC transporter, UDPGT (UDP-glucoronosyl and UDP-Glucosyltransferase domain) and WRKY domain. The most common gene family was NB-LRR, contributing with 18 of the 31 genes.

DISCUSSION

This work reports the first application of the Nested Association Mapping approach in *A. sativa* to identify genomic regions contributing to resistance to the oat crown rust pathogen *Pca*. The AsNAM consisted of 14 subpopulations carrying 13 catalogued *Pc* genes and one uncharacterized source of resistance from the variety Ensiler, which is resistant to most *Pca* isolates from Australia.

Within the eight identified QTLs (Figure 4 and 5), we found four *Pc* genes that were previously mapped, confirming and refining the genomic location of ***Pc38***, ***Pc45***, ***Pc46***, and ***Pc50*** (Table S2). We also identified the chromosomic location of five *Pc* genes (***Pc13***, ***Pc36***, ***Pc60***, ***Pc64***, and ***Pc70***) whose genomic locations were previously unknown (Table S2).

The identified QTL explained a significant portion of the phenotypic variance (R^2), ranging from 2 to 21 %, suggesting QTL of small to medium effects were contributing to crown rust resistance in the AsNAM population. The marker effects from the AsNAM combined with the genetic analyses revealed the number of genes likely controlling crown rust resistance in each biparental subpopulation (Tables S3 and S4). Five of the eight QTL

were detected on the D genome. The origin of the D genome in hexaploid oats remains uncertain; it is believed that the D-genome progenitor is more closely related to the A-genome than the C-genome, and that the diploid D genome donor species may be extinct (Peng *et al.*, 2022).

QTL **Qpc.NAM.04** accounted for the highest variance in phenotypic data in this study, 21 %. The PC13 donor showed a strong effect at this QTL, jointly with PC46, PC50, and Ensiler. The position genomic location of ***Pc13*** on **chr3D** is reported for the first time. According to the best-fit model, five loci were segregating for the resistance in the PC13 subpopulation (Table 2). Therefore, the resistance present in 'Clinton', the ***Pc13*** donor, is more complex than previously thought. This complexity also comes from the fact that, in addition to have five independent loci, the ***Pc13*** gene was previously described as carrying multiple alleles for resistance (***Pc13***, ***Pc13c*** e ***Pc13d***) (Finkner *et al.*, 1955; Chang 1959). Even though it cannot be ruled out, the possibility of these authors in fact, had found closely linked genes instead of multiple alleles of the ***Pc13*** gene. In contrast to the dominant gene action found for the five loci detected in our study, Chang (1959) showed that the resistance alleles ***Pc13c*** and ***Pc13d*** were recessive in other genetic backgrounds ('Argelia' and 'Dom Pedrito' varieties). The PC46 and PC50 subpopulations showed segregation for on single dominant gene, as described by Fleischmann *et al*. (1971b). It was previously reported that genes ***Pc44***, ***Pc46***, ***Pc50***, ***Pc68***, ***Pc95***, ***PcX*** are linked to each other (Martens *et al.*, 1968; Harder *et al.*, 1995; Wong *et al.*, 1983; Fleischmann *et al.*, 1971b) and with stem rust resistance genes *Pg3* and *Pg9* (McKenzie and Green 1965) (Table 1). According to Chong *et al*. (1994), ***PcX*** could be either ***Pc6c*** or ***Pc9***. Given that both the ***Pc6*** and ***Pc9*** loci comprise multiple alleles, either would add more complexity to this resistance linkage group (Park *et al*., 2022).

We were not able to detect the presence of ***Pc68*** in the linkage group of **Qpc.NAM.04**, **in ch.3D**, not being possible to confirm its position, probably due to the small size of the subpopulation and the absence of *Pc68* in another donor parent of the AsNAM population. Also, it was observed that PC46, PC50, and Ensiler were segregating for a single gene, which could either be the same gene or linked in **Qpc.NAM.04**. To further analyze this, we examined the pathotypes that were used to challenge PC13. We found that the reactions against these pathotypes were contrasting taking into account all the four donor parents PC13, PC46, PC50, and Ensiler (as detailed in Table S1). In addition, considering several pathotypes tests conducted at the Plant Breeding Institute (Table S4), it may be concluded that the ***Pc46***, ***Pc50***, and the gene present on Ensiler are not the same

and they are linked on the **Qpc.NAM.04**, although multiple allelism cannot be discarded. In addition, PC13 was segregating for 5 genes, which are all different from ***Pc46***, ***Pc50***, and Ensiler genes, according to the pathotypes reaction in Tables S2 and S7. As an example, pathotypes 500, 501, 503, 513, and 526 were all virulent on PC13 donor, but were avirulent on Ensiler, PC46 and PC50 donors (Table S4). The location of **Qpc.NAM.04** makers were analyzed, and we found six putative resistance genes on the markers' region. All of them encode the important Leucine-Rich Repeat (LRR) resistance protein family (Table 4). The NB-LRR (Nucleotide-Binding) proteins consist of the largest class in the category of all known resistance genes (R), which encompasses more than 80 % of characterized R genes (Song *et al*., 2017).

Qpc.NAM.03 has a strong allelic effect (0.82) in the PC45 subpopulation. This QTL also presents a high LOD score (18.24) and explains 14 % of the phenotypic variance. ***Pc45*** is potentially the candidate gene for this QTL and was mapped on **chr2D**, confirming its location with previous studies of Gnanesh *et al*., (2015) and Kebede *et al*., (2019). According to the best-fitted genetic model, based on the phenotypic data, PC45 subpopulation segregates for one single dominant gene, as reported also by Fleischmann *et al*., (1971a). In the region of this QTL, at the positions of the significant SNP marker (179.01Mb) and of the one of the marker within the QTL window (183.99 Mb) ten annotated disease resistance genes were identified, being six from the NB-LRR protein family and four from other four protein families (Table 4).

Qpc.NAM.01 was mapped on **chr1C** (457.34 Mbp). The highest effect of this QTL was identified in the subpopulations with PC36 (0.64) and PC60 (0.26) donors (Table 3). Therefore, ***Pc36*** and ***Pc60*** are potential candidate genes for this QTL. According to the results from Tables S2 and S7, these genes cannot be the same gene, based on infection type response to several pathotypes. It is the first time that the genomic location of ***Pc36*** and ***Pc60*** have being found. Swan/PC36 subpopulation fitted the expected Mendelian ratio of 1:2:1, showing that a single dominant gene was segregating in this subpopulation, corroborating with Simon's (1965) findings. However, Swan/PC60 subpopulation shows more complex resistance, revealing three or four segregating genes, according to the pathotype tested (Table 2). According to data from Plant Breeding Institute, on the differential collection some genes are not single. This is the case of ***Pc60***, which is reported to carry also the ***Pc61*** (Park *et al.*, 2022), which can explain part of the complex segregation. Our study does not allow to distinguish between ***Pc36*** and ***Pc60*** being alleles of the same gene or being tightly linked genes. This second hypothesis is more plausible

due to the different origins of the sources of these two genes. ***Pc36*** is derived from PI267989 (Table 1), which is an *Avena sterilis* strain from Portugal (Simons, 1965), while ***Pc60*** is derived from PI 287211 (Table 1), which is also an *Avena sterilis* line, but collect in Israel (https://triticeaetoolbox.org/POOL/index_db.php). On the region of the significant markers, two annotated disease resistance genes were identified, being one from the NB-LRR protein family and another one Cysteine proteinase inhibitor family (Table 4).

According to the study, **Qpc.NAM.07**, located on **chr5D**, has a strong positive effect of 0.70 in the PC70 donor subpopulation (Table 3). It confirms the tested genetic model, suggesting that a single gene is accountable for the resistance observed in this subpopulation (Table 2). Notably, this gene location has not been identified in any previous research, making this study the first to report its position. This QTL explained 11 % of the phenotypic variance and had one significant SNP marker associated (SNP_16624935) with it. This SNP position was associated with a coding protein kinase (PKA) gene. Protein kinases are well-known family proteins with functions to signal and respond to signals that regulate defense response pathways in plants (Zhang *et al*,. 2018).

The **Qpc.NAM.08** QTL was identified on **chr7D** in two subpopulations. The possible candidate genes are ***Pc38*** and ***Pc64*** for this QTL. This study has identified the probable position of ***Pc64***, which was reported for the first time, while ***Pc38*** was found in the same position on **Chr7D**, as reported by Wight *et al.* (2004). The best fit model for Swan/PC38 subpopulation was 1:2:1 in agreement with Chong *et al*. (2000), and Swan/PC64 was 1:8:7, in which the resistance is controlled by at least two dominant genes acting in an epistatic way. Unfortunately, because of the same chromosome location and the two genes segregating in the subpopulation Swan/PC64 it is not possible to distinguish among three hypotheses for *Pc38* and *Pc64*: i) both the same gene and same alleles; ii) they are different alleles of the same gene; and iii) they are different genes tightly linked on QTL. **Qpc.NAM.08**. ***Pc38*** and ***Pc64*** showed different infection types when challenged by the same pathotypes (Tables S2 and S7). This would be an indication of different genes or different alleles of the same gene, but because the donor parent PC64 has a second gene controlling the resistance to crown rust (Table S1), the reactions against the same pathotypes may differ even if both ***Pc38*** and ***Pc64*** are the allele of the same gene. Also, this QTL may be associated with other resistance genes, such as *Pc62* and *Pc63*, which have been reported to be clustered with *Pc38* (Harder *et al,*. 1980). In addition, our findings reported one SNP marker significantly associated to the QTL **Qpc.NAM.08**.

Based on the Esembl Plants database analysis, the position of this SNP is very close to a UDP-glycosyltransferase activity (UGT) coding protein gene, in addition to a NB-LRR gene close by (Table 4). UGT proteins are well-known to be involved on the resistance response in plants (Gharabli *et al.*, 2023), playing an important indirect role in regulating biotic stress responses *via* the glycosylation of phytohormones (Gharabli *et al.*, 2023).

It was not possible to associate **Qpc.NAM.02** (**chr1D**) and **Qpc.NAM.06** (**chr4C**), with any specific gene donor subpopulation. In the NAM analyses, the QTLs are detected due to the strong effect in one donor or small effects in several donors, the last one being the case on these QTL. Probably the resistance detected by these QTL is quantitative, with several genes with small effects in many donors. This can be explained also by the low LOD score and very low percentage of variance explained or sometimes absent (Table 3). It is also important to highlight that the donor PC45 might carry a susceptibility gene, *i.e.*, a plant gene that is necessary for pathogen infection and/or colonization, once it shows a high negative effect on **Qpc.NAM.05** (**chr4A**), and it is the main responsible for the detection of this QTL (Table 3).

From the donors PC51, PC55, PC58, and PC68 subpopulations, it was not possible to map any QTL. The swan/PC51 subpopulation segregates for one or three genes, according to the pathotyped tested. According to Browning and Frey (1962), ***Pc51*** is a single dominant gene.

Swan/PC55 is another subpopulation that exhibited complex segregation, in which there are three dominant genes segregating for the resistance (Table 2). According to Kiehn *et al.* (1976), ***Pc55*** is an incompletely dominant gene that is influenced by genetic background, being recessive in crosses with Pendek (Kiehn *et al.*, 1976). ***Pc55*** also have been reported as a dominant gene in crosses with Algerian, TAM-O-312, and several Pendek isolines carrying other genes (Brouwer, 1983), which agrees with our findings, since we used ***Pc55*** in a Pendek background (Table 1). The more complex inheritance of PC55 crown rust resistance may explain why the subpopulation Swan/PC55 showed a small positive effect of 0.24 in two QTL, **Qpc.NAM.06** and **Qpc.NAM.07**, located on **chr4C** and **chr5D**, respectively (Table 3). Research by Kiehn *et al*. (1976) and Leonard *et al*. (2005) suggests that ***Pc55*** and ***Pc39*** are either very closely linked or allelic. Similarly, Chong and Seaman (1989) reported an association between virulence for ***Pc39*** and ***Pc55*** in Canada (Table S2). Wight *et al*. (2004) mapped *Pc39* to a region homologous to KO group 37, while more recent studies by Sowa and Paczos-Grzeda (2020) and Zhao *et al*. (2020) both mapped ***Pc39*** to Mrg11, on **chr4C**.

The best-fitted model for Swan/PC68 followed the Mendelian ratio of 1:2:1, for a single dominant gene segregation. While the Swan/PC58 subpopulation best-fitted model was 10:44:10, which predicts three epistatic dominant gene segregating for resistance, only two of them are needed at once for the resistant phenotype (Table 2). Even though the Swan/PC58 and Swan/PC68 subpopulations did not reveal any significant QTL, the resistance genes these subpopulations must carry have been previously mapped. ***Pc58*** was already mapped (Hoffman *et al*., 2006; Jackson *et al*., 2007; Klos *et al*., 2017) on Mrg02 (**chr7D**) (Table S2). ***Pc68*** was mapped by Chen *et al*. (2006), Kulchelski *et al*. (2010) and Klos *et al*. (2017) on Mrg19 (**chr3D**). The fact of these genes could not be mapped might be due to the low size of the subpopulations and the probable absence of ***Pc58*** e ***Pc68*** from other parental donors included in the AsNAM population. Also, the fact no QTL could be mapped from donors PC58 and PC68 is not related to marker coverage in the region where their resistance genes must be located, once ***Pc58*** and ***Pc68*** were previously mapped in the genome D, and is the genome with higher marker coverage in the AsNAM population, with 6,690 SNP Markers. Another studies by Wong *et al.* (1983) and Fleischmann *et al.* (1971b) suggests that ***Pc46*** and ***Pc50*** are also linked with ***Pc68***, showing that the low marker coverage of the genome is a false hypothesis for the failure of mapping ***Pc58*** e ***Pc68***, once ***Pc46*** and ***Pc50*** were successfully mapped on this study (Figure 5).

The absence of single gene reference stocks makes it very difficult to resolve the genetic basis of the resistance to crown rust and to identify with *Pc* gene is present in an accession with more complex resistance inheritance (Park *et al.,* 2022). In our study, the donors of ***Pc13***, ***Pc51***, ***Pc55***, ***Pc58***, ***Pc60,*** and ***Pc64*** do not carry single resistance genes (Table 2). According to Park *et al.,* (2022), reference stocks have been identified for all designated *Pc* genes, however, 35 of them are not currently accessible as single genes or with single resistance specificity. ***Pc64*** is known to also carry ***Pc65***, *and* ***Pc66*** (Park, RF unpublished), agreeing with findings that more than one gene is segregating. Also, ***Pc60*** was reported to carry ***Pc61*** by Park *et al.*, (2022). A further complicating factor with the designation of *Pc* genes is that the allelic relationships between many of them are unknown. Also, it is reported that several Pendek isolines carry other genes (Brouwer 1983), being the case of some of the genotypes evaluated in this study (Table 1). Similarly, oat crown rust pathotypes are known to be very complex, with many virulent genes. Therefore, it is not unexpected that genotypes previously identified as having just one resistance gene may carry more than one. This is determined by the pathotype utilized

to challenge these oat genotypes. In a study by Leonard and Martinelli (2005) they found minimum of two virulent genes and a maximum of 22 virulent genes in each pathotype evaluated. This scenario can be even worse once populations of this pathogen exhibit high variability in virulence phenotypes on oat varieties and rapid emergence of new virulence pathotypes (Park, 2008; Nazareno, 2018). Several decades ago, more than 400 physiological races of *Pca* had been already identified worldwide (Harder and Haber, 1992). In order to overcome these obstacles, when more than one resistance gene is found in a specific genotype, single gene isolines should be generated for each and identified as single gene reference stocks. Also, genome sequencing and mutational genetics approaches may resolve the situation in the future (Park *et al.,* 2022).

As confirmed in this study, it is not unusual for gene clustering to occur in oats. The hexaploidy oat genome contains multiple clusters of crown rust resistance genes. According to Kamal *et al*. (2022), around 1,269 R-genes across the three hexaploidy oat subgenomes, most of them occur in clusters. The R gene family has been widely expanded in several plant species, originating from duplication or alterations in DNA sequences (Michelmore and Meyers, 1998). From an evolutionary point of view, cluster organization is regarded as a reservoir of genetic variation, suggesting how plants can generate and maintain a large number of resistance genes against changing pathogen populations (Graham *et al*., 2002).

Genes that surely are related or might be related to disease resistance were found nearby of all eight detected QTL, even the ones that explained few amounts of the phenotypic variance.

Nearby the QTL **Qpc.NAM.01** (**chr1C**), there are two candidate genes to be involved in the resistance to crown rust (Table 4), where ***Pc36*** and ***Pc60*** can be the candidates (Table 3). One of the genes codes for a RAN GTPase-activating protein 2, which contains a WPP domain and LRR domains, and the other one codes for a Cysteine proteinase inhibitor 3, a gene from the Cystatin protein family (Table 4). The resistance to the potato virus X, a pathogen *Solanum tuberosum* and tobacco (*Nicotiana* spp.), is controlled by the resistance gene Rx, which belongs to NB-LRR family. The Rx protein interacts with a cofactor that is a Ran GTPase-activating protein 2 (RanGAP2) (Hao *et al.,* 2013). RanGAP2 is involved in nucleocytoplasmic trafficking of macromolecules through the nuclear pores, and the physical interaction between these two proteins is essential to the activation of the resistance mediated by Rx (Tameling and Baulcombe 2007). Protease inhibitors, besides their role in endogenous regulation, also play a role in the defense against pests

and pathogens (Habib and Fazili, 2007). In *Solanum tuberosum*, the cysteine proteinase inhibitor (CPI) helps the plant resist *Phytophthora infestans*. When CPI is silenced, there is a significant increase in lesion size and water soaking, indicating that the protein plays a critical role in limiting lesion expansion (El-Banna and Taller, 2017).

On chromosome **chr1D**, four candidate genes were found nearby the significant marker for the QTL **Qpc.NAM.02** (Table 4), which is associated to the PC36 donor (Table 2). each of these genes was from a different gene family (NB-LRR, Protein kinase, Cytochrome P450 and ABC transporter). Most resistance proteins (R) contain a central nucleotide-binding domain (NB). These R proteins contain a C-terminal leucine-rich repeat (LRR) domain fused to a central nucleotide-binding (NB) domain (NB-LRR proteins). The core nucleotide-binding fold in NB-LRR proteins is part of a larger entity called the NB-ARC domain, known to be involved in pathogen recognition and activation of immune responses (Van Ooijen *et al.,* 2008). The other three genes may be involved in the response to the pathogen attack and not directly to its recognition, making them also candidates for the **Qpc.NAM.02** QTL, because there was no strong phenotypic effect from any NAM subpopulation (Table 3), and many genes may be quantitatively involved with the resistance in this QTL.

Protein kinases catalyze the transfer of phosphate between their substrates (phosphorylation). The protein kinase pathways work together in signaling and responding to signals that regulate cellular processes. Phosphorylation is crucial in plant immune responses. In *Arabidopsis thaliana* with over 1,170 phosphopeptides have been identified (Benschop *et al*., 2007). Many phosphorylated proteins are key signal transduction components of defense responses, such as receptor-like kinases (RLKs), mitogen activated protein kinases (MAPKs) and calcium-dependent protein kinases (CDPKs) (Zhang *et al*., 2018).

CYTOCROMO P450 (CYP) proteins are responsible for regulating the expression of certain genes, to adapt to environmental stresses such as diseases (Pandian *et al.,* 2020). This is the case of avenacins in oats (*Avena spp.),* an antimicrobial compound well known to provide protection against disease. Avenacins are synthesized from a gene encoding a member of the CYP51 family of cytochromes P450 (AsCyp51H10) (Geisler *et al.,* 2013). This was because the CYP gene family has many important functions in plants, particularly in the detoxification of external or stress-induced compounds during secondary metabolism (Pandian *et al.*, 2020).

While NB-LRR, usually, remains effective for short periods after being deployed in agricultural systems, *Lr34*, a wheat gene durable gene, which confers partial resistance against leaf rust (*Puccinia recondita* f.sp. *tritici*), stem rust (*Puccinia graminis* f. sp. *tritici*) and powdery mildew (*Blumeria graminis* f. sp. *tritici*). *Lr34* codes an ABC transporter protein, which differs only in aminoacids at the first transmembrane domain between the susceptible and the resistance alleles. The closest orthologues to *Lr34* are two ABCG transporter genes from *Oryza sativa* and *Sorghum bicolor* (Deppe *et al.*, 2018). The gene found closest to the significant marker associated with **Qpc.NAM.02** is also an ABC transporter of the G subfamily (Table 4), a gene subfamily known to be involved in the transport of plant hormones, such as abscisic acid (ABA) and jasmonate, among other substrates, and there is strong evidence that ABA is a substrate of *Lr34* (Do *et al*., 2021).

Genes of the LRR protein family were also found to be linked with significant markers on QTLs **Qpc.NAM.01** (**chr1C**) and **Qpc.NAM.03** (**chr2D**). One LRR gene was found in **Qpc.NAM.01** (Table 4), associated with the PC36 donor (Table 3), making this gene (AVESA.00001b.r3.1Cg0001866) a strong candidate for the ***Pc36*** gene, on **chr1C** (Table 4).

A total of ten candidate genes were found nearby the location of two markers associated with the **Qpc.NAM.03** QTL, six of them are located closer to the significant marker SNP_20625033_1, at position 179.01 Mb of **chr2D**, and four are located near the non-significant marker SNP_5440447, within the QTL window, at position 183.99 Mb of **chr2D** (Table 4). Among the ten genes, six belong to the NB-LRR families, being the strongest candidates for **Qpc.NAM.03**. The other genes present very different molecular functions (protein kinase, monooxygenase, ATP-binding, and UDP-glycosyltransferase activities), but all are possibly involved with biotic stress response (Table 4). Monooxygenase is an activity expressed by protein from the Cytochrome P450 family, already discussed above. The ATP binding activity was found in a protein that is probably a LRR receptor and exhibits a kinase activity (Table 4), being also possibly associated with biotic stress response. The presence of many genes in the region of **Qpc.NAM.03** and the very strong phenotypic effect from the PC45 donor at this QTL (Table 3), indicating the possibility of ***pc45*** being, in fact, a complex locus, comprising many genes involved with pathogen recognition and pathogen response.

Many examples of resistance related to proteins with UDP-glycosyltransferase activity, which belong to the UDPGT domain family, were reviewed by (Gharabli *et al.*, 2023).

Plants utilize UDPGTs for multiple functions, including growth regulation and development, protection against pathogens and abiotic stresses, and adaptation to changing environments. The endogenous substrates of UDPGTs in plants include phytohormones, defensive compounds, and other secondary metabolites. In addition, UDPGTs can glycosylate exogenous foreign compounds, *i.e.*, xenobiotics, such as toxins from pathogens and pollutants (Gharabli *et al.,* 2023). UDP-glucosyltransferase was reported to be evolved on Fusarium Head Bligh (FHB) resistance and decreasing of mycotoxins caused by these fungi in Wheat (Li *et al*., 2015, Xing *et al*., 2018) and resistance against *Magnaporthe grisea* in rice (Tezuca *et al.,* 2021). Tezuca *et al.* (2021) reported UDP-glucosyltransferase gene mutants accumulated high levels of salicylic acid (SA) under non-stressed conditions. SA is a phytohormone that regulates a variety of physiological and developmental processes, including disease resistance being a key signaling component in the immune response of many plant species (Nandety *et al*., 2013).

In the region of **Qpc.NAM.04** (**chr3D**), six resistance genes were identified, all of them encoding the NB-LRR (Table 4), known to be involved in pathogen recognition and activation of immune responses (van Ooijen *et al.,* 2008). Four parental donors had positive effects on the **Qpc.NAM.04** (Ensiler, PC13, PC46, and PC50). Of these donors, only PC13 had a complex inheritance, with five genes segregating for crown rust resistance, and the other three segregated for a single resistance gene (Table 2). Therefore, there are more candidate genes in this QTL region than the number of effects detected from the parental donor (Table 3). These findings added to different response to pathotypes (Tables S2 and S7) allow to infer that Ensiler must carry a different gene them ***Pc46*** and ***Pc50***, and possible from ***Pc13***. Although PC13 donor may have different response to the tested pathotypes than Ensiler due to its complex inheritance (Table 2).

QTL **Qpc.NAM.05** (**chr4A**) explained just a low amount of the phenotypic variance (6 %) but is of interest because there is a strong negative effect in Swan/PC45 subpopulation (Table 3), indicating that it may be a susceptibility gene, as already pointed out above. In the genome region of the significant marker associated to this QTL there are many unknown genes and many identified ones that must not be involved with pathogen resistance. However, nearby there is a gene from the WRKY family that may be a candidate gene for the **Qpc.NAM.05**. WRKY proteins are transcription factors associated with a variety of regulatory functions, including disease resistance. Although most WRKY related to pathogen response are positive regulators of disease resistance,

there are examples, such as *WRKY38* and *WRKY62*, from *Arabidopsis thaliana*, that are negative regulators of the resistance against the bacteria *Pseudomonas syringae* (Kim *et al.*, 2008). *Ta*WRKY49, from *Triticum aestivum*, is associated with negative regulation of seedling resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*), under high temperature (Wang *et al*., 2017).

Similar to the findings for **Qpc.NAM.04**, at the proximity of QTL **Qpc.NAM.06** (**chr4C**) there are four NB-LRR coding protein genes. There was no strong positive phenotypic effect coming from any parental donors at **Qpc.NAM.06**, only weak effects were detected from PC13, PC55 and PC70 (Table 3). PC70 subpopulation segregated for only one resistance gene, while PC13 and PC55 had more complex resistance inheritance (Table 2). The PC70 resistance gene must be located on **Qpc.NAM.07** (**chr5D**). Therefore, it is possible that the NB-LRR genes in the region of **Qpc.NAM.06** have residual effects on the crown rust resistance and/or genes contributing for the resistance of PC13 and PC55. Residual effects of major resistance genes are observed when the gene is already defeated by virulent pathogen races but still present an incomplete resistance response to these races. Genes with residual effects may contribute to resistance durability when pyramid in a given genotype (Pedersen and Leath 1988).

It is not a surprise to find many NB-LRR genes nearby the same QTL as for **Qpc.NAM.03**, **Qpc.NAM.04** and **Qpc.NAM.06** (Table 4). The NB-LRR (Nucleotide-Binding) proteins consist of the largest class in the category of all known R genes (Song *et al*., 2017). NB-LRR domain proteins can detect pathogen secretions and trigger signaling pathways that activate plant defenses against a variety of pathogens (Dubey and Singh 2018). Based on the sequenced genomes of various plant species, hundreds of NBS-LRR genes have been identified (McHale *et al.,* 2006). These classes of R genes evolve rapidly, and the number of genes varies among different plant species (Rafiqi *et al*., 2009). *Arabidopsis thaliana*, the first sequenced plant species, has 165 NBS-LRR genes (Zhang *et al*., 2016). Plants that belong to the *Solanaceae* family like *Solanum tuberosum* (potato) and *Solanum lycopersicum* (tomato) have more than twice the number of NBS-LRR genes than Arabidopsis (Jupe *et al.,* 2012). Recently, Kamal *et al.* (2022) identified 1,269 R-genes across the three subgenomes in *A. sativa*. This number is bigger when compared to Arabidopsis genome, once *A. sativa* is a hexaploidy and suffered duplication events during its evolution. Our findings are in line with the discoveries made by Kamal *et al*. (2022).

At the genomic regions of QTL **Qpc.NAM.07** (**chr5D**) and **Qpc.NAM.08** (**chr7D**) there were identified four candidate genes, two in each QTL, having in common that there is a NB-LRR gene in each QTL region, while the other two genes belong to different protein families. At **Qpc.NAM.07** (**chr5D**) it was found a gene coding for G-type lectin S-receptor-like serine/threonine-protein kinase SRK, that is a protein kinase, while at there is gene coding for a putative UDP-rhamnose:rhamnosyltransferase 1, from the UDPGT protein family, with UDP-glycosyltransferase activity (Table 4). The NB-LRR genes have a higher probability of being the candidate genes for *Pc70* in **Qpc.NAM.07** (**chr5D**) and *Pc38* in **Qpc.NAM.08** (**chr7D**), which donor parents had the strongest phenotypic effect at each QTL (Table 3). The play of the two other candidate genes cannot be ruled out because genes from protein kinase and UDPGT protein families have been implicated with biotic stress, as already wrote. They could be playing a role in crown rust resistance in parental donors PC55 e PC64, that have a mild phenotypic effect on **Qpc.NAM.07** and **Qpc.NAM.08**, respectively (Table 3) and were segregating for more than one resistance gene in their NAM subpopulation (Table 2).

Based on the QTLs, we found 31 genes belonging to different gene families, consequently to different mechanisms of resistance. These findings can be really useful in breeding programs. Once a resistance with different mechanisms of resistance is present, the pathogen finds it harder to overcome it. An example is join the ***Pc45*** (**Qpc.NAM.03)** with ***Pc70*** and ***Pc38*** **(Qpc.NAM.08)** which have NBLRR protein, but also encode UDP proteins, probably being different mechanisms of resistance, and a good combination of resistance genes.

The nested association mapping requires specially designed statistical tools to deal with complex traits. For this reason, the IBD-based model was implemented in this study, allowing to estimate random QTL effects, by analyzing the IBD probabilities of parental origins throughout the offspring genome. This method accounts for both polygenic and family background genetic variation, resulting in increased mapping power (Li *et al.*, 2021). Another advantage of NAM analysis is, when a joint analysis of subpopulations is made, the chance of finding new QTL is higher than analyzing each biparental population individually. In a study on *Arabidopsis thaliana*, among the nine QTLs identified for flowering time, four QTLs were significant only when analyzed in a multiple-cross mating design sharing one common parent (Li *et al.*, 2011).

In our design, we used a balanced size of subpopulations to not cause any bias on the effect of the detected QTL. Even though we were not able to find strong phenotypic

effects, on the mapped QTL, from four subpopulations: Swan/PC51, Swan/PC55, Swan/PC58, and Swan/PC68. This may be due to the small size of our subpopulations (50 plants), besides the possible absence of the resistance genes carried by these donors by any other resistant parents, reducing the power to detect existing QTLs in the AsNAM population.

In one hand, larger population size leads to higher genotyping costs and turns phenotyping even more challenging when compared to biparental populations. On the other hand, it increases the number of recombinations and improves the ability to detect QTL, while providing more precise estimations of allelic effects (Cockram and Mackay, 2018). Our population design tried to reach a balance among population size, cost, and amount of work required for phenotyping.

The selection of donor parents is another critical factor in the development of the NAM population. Donor parents should be as diverse as possible and should represent the maximum genetic diversity for target traits, enhancing the chances of multiple groups segregating for a specific trait or allele (Chidzanga *et al.*, 2021). In this present study, we select donors who were predicted to carry *Pca* resistance genes (Table 1). We use 14 donors to build our AsNAM population. The population structure was week to intermediary due the presence of many donors with Pendek on its background (Table 1 and Figure 3). A study of Stich (2009) in maize and *Arabidopsis thaliana* revealed that the higher the number of founder parents of the NAM population the higher the power of QTL detection. However, this can increase the difficulty of obtaining the NAM population because of the greater number of crosses necessary to build the population.

It has also been observed that the NAM method is more effective in detecting QTLs than GWAS in situations under low heritability, small population size, and small effects of QTLs. However, GWAS has greater QTL detection power than NAM when dealing with large population sizes and high heritability. This could be attributed to the loss of allelic diversity in the NAM population due to its balanced population structure with fewer crossovers, as compared to an association panel (Bouchet *et al.*, 2017).

The NAM mapping coupled with IBD-approach allowed to find eight QTL and associated significant SNP markers to them (Table S3). In the genomic regions of these QTL, 31 candidate genes were located, which may play direct or indirect roles on crown rust resistance (Table 4). These candidate genes are a starting point for future research to investigate their possible roles in disease resistance, using techniques such as gene cloning or genome edition. Also, molecular markers may be specially designed to validate

the identified QTL e to implement marker assisted selection for crown rust resistance in oat breeding programs.

ACKNOWLEDGMENTS

The first author was a recipient of a scholarship from the Coordination for the Improvement of Higher Education Personnel (CAPES) and Institutional Internationalization Program (CAPES/PrInt).

REFERENCES

Admassu-Yimer B, Bonman, M. J, Klos K. E. (2018) Mapping of crown rust resistance gene *Pc53* in oat (*Avena sativa*). *PLOS One*, 13(12), e 0209105. https://doi.org/10.1371/journal.pone.0209105

Avena sativa – OT3098 v2, PepsiCo, https:// wheat. Pw. Usda. Gov/ jb?data=/ ggds/ oat- ot309 8v2- pepsico

Bajgain P., Rouse M. N., Tsilo T. J., Macharia, G. K., Bhavani, S., Jin, Y., Anderson, J. A. (2016) Nested association mapping of stem rust resistance in wheat using genotyping by sequencing. *PLOS One*, 11(5), e0155760. https://doi.org/10.1371/journal.pone.0155760

Benschop J. J., Mohammed S., O'Flaherty M., Heck A. J. R., Slijper, M., Menke, F. L. H. (2007) Quantitative phosphoproteomics of early elicitor signaling in Arabidopsis. *Molecular and Cellular Proteomics*, 6(7), 1198-1214. https://doi.org/10.1074/mcp.M600429-MCP200

Blanc G., Charcosset A., Mangin B., Gallais A., Moreau, L. (2006) Connected populations for detecting quantitative trait loci and testing for epistasis: An application in maize. *Theoretical and Applied Genetics*, 113(2), 206-224. https://doi.org/10.1007/s00122-006-0287-1

Bouchet S., Olatoye M. O., Marla S. R., Perumal R., Tesso T., Yu J., Tuinstra M., Morris G. P. (2017) Increased power to dissect adaptive traits in global sorghum diversity using a nested association mapping population. *Genetics*, 206(2), 573-585. https://doi.org/10.1534/genetics.116.198499

Brinkman M. A., Forsberg R.A., Stevens J. B., Duerst R. D. (1990) Ensiler oats. *In*: 1989 *Oat Newsletter*, 40, 87 (Ed. M.S. McMullen).

Brouwer J. B. (1983) *Inheritance and breeding potential of resistances to Puccinia coronata avenae in oats*. PhD Thesis. University of Sydney, Australia, 370 pp.

Browning J. A., Frey K. J. (1962) Genetics of oat stem rust resistance. 1. Inheritance of reaction to races 6, 8, and I3A in C.I. 3039. *Iowa State Journal of Science*, 36, 483-489.

Buckler E., Gore M. (2007) An Arabidopsis haplotype map takes root. *Nature Genetics*, 39,(9), 1056-1057. https://doi.org/10.1038/ng0907-1056

Chang T. T. (1959) *Analysis of genes conditioning resistance of oat varieties to races of Puccinia coronata Cda. var. avenae F. and L.* Ph.D. thesis. University of Minnesota, St. Paul. 1959.

Chen G., Chong J., Gray M., Prashar S., Procunier, D. J. (2006) Identification of single-nucleotide polymorphisms linked to resistance gene *Pc68* to crown rust in cultivated oat. *Canadian Journal of Plant Pathology*, 28(2), 214-222. https://doi.org/10.1080/07060660609507289

Chidzanga C., Fleury D., Baumann U., Mullan D., Watanabe S., Kalambettu P., Pontre R., Edwards J., Forrest K., Wong D., Langridge P., Chalmers K., Garcia M. (2021) Development of an australian bread wheat nested association mapping population, a new genetic diversity resource for breeding under dry and hot climates. *International Journal of Molecular Sciences*, 22(9), e4348. https://doi.org/10.3390/ijms22094348

Chong J., Seaman W. L. (1989) Virulence and distribution of *Puccinia coronata* in Canada in 1988. *Canadian Journal of Plant Pathology*, 11(4), 439-442. https://doi.org/10.1080/07060668909501094

Chong J., Howes N. K., Brown P. D., Harder D. E. (1994) Identification of the stem rust resistance gene Pg9 and its association with crown rust resistance and endosperm proteins in “Dumont” oat. *Genome*, 37(3), 440-447. https://doi.org/10.1139/g94-062

Chong J., Leonard K. J., Salmeron J. J. (2000) A north American system of nomenclature for *Puccinia coronata* f. sp. *avenae*. *Plant Disease*, 84(5), 580-585. https://doi.org/10.1094/PDIS.2000.84.5.580

Cockram J., Mackay, I. (2018) Genetic mapping populations for conducting high-resolution trait mapping in plants. *Advances in Biochemical Engineering/Biotechnology*, 164, 109-138. https://doi.org/10.1007/10_2017_48

Deppe J. P., Rabbat R., Hörtensteiner S., Keller B., Martinoia E., Lopéz-Marqués, R. L. (2018) The wheat ABC transporter Lr34 modifies the lipid environment at the plasma membrane. *Journal of Biological Chemistry*, 293(48), 18667–18679. https://doi.org/10.1074/jbc.RA118.002532

Díaz-Lago J. E., Stuthman D. D., Leonard K. J. (2003) Evaluation of components of partial resistance to oat crown rust using digital image analysis. *Plant Disease*, 87(6), 667-674. https://doi.org/10.1094/PDIS.2003.87.6.667

Do T. H. T., Martinoia E., Lee Y., Hwang J. U. (2021) 2021 update on ATP-binding cassette (ABC) transporters: how they meet the needs of plants. *Plant Physiology*,187, 1876-1892. https:// doi.org/10.1093/plphys/kiab193

Doyle J. J., Doyle J. L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19(1), 11-15. Available at < https://webpages.charlotte.edu/~jweller2/pages/BINF8350f2011/BINF8350_Readings/Doyle_plantDNAextractCTAB_1987.pdf>

Dubey N., Singh K. (2018) Role of NBS-LRR Proteins in Plant Defense. *In*: Singh, A., Singh, I. (eds) *Molecular Aspects of Plant-Pathogen Interaction*. Springer, Singapore.

El-Banna A., Taller J. B. (2017) Functional characterization of the silenced potato cysteine proteinase inhibitor gene (PCPI) in *Phytophthora infestans* resistance. *Physiological and Molecular Plant Pathology*, 100, 23-29. https://doi.org/10.1016/j.pmpp.2017.05.002

Finkner R. E., Atkins R. E, Murphy H. C. (1955) Inheritance of resistance to two races of crown rust in oats. *Iowa State College Journal of Science*, 30, 211-228.

Fleischmann G., McKenzie R. I. H., Shipton W. A. (1971a) Inheritance of crown rust resistance in *Avena sterilis* L. from Israel. *Crop Science*, 11, 451-453. https://doi.org/10.2135/cropsci1971.0011183X001100030044x

Fleischmann G., McKenzie R. I. H., Shipton W. A. (1971b) Inheritance of crown rust resistance in *Avena sterilis* L. from Israel, Portugal and Tunisia. *Canadian Journal of Genetics and Cytology*, 13, 251-255. https://doi.org/10.1139/g71-042

Flor H. H. (1956) The Complementary Genic Systems in Flax and Flax Rust. *Advances in Genetics*, 8(C), 29-54.

Fu D., Uauy C., Distelfeld A., Blechl A., Epstein L., Chen X., Sela H., Fahima T., Dubcovsky J. (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science*, 323(5919), 1357-1360.

https://doi.org/10.1126/science.1166289

Geisler K., Hughes R. K., Sainsbury F., Lomonossoff G. P., Rejzek M., Fairhurst S., Olsen C. E., Motawia M. S., Melton R. E., Hemmings A. M., Bak S., Osbourn A. (2013) Biochemical analysis of a multifunctional cytochrome P450 (CYP51) enzyme required for synthesis of antimicrobial triterpenes in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 110(35), E3360-E3367. https://doi.org/10.1073/pnas.1309157110

Gharabli H., Della Gala V., Welner D. H. (2023) The function of UDP-glycosyltransferases in plants and their possible use in crop protection. *Biotechnology Advances,* 67, e108182. https://doi.org/10.1016/j.biotechadv.2023.108182

Gireesh C., Sundaram R. M., Anantha S. M., Pandey M. K., Madhav M. S., Rathod S., Yathish K. R., Senguttuvel P., Kalyani B. M., Ranjith E., Venkata Subbarao L., Kumar Mondal T., Swamy M., Rakshit S. (2021) Nested Association Mapping (NAM) Populations: Present Status and Future Prospects in the Genomics Era. *Critical Reviews in Plant Sciences*, 40(1), 49-67. https://doi.org/10.1080/07352689.2021.1880019

Gnanesh B. N., McCartney C. A., Eckstein P. E., Mitchell Fetch J. W., Menzies J. G., Beattie A. D. (2014) Genetic analysis and molecular mapping of a seedling crown rust resistance gene in oat. *Theoretical and Applied Genetics*, 128(2), 247-258. https://doi.org/10.1007/s00122-014-2425-5

Graham M. A., Marek L. F., Shoemaker R. C. (2002) Organization, expression and evolution of a disease resistance gene cluster in soybean. *Genetics*, 162(4), 1961-1977. https://doi.org/10.1093/genetics/162.4.1961

Habib H., Fazili K. M. (2007) Plant protease inhibitors: a defense strategy in plants. *Biotechnology and Molecular Biology Review*, 2 (3), 068-085. https://academicjournals.org/article/article1380100578_Habeeb%2520and%2520Khalid.pdf

Hao W., Collier S. M., Moffett P., Chai J. (2013) Structural basis for the interaction between the potato virus X resistance protein (Rx) and its cofactor Ran GTPase-activating protein 2 (RanGAP2). *Journal of Biological Chemistry*, 288(50), 35868-35876. https://doi.org/10.1074/jbc.M113.517417

Harder D. E., McKenzie R. I. H., Martens J. W. (1980) Inheritance of crown rust resistance in three accessions of *Avena sterilis*. *Canadian Journal of Genetics and*

Cytology, 22(1), 27-33. https://doi.org/10.1139/g80-005

Heath M. C. (2000) Nonhost resistance and nonspecific plant defenses. *Current Opinion in Plant Biology* 2000, 3(4), 315-319. https://doi.org/10.1016/S1369-5266(00)00087-X

Hoffman D. L., Chong J., Jackson E. W., Obert D. E. (2006) Characterization and mapping of a crown rust resistance gene complex (*Pc58*) in TAM O-301. *Crop Science*, 46(6), 2630-2635. https://doi.org/10.2135/cropsci2006.01.0014 https://doi.org/10.1016/S0065-2660(08)60498-8

Jackson E. W., Obert D. E., Menz M., Hu G., Avant J. B., Chong J., Bonman J. M. (2007) Characterization and mapping of oat crown rust resistance genes using three assessment methods. *Phytopathology*, 97(9), 1063-1070. https://doi.org/10.1094/PHYTO-97-9-1063

Jupe F., Pritchard L., Etherington G. J., MacKenzie K., Cock P. J. A., Wright F., Sharma S. K., Bolser D., Bryan G. J., Jones J. D. G., Hein I. (2012) Identification and localization of the NB-LRR gene family within the potato genome. *BMC Genomics*, 13(1), e75. https://doi.org/10.1186/1471-2164-13-75

Kamal N., Tsardakas Renhuldt N., Bentzer J., Gundlach H., Haberer G., Juhász A., Lux T., Bose U., Tye-Din J. A., Lang D., van Gessel N., Reski R., Fu Y. B., Spégel P., Ceplitis A., Himmelbach A., Waters A. J., Bekele W. A., Colgrave M. L., […] Sirijovski N. (2022) The mosaic oat genome gives insights into a uniquely healthy cereal crop. *Nature*, 606, 113-119. https://doi.org/10.1038/s41586-022-04732-y

Kebede A. Z., Friesen-Enns J., Gnanesh B. N., Menzies J. G., Fetch J. W. M., Chong J., Beattie A. D., Paczos-Grzeda E., McCartney C. A. (2019) Mapping oat crown rust resistance gene *Pc45* confirms association with *PcKM*. *G3: Genes, Genomes, Genetics*, 9(2), 505-511. https://doi.org/10.1534/g3.118.200757

Kiehn F. A., McKenzie R. I. H., Harder D. E. (1976) Inheritance of resistance to *Puccinia coronata avenae* and its association with seed characteristics in four accessions of *Avena sterilis*. *Canadian Journal of Genetics and Cytology*, 18(4), 717-726. https://doi.org/10.1139/g76-084

Kim K. C., Lai Z., Fan B., Chen Z. (2008) Arabidopsis WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. *Plant Cell*, 20(9), 2357-2371. https:// doi.org/10.1105/tpc.107.055566

Klos K. E., Yimer B. A., Babiker E. M., Beattie A. D., Bonman J. M., Carson M. L., Chong J., Harrison S. A., Ibrahim A. M. H., Kolb F. L., McCartney C. A.,

McMullen M., Mitchell Fetch J., Mohammadi M., Murphy J. P., Tinker N. A. (2017) Genome-wide association mapping of crown rust resistance in oat elite germplasm. *The Plant Genome*, 10(2), plantgenome2016.10.0107. https://doi.org/10.3835/plantgenome2016.10.0107

Krattinger S. G., Lagudah E. S., Spielmeyer W., Singh R. P., Huerta-Espino J., McFadden H., Bossolini E., Selter L. L., Keller B. (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science*, 323(5919), 1360-1363. https://doi.org/10.1126/science.1166453

Kulchelski F. R., Graichen F. A. S., Martinelli J. A., Locatelli A. B., Federizzi L. C., Delatorre CA (2010) Molecular mapping of *Pc68*, a crown rust resistance genes in *Avena sativa*. *Euphytica* 175:423-432. https://doi.org/10.1007/s10681-010-0198-8

leischmann G., McKenzie R. I. H. (1968) Inheritance of crown rust resistance in *Avena sterilis*. *Crop Science*, 8:710-713. https://doi.org/10.2135/cropsci1971.0011183X001100030044x

Leonard KJ, Martinelli JA (2005) Virulence of oat crown rust in Brazil and Uruguay. *Plant Disease*, 89(8), 802-808.

Leonard, K. J., Anikster, Y., Manisterski, J. (2005) Virulence associations in oat crown rust. *Phytopathology*, 95(1), 53-61. https://doi.org/10.1094/PHYTO-95-0053

Li C., Sun B., Li Y., Liu C., Wu X., Zhang D., Shi Y., Song Y., Buckler E. S., Zhang Z., Wang T., Li Y. (2016) Numerous genetic loci identified for drought tolerance in the maize nested association mapping populations. *BMC Genomics*, 17(1), e894. https://doi.org/10.1186/s12864-016-3170-8

Li H., Bradbury P., Ersoz E., Buckler E. S., Wang J. (2011) Joint QTL linkage mapping for multiple-cross mating design sharing one common parent. *PLoS ONE*, 6(3), e17573. https://doi.org/10.1371/journal.pone.0017573

Li W., Boer M. P., van Rossum B. J., Zheng C., Joosen R. V. L., van Eeuwijk F. A. (2022) statgenMPP: an R package implementing an IBD-based mixed model approach for QTL mapping in a wide range of multi-parent populations. *Bioinformatics*, 38(22), 5134-5136. https://doi.org/10.1093/bioinformatics/btac662

Li X., Shin S., Heinen S., Dill-Macky R., Berthiller F., Nersesian N., Clemente T., McCormick S., Muehlbauer G. J. (2015) Transgenic wheat expressing a barley UDP-glucosyltransferase detoxifies deoxynivalenol and provides high levels of resistance to *Fusarium graminearum*. *Molecular Plant-Microbe Interactions*, 28(11), 1237-46. https://doi.org/10.1094/MPMI-03-15-0062-R

Lin Y., Gnanesh B. N., Chong J., Chen G., Beattie A. D., Mitchell Fetch J. W., Kutcher H. R., Eckstein P. E., Menzies J. G., Jackson E. W., McCartney C. A. (2014) A major quantitative trait locus conferring adult plant partial resistance to crown rust in oat. *BMC Plant Biology*, 14(1), 1-11. https://doi.org/10.1186/s12870-014-0250-2

Martinelli J. A.; Federizzi L.C.; Bennedetti A. C. (1994) Yield reductions of oat grains due to crown rust severity. *Summa Phytopathologica*, 20(2), 116-118.

Maughan P. J., Lee R., Walstead R., Vickerstaff R. J., Fogarty M. C., Brouwer C. R., Reid R. R., Jay J. J., Bekele W. A., Jackson E. W., Tinker N. A., Langdon T., Schlueter J. A., Jellen E. N. (2019) Genomic insights from the first chromosome-scale assemblies of oat (*Avena spp*.) diploid species. *BMC Biology*, 17(1), e92. https://doi.org/10.1186/s12915-019-0712-y

McHale L., Tan X., Koehl P., Michelmore R. W. (2006) Plant NBS-LRR proteins: Adaptable guards. *Genome Biology*, 7(4), e212. https://doi.org/10.1186/gb-2006-7-4-212

McKenzie R. I. H., Green G. J. (1965) Stem rust resistance in oats. I. The inheritance of resistance to race 6AF in six varieties of oats. *Canadian Journal of Genetics and Cytology*, 7, 268-274. https://doi.org/10.1139/g65-037

McMullen M. D., Kresovich S., Villeda H. S., Bradbury P., Li H., Sun Q., Flint-Garcia S., Thornsberry J., Acharya C., Bottoms C., Brown P., Browne C., Eller M., Guill K., Harjes C., Kroon D., Lepak N., Mitchell S. E., Peterson B., […] Buckler E. S. (2009) Genetic properties of the maize nested association mapping population. *Science*, 325(5941), 737-740. https://doi.org/10.1126/science.1174320

Michelmore R. W., Meyers B. C. (1998) Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. *Genome Research*, 8(11), 1113-1130. https://doi.org/10.1101/gr.8.11.1113

Moore J. W., Herrera-Foessel S., Lan C., Schnippenkoetter W., Ayliffe M., Huerta-Espino J., [...] and Lagudah E. (2015) A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. *Nature genetics*, 47(12), 1494-1498. https://doi.org/10.1038/ng.3439

Nandety R. S., Caplan J. L., Cavanaugh K., Perroud B., Wroblewski T., Michelmore R. W., Meyers B. C. (2013) The role of TIR-NBS and TIR-X proteins in plant basal defense responses. *Plant physiology*, 162(3), 1459-1472. https://doi.org/10.1104/pp.113.219162

Nazareno E. S., Li F., Smith M., Park R. F., Kianian S. F., Figueroa M. (2018) *Puccinia coronata* f. sp. *avenae*: a threat to global oat production. *Molecular Plant Pathology*, 19(5), 1047-1060. https://doi.org/10.1111/mpp.12608

Pandian B. A., Sathishraj R., Djanaguiraman M., Prasad P. V. V., Jugulam M. (2020) Role of cytochrome P450 enzymes in plant stress response. *Antioxidants*, 9(5), e454. https://doi.org/10.3390/antiox9050454

Park R. F. (2008) Breeding cereals for rust resistance in Australia. *Plant Pathology*, 57(4), 591-602. https://doi.org/10.1111/j.1365-3059.2008.01836.x

Park R. F., Boshoff W. H. P., Cabral A. L., Chong J., Martinelli J. A., McMullen M. S., Fetch J. W. M., Paczos-Grzęda E., Prats E., Roake J., Sowa S., Ziems L., Singh D. (2022) Breeding oat for resistance to the crown rust pathogen *Puccinia coronata* f. sp. *avenae*: achievements and prospects. *Theoretical and Applied Genetics*, 135(11), 3709-3734. https://doi.org/10.1007/s00122-022-04121-z

Parlevliet J. E. (2002) Durability of resistance against fungal, bacterial and viral pathogens; present situation. *Euphytica*, 124(2), 147-156. https://doi.org/10.1023/A:1015601731446

Pedersen W. L., Leath S. (1988) Pyramiding major genes for resistance to maintain residual effects. *Annual Review of Phytopathology*, 26, 369-378. https://doi.org/10.1146/annurev.py.26.090188.002101

Peng Y., Yan H., Guo L., Deng C., Wang C., Wang Y., Kang L., Zhou P., Yu K., Dong X., Liu X., Sun Z., Peng Y., Zhao J., Deng D., Xu Y., Li Y., Jiang Q., Li Y., […] Ren C. (2022) Reference genome assemblies reveal the origin and evolution of allohexaploid oat. *Nature Genetics*, 54(8), 1248-1258. https://doi.org/10.1038/s41588-022-01127-7

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rafiqi M., Bernoux M., Ellis J. G., Dodds P. N. (2009) In the trenches of plant pathogen recognition: Role of NB-LRR proteins. *Seminars in Cell and Developmental Biology*, 20(9), 1017-1024. https://doi.org/10.1016/j.semcdb.2009.04.010

Sanz M. J., Loarce Y., Fominaya A., Vossen J. H., Ferrer E. (2013) Identification of RFLP and NBS/PK profiling markers for disease resistance loci in genetic maps of oats. *Theoretical and Applied Genetics*, 126(1), 203-218. https://doi.org/10.1007/s00122-012-1974-8

Simons M. D. (1965) Seedling resistance to *Puccinia coronata avenae* race 264 found

in *Avena sterilis*. *Phytopathology*, 55,700-701.

Simons M. D., Martens J. W., McKenzie R. I. H., Nishiyama I., Sadanaga K., Sebesta J., Thomas H. (1978) *Oats: a standardized system of nomenclature for genes and chromosomes and catalog of genes governing characters*. USDA Agriculture Handbook, 509:43.

Simons M.D. (1970) *Crown rust of oats and grasses*. Monograph Number 5, The American Phytopathological Society . The Heffernan Press Inc: Worcester Massechusets USA. 47 pp.

Song Q., Yan L., Quigley C., Jordan B. D., Fickus E., Schroeder S., Song B. H., Charles An Y.Q, Hyten D., Nelson R., Rainey K., Beavis W. D., Specht J., Diers B., Cregan P. (2017) Genetic Characterization of the Soybean Nested Association Mapping Population. *The Plant Genome*, 10(2), plantgenome2016.10.0109. https://doi.org/10.3835/plantgenome2016.10.0109

Sowa S., Paczos-Grzęda E. (2020) Identification of molecular markers for the *Pc39* gene conferring resistance to crown rust in oat. *Theoretical and Applied Genetics*, 133(4), 1081-1094. https://doi.org/10.1007/s00122-020-03533-z

Steel R. G. D., Torrie J. H., Dickey D. A. (1997) *Principles and procedures of statistics: A biometrical approach*. 3rd ed., McGraw Hill, New York, USA, 666 pp.

Stich B. (2009) Comparison of mating designs for establishing nested association mapping populations in maize and *Arabidopsis thaliana. Genetics*, 183(4), 1525-1534. https://doi.org/10.1534/genetics.109.108449

Tameling W. I. L., Baulcombe D. C. (2007) Physical Association of the NB-LRR Resistance Protein Rx with a Ran GTPase–Activating Protein Is Required for Extreme Resistance to Potato virus X. *The Plant Cell*, 19(5), 1682-1694. https://doi.org/10.1105/tpc.107.050880

Tezuka D., Matsuura H., Saburi W., Mori H., Imai R. (2021) A ubiquitously expressed UDP-glucosyltransferase, uGT74J1, controls basal salicylic acid levels in rice. *Plants*, 10(9), e.1875. https://doi.org/10.3390/plants10091875

Thomas P. D., Ebert D., Muruganujan A., Mushayahama T., Albou L. P., Mi H. (2022) PANTHER: Making genome-scale phylogenetics accessible to all. Protein *Science*, 31(1), 8-22.

USDA-ARS CDL. *Oat Crown Rust Resistance Genes*. USDA-ARS Cereal Disease Laboratory. St. Paul, 2022. Available at: http://www.ars.usda.gov/Main/docs.htm?docid=10342>. Accessed 28 sep 2022.

van Ooijen G., Mayr G., Kasiem M. M. A., Albrecht M., Cornelissen B. J. C., Takken F. L. W. (2008) Structure–function analysis of the NB-ARC domain of plant disease resistance proteins. *Journal of Experimental Botany*, 59(6), 1383-1397. https://doi.org/10.1093/jxb/ern045

Voorrips R. E. (2002) Mapchart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, 93(1), 77-78. https://doi.org/10.1093/jhered/93.1.77

Wang J., Tao F., Tian W., Guo Z, Chen X, Xu X., Shang H., Hu X. (2017) The wheat WRKY transcription factors TaWRKY49 and TaWRKY62 confer differential high-temperature seedling-plant resistance to *Puccinia striiformis* f. sp. *tritici*. *PloS One*, 12(7), e0181963. https://doi.org/10.1371/journal.pone.0181963

Wickham H. (2016) Package `ggplot2`: Elegant Graphics for Data Analysis. *Springer-Verlag* New York.

Wight C. P., O'Donoughue L. S., Chong J., Tinker N. A., Molnar S. J. (2005) Discovery, localization, and sequence characterization of molecular markers for the crown rust resistance genes *Pc38*, *Pc39*, and *Pc48* in cultivated oat (*Avena sativa* L.). *Molecular Breeding*, 14(4), 349-361. https://doi.org/10.1007/s11032-005-0148-7

Wimmer V., Albrecht T., Auinger H. J., Schön C. C. (2012) Synbreed: A framework for the analysis of genomic prediction data using R. Bioinformatics, 28(15), 2086-2087. https://doi.org/10.1093/bioinformatics/bts335

Wong L. S. L., McKenzie R. I. H., Harder D. E., Martens J. W. (1983) The inheritance of resistance to *Puccinia coronata* and of floret characters in *Avena sterilis*. *Canadian Journal of Genetics and Cytology*, 25(4), 329-335. https://doi.org/10.1139/g83-052

Xing L., Gao L., Chen Q., Pei H., Di Z., Xiao J., Wang H., Ma L., Chen P., Cao A., Wang X. (2018) Over-expressing a UDP-glucosyltransferase gene (Ta-UGT3) enhances Fusarium Head Blight resistance of wheat. *Plant Growth* Regulation, 84(3), 561-571. https://doi.org/10.1007/s10725-017-0361-5

Yao E., Blake V. C., Cooper L., Wight C. P., Michel S., Cagirici H. B., Lazo G. R., Birkett C. L., Waring D. J, Jannink J., Holmes I., Waters A.J., Eickholt D. P., Sen, T. Z. (2022) GrainGenes: a data-rich repository for small grains genetics and genomics. Database, 2022, BAAC034. https://doi.org/10.1093/database/baac034

Yates A. D., Allen J., Amode R. M., Azov A. G., Barba M., Becerra A., Bhai J.,

Campbell L. I., Carbajo Martinez M., Chakiachvili M., Chougule K., Christensen, M., Contreras-Moreira B., Cuzick A., da Rin Fioretto, L., Davis, P., de Silva, N. H., Diamantakis, S., Dyer, S., […] Flicek, P. (2022) Ensembl Genomes 2022: An expanding genome resource for non-vertebrates. *Nucleic Acids Research*, 50(D1), D996-D1003. https://doi.org/10.1093/nar/gkab1007

Yu J., Holland J. B., McMullen M. D., Buckler E. S. (2008) Genetic design and statistical power of nested association mapping in maize. *Genetics*, 178(1), 539-551. https://doi.org/10.1534/genetics.107.074245

Zhang M., Su J., Zhang Y., Xu J., and Zhang S. (2018) Conveying endogenous and exogenous signals: MAPK cascades in plant growth and defense. *Current Opinion in Plant Biology*, 45(A), 1-10. https://doi.org/10.1016/j.pbi.2018.04.012

Zhang Y. M., Shao Z. Q., Wang Q., Hang Y. Y., Xue J. Y., Wang B., Chen J. Q. (2016) Uncovering the dynamic evolution of nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes in Brassicaceae. *Journal of Integrative Plant Biology*, 58(2), 165-177. https://doi.org/10.1111/jipb.12365

Zhao J., Kebede A. Z., Bekele W. A., Menzies J. G., Chong J., Mitchell Fetch, J. W., Tinker, N. A., Beattie, A. D., Peng, Y. Y., McCartney, C. A. (2020) Mapping of the oat crown rust resistance gene *Pc39* relative to single nucleotide polymorphism markers. *Plant Disease*, 104(5), 1507-1513. https://doi.org/10.1094/PDIS-09-19-2002-RE

Ziems L. A., Hickey L. T., Platz G. J., Franckowiak J. D., Dracatos P. M., Singh D., Park R. F. (2017) Characterization of Rph24: A gene conferring adult plant resistance to *Puccinia hordei* in Barley. *Phytopathology*, 107(7), 834-841. https://doi.org/10.1094/PHYTO-08-16-0295-R

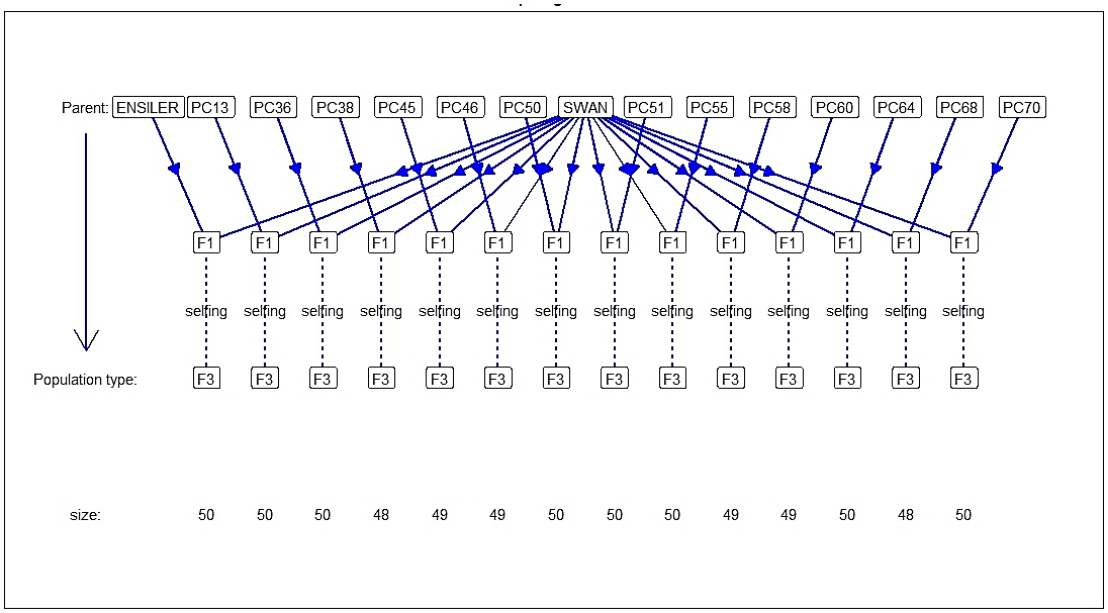


Figure 1 Schematic diagram of nested association mapping (AsNAM) analysis design, consisting of fourteen subpopulations. segregating for resistance to Pc where the highly susceptible genotype 'Swan,' was the ‘founder’ and lines predicted to carry ASR genes were the ‘donors’.50 genotypes were selected for each subpopulation, 25 NSR (Non-segregating Resistant) and 25 NSS (Non-segregating Susceptible), to integrate the analysis. The final AsNAM population consisted of 692 genotypes and 15 parents, totalizing 707 genotypes.

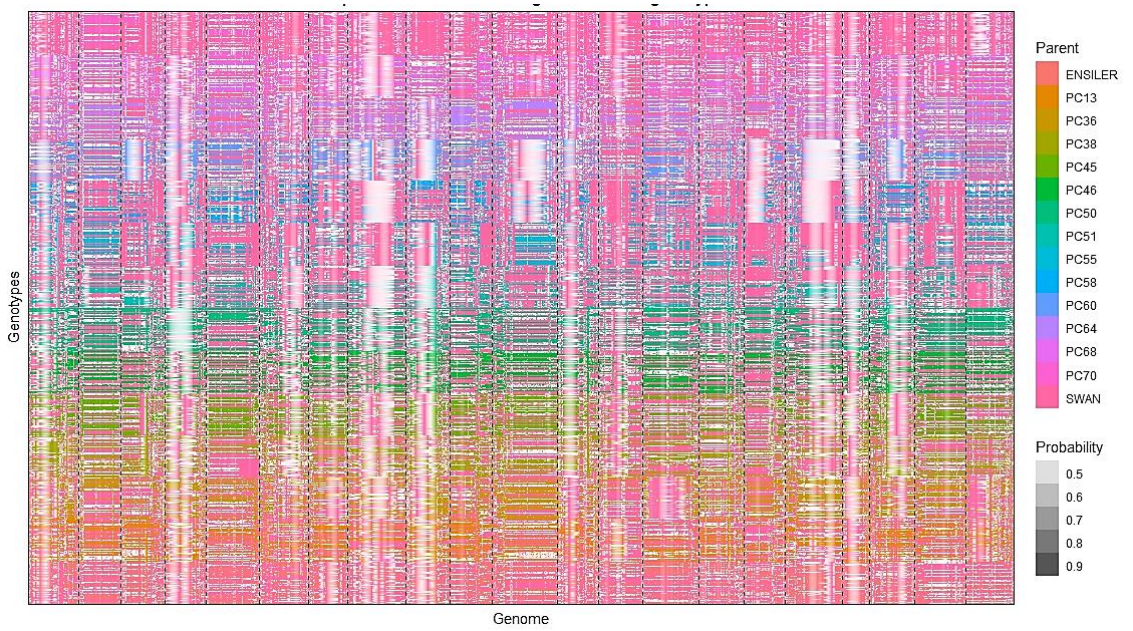


Figure 2 A graphical genotype heat map showing parent of origin for each marker in the AsNAM population obtained from thresholding IBD probabilities. The colors indicate the parental origins, and the color intensities indicate confidence.

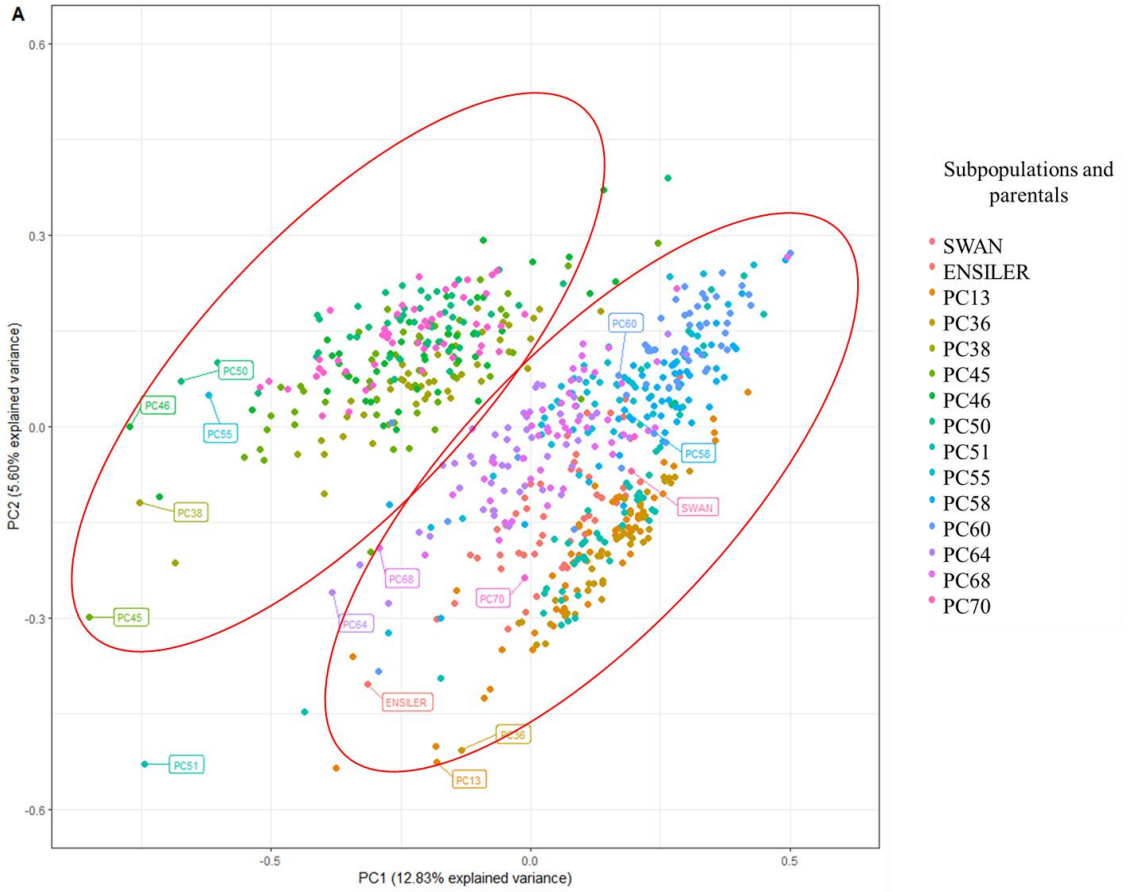


Figure 3 Principal component analysis of the kinship matrix visualizing the genetic relationships between 707 genotypes. The figure represents the first principal component (PC1; x-axis) and the second principal component (PC2; y-axis). Genotypes are colored according to donors ID.

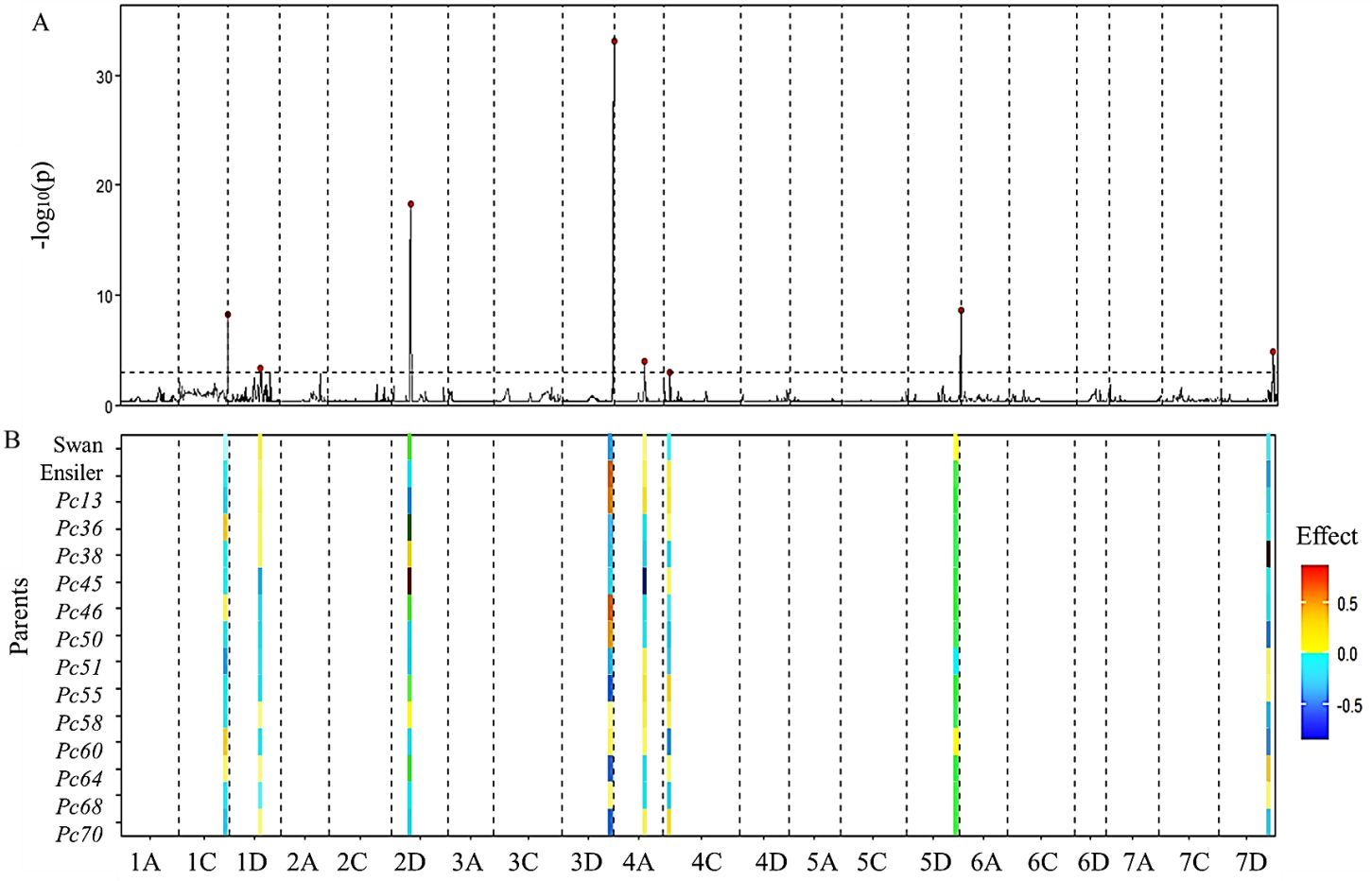


Figure 4 QTLs associated with Oat Crown Rust resistance in AsNAM population. A) Manhattan plots representing QTL profiles. The grey horizontal line represents a significance threshold of −log10(p) of 2 (>1%) B) Estimation of Parental effects at QTLs, associated with OCR resistance in a AsNAM population. Colors represent effects of each parental at QTL. Red gradient is associated with a positive effect on phenotype (resistance) and Blue the one with a negative (susceptibility).

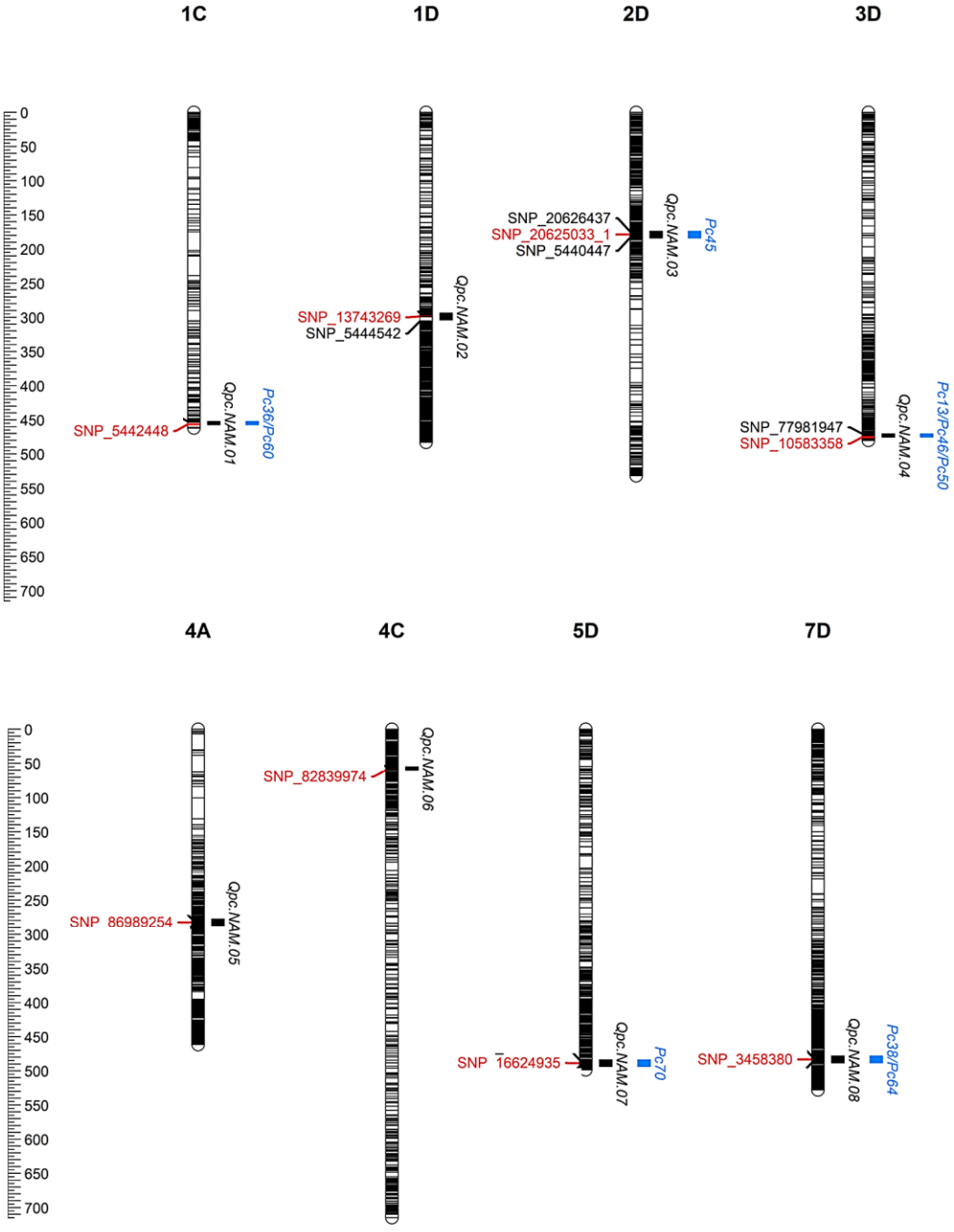


Figure 5 The positions (Mbp) of quantitative trait loci (QTL) identified in this study, and SNPs significantly associated to the QTL to crown rust resistance based on OT3098 v2 Oat genome. The significant SNP markers are in red. The SNP markers within the QTL window of significant SNP markers are in black. The candidate genes in each QTL are in blue.

Table 1 Origin, predicted gene, pedigree, and linked genes of oat crown rust resistant donor used to develop the AsNAM population.

Name	Pedigree	Abbreviated name	Gene	Origin	Reference
Ensiler	Otee/4/X666-2/Lodi/2/PI295909/3/CI7463/Lodi/2/PI295909	Ensiler	?	*A.sativa*	Brinkman *et al*. (1990)
Clinton	Iowa No. D69 (Richland/Green Russian) / Bond (*Avena sterilis* 1918 / Golden Rain)	PC13	*Pc*13	*A. sativa*	Finkner *et al*. (1955)
Pc36	Lang*3 // Clinton / PI 267989-1	PC36	*Pc*36	*A. sterilis*	Kurt Leonard (unpublished)
Pc38	CAV 2648-4/4*Pendek	PC38	*Pc*38*	*A. sterilis*	Fleischmann and McKenzie (1968)
Pc45	Pendek*4/CAV 5050	PC45	*Pc*45	*A. sterilis*	Fleischmann *et al*. (1971a)
Pc46	Pendek*4/CAV5115	PC46	*Pc*46**	*A. sterilis*	Fleischmann *et al*. (1971a)
Pc50	CAV 2643/4*Pendek	PC50	*Pc*50**	*A. sterilis*	Fleischmann *et al*. (1971b)
Iowa isoline X270	CI 7555*6/CI 8079	PC51	*Pc*51	*A. sterilis*	Simons *et al*. (1978)
Pc55	Pendek*4/CAV 4963	PC55	*Pc*55***	*A. sterilis*	Kiehn *et al*. (1976)
TAM O-301	AB555 (Coronado/Cortez) /3/ Ora / 63C3868-4-2 (Coronado/Cortez) // Ora / 3*PI 295919	PC58	*Pc*58	*A. sterilis*	Simons *et al*. (1978)
Coker 227	FL500 // Woodgrain / CI7330 /3/ Moregrain / Milford sel. /4/ 2*Suregrain / PI 287211	PC60	*Pc*60	*A. sterilis*	Simons *et al*. (1978)
CAV 4248	Makuru//SUN II isoline	PC64	*Pc*64	*A. sterilis*	Wong *et al*. (1983)
CAV 4904	Makuru//SUN II isoline	PC68	*Pc*68**	*A. sterilis*	Wong *et al*. (1983)
H547	PI318282	PC70	*Pc*70	*A. sterilis*	CDL (2010)

Adapted from Gnanesh *et al*. (2014) and Park *et al*. (2022)
§Linked genes as listed by Park *et al.* (2022)
* Linked to *Pc62* and *Pc63*
** Member of a linkage group comprising *Pc*44, *Pc46*, *Pc*50, *Pc*68, *Pc*95, *Pc*X and the stem rust resistance genes *Pg3* and *Pg9*
*** Linked to *Pc39*

Table 2 Infection type and segregation analysis of F_3 subpopulations for crown rust resistance.

Subpopulation	Pathotype[†]	Infection Type		Number of observed plants							Expected genetic model				
		NSR	NSS	NSR	(%)	SEG	(%)	NSS	(%)	Total	Nº segr. genes[§]	Nº of genes for resistance[‡]	Ratio[‡]	χ^2	p-value
Swan / Ensiler	649	;N	3+	33	(25)	69	(53)	29	(22)	131	1	1	1:2:1	0.62	0.73
Swan / PC13	135	;1N	3+	2	(2)	59	(62)	34	(36)	95	5	4	16:630:378	0.22	0.90
	159	12N	3+	2	(2)	49	(56)	37	(42)	88	5	4	16:630:378	1.40	0.50
Swan / PC36	567	;1N	3+	22	(25)	48	(55)	17	(20)	87	1	1	1:2:1	1.51	0.47
	501	;1N	3+	25	(27)	47	(52)	19	(21)	91	1	1	1:2:1	0.89	0.64
Swan / PC38	271	12N	3+	21	(20)	58	(56)	25	(24)	104	1	1	1:2:1	1.69	0.43
	559	;12CN	3+	21	(22)	49	(51)	27	(28)	97	1	1	1:2:1	0.75	0.69
Swan / PC45	548	;1CN	3+	23	(19)	73	(59)	27	(22)	123	1	1	1:2:1	4.56	0.10
	135	;N	3+	30	(26)	67	(57)	20	(17)	117	1	1	1:2:1	4.18	0.12
Swan / PC46	684	;N	3+	27	(21)	71	(55)	32	(25)	130	1	1	1:2:1	1.49	0.47
Swan / PC50	684	0;	3+	27	(26)	51	(49)	27	(26)	105	1	1	1:2:1	0.09	0.96
Swan / PC51	500	1N	3+	19	(20)	56	(60)	18	(19)	93	1	1	1:2:1	3.90	0.14
	271	;12N	3+	9	(10)	68	(75)	14	(15)	91	3	2	10:44:10	2.39	0.30
Swan / PC55	271	;N	3+	19	(21)	59	(66)	12	(13)	90	3	2	10:44:10	2.17	0.34
	135	;N	3+	15	(22)	42	(63)	10	(15)	67	3	2	10:44:10	2.34	0.31
Swan / PC58	501	;N	3+	12	(12)	69	(67)	22	(21)	103	3	2	10:44:10	3.26	0.20
	135	N	3+	9	(10)	64	(73)	15	(17)	88	3	2	10:44:10	1.96	0.38
Swan / PC60	501	1N	3+	11	(9)	88	(75)	19	(16)	118	3	2	10:44:10	3.60	0.17
	271	12N	3+	4	(5)	57	(73)	17	(22)	78	4	3	12:177:67	0.78	0.68
Swan / PC64	271	;N	3+	4	(6)	36	(58)	22	(35)	62	2	2	1:8:7	1.78	0.41
	135	;1N	3+	4	(11)	20	(54)	13	(35)	37	2	2	1:8:7	1.98	0.37

Continuation…**Table 2** Infection type and segregation analysis of F_3 populations for crown rust resistance.

Subpopulation	Pathotype[†]	Infection Type		Number of observed plants							Expected genetic model				
		NSR	NSS	NSR	(%)	SEG	(%)	NSS	(%)	Total	Nº segr. genes[§]	Nº of genes for resistance[‡]	Ratio[⁑]	χ^2	p-value
Swan / PC68	548	0	3+	28	(23)	67	(54)	29	(23)	124	1	1	1:2:1	0.82	0.66
Swan / PC70	271	;N	3+	30	(25)	62	(51)	30	(25)	122	1	1	1:2:1	0.03	0.98
	135	;N	3+	29	(25)	58	(50)	28	(24)	115	1	1	1:2:1	0.03	0.99
Total	11			Total						2,356					

IT: scored 10 days after inoculation on a 0-4 scale described by Nazareno *et al*. (2018). NSR: Non segregating resistant plants. SEG: Segregating plants. NSS: Non-segregating susceptible plants. [†]Pathotypes were from a wide collection of single pustule isolates from the Cereal Rust Collection in the Plant Breeding Institute, University of Sydney – Camden campus, NSW, Australia. [§]Number of genes segregating for resistance when challenged by that specific pathotype. [‡]Number of genes that are needed, at single time, to be present in the genotype, in order to show resistance to that specific pathotype. [⁑]Expected ratio of NSR:SEG: NSS plants in the subpopulation, challenged by that specific pathotype.

Table 3 QTL and their position on the chromosome, nearest marker on the original map, explained variance and the effects of all parents identified in this study.

				Effect on QTL															
QTL	Chr	Pos (Mbp)	pValue	SWAN	ENSILER	PC13	PC36	PC38	PC45	PC46	PC50	PC51	PC55	PC58	PC60	PC64	PC68	PC70	LOD	R^2
Qpc.NAM.01	1C	457.34	6.24E-09	-0.14	0.13	-0.18	0.64	0.01	0.01	0.00	-0.06	-0.31	0.08	-0.13	0.26	0.01	-0.08	-0.24	8.20	0.10
Qpc.NAM.02	1D	298.81	4.23E-04	0.10	0.12	-0.02	0.03	0.06	-0.13	-0.07	-0.07	-0.05	-0.04	0.03	-0.03	0.05	0.01	0.01	3.37	0.02
Qpc.NAM.02	1D	303.79*	8.67E-04	0.10	0.16	0.06	0.05	0.03	-0.18	-0.11	-0.07	-0.07	-0.05	0.06	-0.03	0.07	-0.03	0.02	3.06	NA
Qpc.NAM.03	2D	174.04*	1.24E-12	0.05	-0.04	-0.15	-0.43	0.25	0.87	0.08	-0.18	-0.20	0.08	0.04	-0.14	-0.09	-0.01	-0.12	11.91	NA
Qpc.NAM.03	2D	179.01	5.78E-19	-0.13	-0.11	-0.31	0.15	0.03	0.82	-0.17	-0.01	0.00	0.00	0.19	-0.12	-0.30	0.08	-0.13	18.24	0.14
Qpc.NAM.03	2D	183.99*	3.22E-09	-0.10	-0.07	-0.30	0.02	0.00	0.77	-0.07	-0.02	0.00	-0.02	0.01	-0.08	0.01	-0.03	-0.13	8.49	NA
Qpc.NAM.04	3D	470.35*	7.46E-23	-0.28	0.36	0.42	-0.22	-0.23	-0.31	0.52	0.38	0.08	-0.06	-0.17	0.01	-0.13	-0.03	-0.35	22.13	NA
Qpc.NAM.04	3D	475.3	8.34E-34	-0.28	0.56	0.49	-0.29	-0.21	-0.16	0.58	0.45	-0.22	-0.35	0.02	0.09	-0.39	0.06	-0.35	33.08	0.21
Qpc.NAM.05	4A	282.94	8.86E-05	0.03	0.07	0.18	-0.01	-0.10	-0.46	-0.04	-0.04	0.08	0.15	0.09	0.02	-0.05	-0.02	0.11	4.05	0.06
Qpc.NAM.06	4C	59.98	9.97E-04	-0.02	0.09	0.15	0.01	-0.08	0.03	-0.09	-0.12	-0.19	0.24	0.09	-0.23	0.02	-0.11	0.21	3.00	0.04
Qpc.NAM.07	5D	488.76	2.10E-09	-0.12	-0.05	0.07	-0.23	0.06	-0.08	-0.06	-0.14	-0.04	0.24	-0.05	-0.14	0.00	-0.24	0.77	8.68	0.11
Qpc.NAM.08	7D	483.3	1.21E-05	-0.09	-0.19	-0.11	-0.01	0.79	-0.01	-0.04	-0.25	0.10	0.03	-0.16	-0.32	0.35	0.03	-0.13	4.92	0.13

Colors represent the effects of each parent at each QTL. A red gradient is associated with positive effects and green to negative effects on the resistance.
NA: Non-significative phenotypic association.
* Within the QTL window of 10Mbp of significant SNP Marker

Table 4 Candidate genes located near the position of SNP markers associated with significant QTL

QTL	Chr	Pos Marker (Mbp)	Transcript ID[§]	Gene map position (Mbp)	Gene description[§]	Protein Family*	Biological process*	Molecular function*
Qpc.NAM.01	1C	457.34	AVESA.00001b.r3.1Cg0001866	457,252,813-457,255,933	RAN GTPase-activating protein 2	WPP domain / LRR domain		GTPase activator activity / Protein binding
			AVESA.00001b.r3.1Cg0001868	457,416,200-457,419,884	Cysteine proteinase inhibitor 3	Cystatin domain		cysteine-type endopeptidase inhibitor activity
Qpc.NAM.02	1D	298.81	AVESA.00001b.r3.1Dg0001261	298,181,783-298,187,206	Probable disease resistance protein RF9	NB-LRR domain	Defense response	ADP binding
			AVESA.00001b.r3.1Dg0001264	298,316,828-298,321,050	Serine/threonine-protein kinase-like protein At3g51990	Protein kinase domain	protein phosphorylation	protein kinase activity
			AVESA.00001b.r3.1Dg0001265	298,411,249-298,412,920	Cytochrome P450 71A25	Cytochrome P450		Monooxygenase activity
			AVESA.00001b.r3.1Dg0001270	298,728,734-298,731,315	ABC transporter G family member 23	ABC transporter-like, ATP-binding domain		ATP binding/ ATP hydrolysis activity/ ABC-2 type transporter
Qpc.NAM.03	2D	179.01	AVESA.00001b.r3.2Dg0002009	178,331,593-178,340,097	Probable LRR receptor-like serine/threonine-protein kinase At1g56140	Protein kinase domain	protein phosphorylation	protein kinase activity
			AVESA.00001b.r3.2Dg0002020	178,832,124-178,836,026	LRR receptor-like serine/threonine-protein kinase FLS2	NB-LRR domain[‡]	Protein phosphorylation	Protein kinase activity
			AVESA.00001b.r3.2Dg0002022	178,936,555-178,939,589	Disease resistance protein Pik-2	NB-LRR domain	Defense response	ADP binding
			AVESA.00001b.r3.2Dg0002029	179,164,920-179,166,843	indolin-2-one monooxygenase	Cytochrome P450		Monooxygenase activity
			AVESA.00001b.r3.2Dg0002036	179,800,856-179,806,880	Putative disease resistance protein RGA1	NB-LRR domain	Defense response	ADP binding
			AVESA.00001b.r3.2Dg0002038	179,917,719-179,923,202	Disease resistance protein RGA2	NB-LRR domain	Defense response	DNA binding/ ADP binding

Continuation…**Table 4** Candidate genes located near the position of SNP markers associated with significant QTL.

Qpc.NAM.03	2D	183.99	AVESA.00001b.r3.2Dg0002069	182,032,813-182,046,776	Disease resistance protein RGA5	NB-LRR domain	Defense response	DNA binding/ Protein binding/ ADP binding
			AVESA.00001b.r3.2Dg0002071	182,085,659-182,090,958	Disease resistance protein RGA4	NB-LRR domain	Defense response	ADP binding
			AVESA.00001b.r3.2Dg0002072	182,089,403-182,101,126	Probable LRR receptor-like serine/threonine-protein kinase At3g47570	Serine-threonine/tyrosine-protein kinase catalytic domain	Protein phosphorylation	protein kinase activity/ ATP binding
			AVESA.00001b.r3.2Dg0002101	183,291,108-183,292,877	UDP-glucose flavonoid 3-O-glucosyltransferase 7	UDPGT domain	Phenylpropanoid metabolism‡	UDP-glycosyltransferase activity
Qpc.NAM.04	3D	470.35	AVESA.00001b.r3.3Dg0002694	470,277,905-470,298,071	Disease resistance protein RGA5	NB-LRR domain	Defense response	ADP binding
			AVESA.00001b.r3.3Dg0002695	470,279,098-470,282,932	Disease resistance protein RGA5	NB-LRR domain	Defense response	ADP binding
			AVESA.00001b.r3.3Dg0002700	470,561,229-470,566,614	Putative disease resistance protein RGA1	Protein kinase domain / NB-LRR domain	protein phosphorylation/ defense response	protein kinase activity/ ATP binding
			AVESA.00001b.r3.3Dg0002702	470,774,629-470,783,736	Putative disease resistance protein RGA1	NB-LRR domain	Defense response	ADP binding
		475.3	AVESA.00001b.r3.3Dg0002753	476,085,954-476,103,958	Disease resistance protein RGA5	NB-LRR domain	Defense response	ADP binding
			AVESA.00001b.r3.3Dg0002755	476,105,843-476,111,177	Disease resistance protein RGA4	NB-LRR domain	Defense response	ADP binding
Qpc.NAM.05	4A	282.94	AVESA.00001b.r3.4Ag0001473	283,449,082-283,452,301	Protein WRKY1	WRKY domain	Regulation of DNA-templated transcription	DNA-binding transcription factor activity/ Sequence-specific DNA binding
Qpc.NAM.06	4C	59.98	AVESA.00001b.r3.4Cg0000795	59,281,763-59,285,467	LRR receptor-like serine/threonine-protein kinase RPK2	NB-LRR domain	Protein phosphorylation	Protein kinase activity/ Protein binding/ ATP binding
			AVESA.00001b.r3.4Cg0000810	60,031,370-60,036,419	Putative disease resistance protein At1g63350	NB-LRR domain‡	Plant defense‡	ATP-binding/ Nucleotide-binding‡

Continuation…**Table 4** Candidate genes located near the position of SNP markers associated with significant QTL.

Qpc.NAM.06	4C	59.98	AVESA.00001b.r3.4Cg0000813	60,105,774-60,112,135	Disease resistance protein Pik-2	NB-LRR domain	Defense response	ADP binding
			AVESA.00001b.r3.4Cg0000817	60,311,274-60,317,065	Putative disease resistance protein RGA1	NB-LRR domain	Defense response to fungus	ADP binding
Qpc.NAM.07	5D	488.76	AVESA.00001b.r3.5Dg0003340	488,805,172-488,821,563	G-type lectin S-receptor-like serine/threonine-protein kinase SRK	Protein kinase domain	protein phosphorylation	protein kinase activity
			AVESA.00001b.r3.5Dg0003341	488,806,572-488,812,267	Disease resistance protein RGA4	NB-LRR domain	Defense response	ADP binding
Qpc.NAM.08	7D	483.3	AVESA.00001b.r3.7Dg0002950	482,277,214-482,285,320	Putative disease resistance protein RGA1	NB-LRR domain	Defense response	ADP binding
			AVESA.00001b.r3.7Dg0002956	483,175,612-483,177,357	Putative UDP-rhamnose:rhamnosyl transferase 1	UDPGT domain		UDP-glycosyltransferase activity

§According to OT3098 oat reference genome v2.
*According to Esembl Plants Database.
‡Information from UniProt (https://www.uniprot.org/)
WPP: tryptophan-proline-proline motif. NB-LRR: nucleotide-binding and leucine-rich repeat. ABC transporter: ATP-binding cassette transporter. UDGPT: UDP-glucoronosyl and UDP-glucosyl transferase domain.

SUPPLEMENTAR MATTERIAL

Table S1 Infection type on the Australian Oat differential set lines to *Puccinia coronata* f. sp. *avenae* pathotypes.

		Pathotype														
Line	Gene	573	889	271§	501§	617	529	135§	559§	500§	548§	684§	552	649§	567§	159§
H458	*PcH548*	N	;N	N	;N	;1N	;N	;N	2N	;N	;N	;N	;N	N	3+	0;-
WIX4361-9	*PcWIX1, WIX2*	;N	;N	;N	;N	;	;N	;N	;N	;N	;N	3+	;1N	;N	;	0;=
AMAGALON	*Pc91*	0	;N	;N	;N	;N	;N	;	N	;N	0	3+	0	0	0;	0;=
CULGOA	Unknown	3+	;2	N	;N	0	N	;N	N	;N	3+	3+	3+	3+	NA	0;=
CLEANLEAF	*Pc38,39,52*	;N	0	;	;N	0	;N	;	;N	;N	3+	3+	;N	3+	;N	0;-
PC68**	*Pc68*	0	N	0	;	1	0	0	0	0	;	3+	0	3+	0;=	0;=
TAM O-301**	*Pc58*	;N	N	;N	;N	0	;N	N	;1N	0	3+	22+	;N	3+	3+	;-
TAM O-312	*Pc59*	;N	N	;N	N	0	;N	;N	1N	;N	;N	2+N	;N	2+	3+	;-
PC61	*Pc61*	;N	12N	;N	3+	3+	N	;N	1N	0	;N	;1N	;N	;N	3+	0;-
PC39	*Pc39*	;1N	;N	;	;	;1N	;N	;	3+	3+	3+	3+	3+	3+	3+	;1
PC38	*Pc38*	;1N	;N	;1N	;N	;N	;2N	3+	12N	3+	3+	3+	3+	3+	;N	0;
SWAN*	*Pc1*	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	;N	3+	3+	3+
PC36**	*Pc36*	;N	;1N	;N	;N	;N	;N	;N	12N	;2N	;1N	3+	;1N	3+	;N	;1
PC46**	*Pc46*	12	3+	3+	;	3+	3+	3+	;N	;1N	;N	;	;N	;N	;N	3+
PC50**	*Pc50*	3+	;N	;	0	;N	0	0	;N	;N	;N	0;=	?	;N	0;=	0;=
PC51**	*Pc51*	;N	;N	;N	;N	;N	;N	;N	;1N	;1N	;N	2+	;2N	12N	;N	;
PC52	*Pc52*	0	0	;	?	0	N	0	;N	0	3+	3+	;N	3+	;N	0;=
PC55**	*Pc55*	;N	;N	;N	;	;	N	;N	;1N	3+	3+	3+	3+	3+	3+	;
PC56	*Pc56*	;N	;N	;N	;N	;N	;N	;N	12N	?	;N	3+	?	3+	1N	0;
PC63	*Pc63*	;N	;N	;N	;N	;N	;N	3+	N	;12N	;N	;	3+	;N	0;	0;=
PC64**	*Pc64*	;1N	3+	3+	3+	;1N	3+	;12N	1N	;N	N	;	;N	N	;N	;1+
PC71	*Pc71*	;N	;N	;N	;	3+	12C	;N	3+	3+	3+	3+	3+	3+	3+	0;-

Continuation… **Table S1** Infection type on the Australian Oat differential set lines to *Puccinia coronata* f. sp. *avenae* pathotypes

		Pathotype														
Line	Gene	573	889	271§	501§	617	529	135§	559§	500§	548§	684§	552	649§	567§	159§
X716	Unknown	;N	;1N	;N	;N	;N	;N	;N	12N	;12N	;1N	3+	3+	3+	NA	0;=
WARREGO	Unknown	N	;N	0	;N	;N	;N	0	;N	;1N	;1N	3+	;N	3+	0;	0;=
BETTONG	Unknown	0	;N	;N	;N	;N	;N	;N	;1N	0	N	0;	;N	N	3+	0;=
BARCOO	Unknown	;N	;	;	;N	;N	;	;	;N	0	0	;1-N	;N	;N	3+	122+
LANDHAFER	*Pc5, Pc4*	;N	3+	3+	3+	0	;N	;N	3+	;N	3+	3+	3+	3+	3+	12C
SANTA FE	*Pc6,7,8,21*	;N	3+	3+	3+	3+	;N	;N	3+	;1N	3+	3+	3+	3+	3+	3+
UKRAINE	*Pc3c,4c,6c,9*	3+	3+	;	3+	3+	;N	;1N	3+	3+	3+	3+	3+	3+	3+	2+3
TRISPERNIA	*Pc6d*	;1N	3+	3+	3+	3+	;N	;N	3+	;N	;1N	3+	3+	3+	3+	2+3C
BONDVIC	*Pc3,4*	;N	;2N	;N	3+	3+	;N	;N	3+	;N	12N	3N	1N	3+	3+	0;=
PC45**	*Pc45*	0	3+	3+	3+	3+	;N	;	3+	;N	;N	;	;N	;1N	3+	0;=
PC48	*Pc48*	0	3+	0	;	3+	;	;	3+	0	3+	3+	;N	3+	22+	0;=
NUGENE	*Pc48,68*	;N	;N	0	0	3+	;N	;N	N	0	3+	3+	;N	3+	0;-	0;=
GWYDIR	Unknown	0	;	;	0	;	;N	;	N	;N	;N	3+	2N	;N	;N	0;=
VOLTA	*Pc50,68*	;N	;	;N	0	;N	0	0	0	;N	;N	0;=	0	0	0;=	0;=
PC92	*Pc 92*	0	3+	0	;	0	0	0	3+	;N	;N	;	;N	;N	3+	0;-
PC94	*Pc 94*	0	;N	0	;N	0	;	;N	;N	;N	;N	;	;N	;N	3+	;B
SAIA	*Pc15,16,17*	;N	N	;	N	N	N	2N	;N	;N	;N	;1N	;N	;N	;1	0;
ENSILER**	Unknown	N	;1	N	0	;N	;	0	;N	;N	;N	;	;N	;N	;N	0;-
GENIE	*Pc48,56*	;	;	0	0	;1N	;	0	;1N	;N	;N	3+	;1N	3+	;N	0;=
DROVER	*Pc91*	0	;	;	0	0	0	0	0	0	-	3+	0	;N	0;=	0;=
ALADDIN	Unknown	0	;	;	0	0	0	0	;N	0	0	3+	?	;N	0;-	0;=

Continuation… **Table S1** Infection type on the Australian Oat differential set lines to *Puccinia coronata* f. sp. *avenae* pathotypes

		Pathotype**														
Line	Gene	573	889	271§	501§	617	529	135§	559§	500§	548§	684§	552	649§	567§	159§
COKER 227	*Pc60*	;N	3+	0	3+	3+	;	;N	3+	;N	N	;11+N	;N	1N	3+	0;
COKER234	*Pc62*	;N	3+	;	;2	2N	;N	;N	12N	0	N	;1N	;N	;N	;1-	3+
PC70**	*Pc70*	0	;N	;	;	;C	;C	0	NA	3+	3+	3+	NA	NA	3+	;
CLINTON **	*Pc13*	;	;N	;N	3+	;1N	1	1N	NA	3+	3+	3+	NA	NA	3+	;CN

IT: according described by Nazareno *et al*. (2018), incompatible reactions include the following: 0, no urediniospores; fleck (;), presence of flecks; 1, few small pustules; 2, small pustules, presence of green islands; all of these may be accompanied by necrosis (N) and/or chlorosis (C). Compatible reactions include the following: 3, large pustules surrounded by chlorotic halos; 4, large pustules, often coalescing. The signal + and – represents more or less symptoms. NA: No data available. The presence of two numbers indicates an intermediary class.

Pathotype 551 utilized to challenge the Swan x PC38 population was used based on previous data from Plant Breeding Institute, Cobbity, NSW, Australia.

*Founder used in the NAM population. **donors used in NAM population. §Pathotypes used in this study

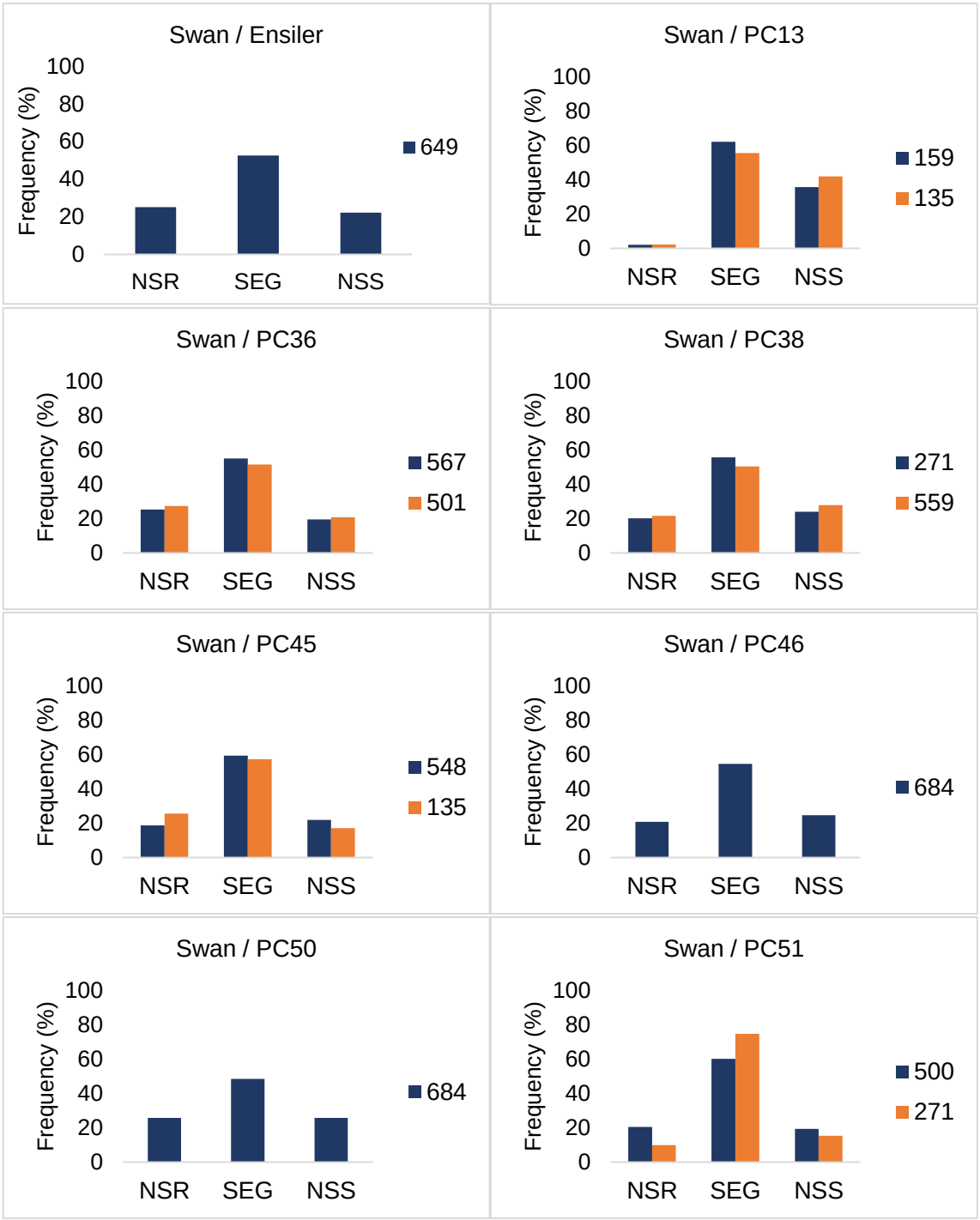
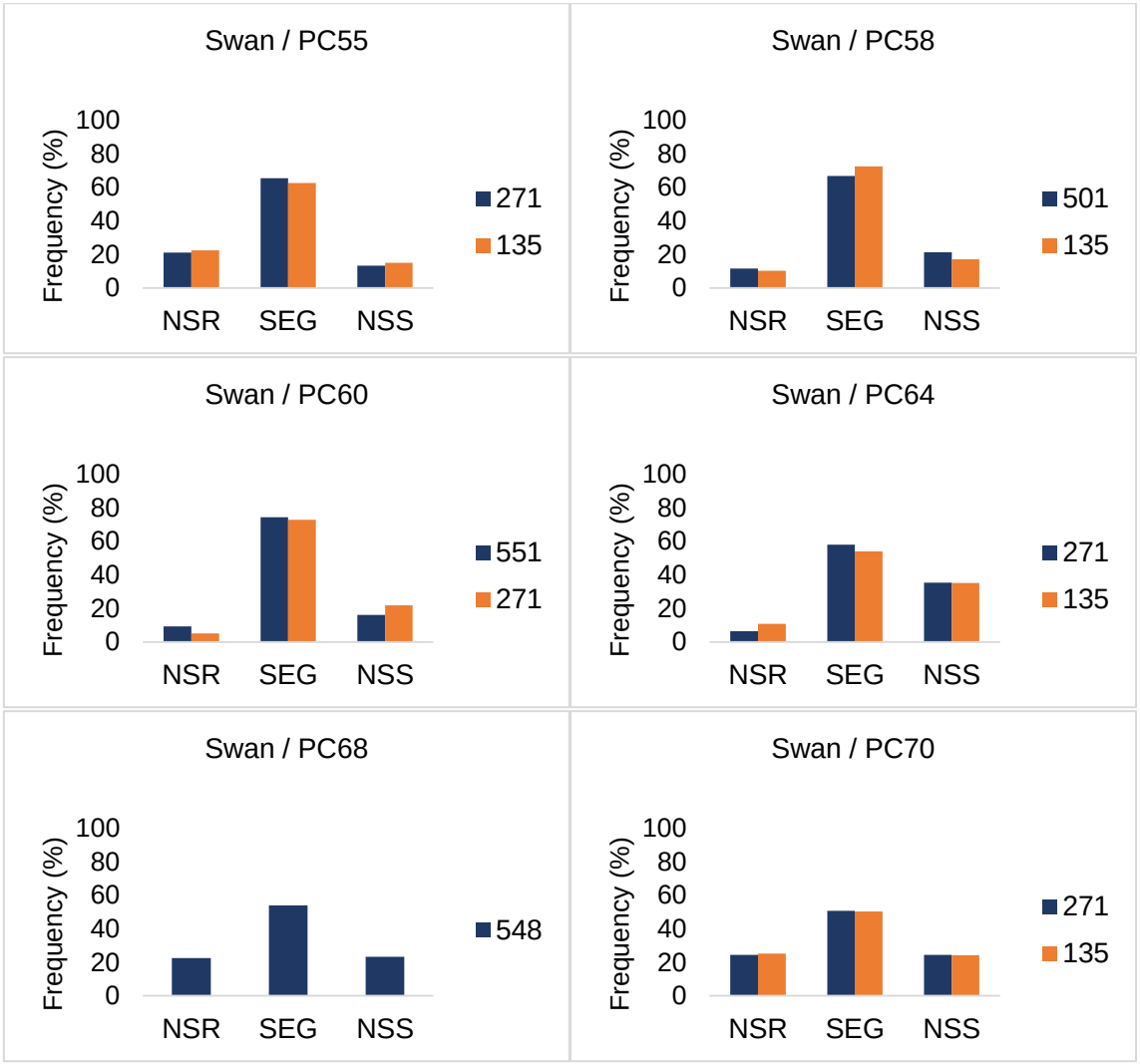


Figure S1 Frequency distribution of oat subpopulations evaluated in the F_3 generation for the percentage of NSR: SEG: NSS plants tested against *Puccinia coronata* f. sp. *avenae* with different pathotypes at the seedling stage.



continuation…**Figure S1** Frequency distribution of oat subpopulation evaluated in the F_3 generation for the percentage of NSR: SEG: NSS plants tested against *Puccinia coronata* f. sp. *avenae* with different pathotypes at the seedling stage.

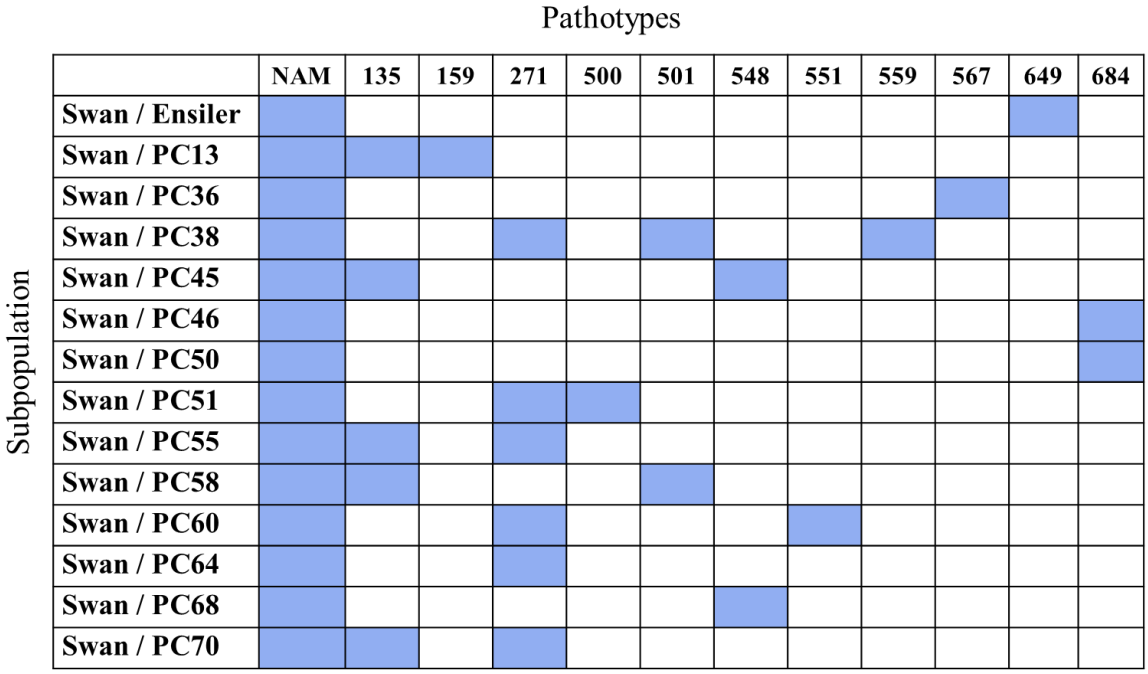


Figure S2 Scheme showing how the NAM analysis was performed. Different Pathotypes were used to challenge the desired genes on the donors.

Table S2 Previously mapped genes and genes mapped on this study.

Population ID	QTL	Chr	Previously mapped	Reference
Swan / Ensiler	Qpc.NAM. 04	3D	No	-
Swan / PC13	Qpc.NAM. 04	3D	No	-
Swan / PC36	Qpc.NAM. 01	1C	No	-
Swan / PC38	Qpc.NAM. 08	7D	7D	Wight *et al.* (2004)
Swan / PC45	Qpc.NAM. 03	2D	2D	Gnanesh *et al.* (2015), Kebede *et al.* (2019)
Swan / PC46	Qpc.NAM. 04	3D	3D	Fleischmann *et al.* (1971), Chong *et al.* (1994)
Swan / PC50	Qpc.NAM. 04	3D	3D	Admassu-Yimer *et al.* (2018)
Swan / PC51	ns	-	No	-
Swan / PC55	Qpc.NAM. 06	-	4C?	Based on the information of linkage with *Pc39* on Chr.4C Wight *et al.* (2004), Zhao *et al.* (2020), Sowa and Paczos-Grzeda (2020)
Swan / PC58	ns	-	7D	Hoffman *et al*. (2006), Jackson *et al.* (2007), Esvelt Klos *et al.* (2017)
Swan / PC60	Qpc.NAM. 01	1C	No	-
Swan / PC64	Qpc.NAM. 07	5D	No	-
Swan / PC68	ns	-	3D	Chen *et al.* (2006), Kulchelski *et al.* (2010), Esvelt Klos *et al.* (2017)
Swan / PC70	Qpc.NAM. 08	7D	No	-

Table S3 Quantitative Trait Loci, chromosome, and sequence of significant SNP markers found in this study.

					Position																																			
QTL	Chr	Marker ID	Pos (Mbp)	SNP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
QpcNAM.01	1C	SNP_5442448*	457.34	28:A>G	G	C	A	G	T	T	T	C	G	C	C	G	A	G	G	G	C	C	G	C	T	G	C	A	T	C	A	A	C	C	A	G	C	C
QpcNAM.02	1D	SNP_13743269*	298.81	38:C>T	G	C	A	G	A	A	T	A	T	T	T	A	G	T	G	C	G	G	A	T	A	G	C	G	A	G	T	G	G	C	A	C	C	C
QpcNAM.02	1D	SNP_5444542**	303.79	65:T>C	G	C	A	G	C	A	G	C	G	A	G	T	C	G	G	A	G	G	A	G	G	A	G	G	C	G	G	G	G	T	T	C	A	G
QpcNAM.03	2D	SNP_20626437**	174.04	19:A>T	G	C	A	G	C	C	C	G	T	G	T	C	G	G	T	G	T	C	A	G	T	G	T	C	T	C	T	G	C	G	G	G	T	G
QpcNAM.03	2D	SNP_20625033_1*	179.01	14:A>G	G	C	A	G	T	T	G	C	A	C	C	C	C	A	C	G	T	G	G	G	G	G	T	C	G	A	G	C	G	C	G	A	G	G
QpcNAM.03	2D	SNP_5440447**	183.99	35:C>G	G	C	A	G	C	A	C	G	A	C	G	A	C	G	G	T	G	G	C	A	G	C	G	C	C	A	T	C	C	A	C	T	C	C
QpcNAM.04	3D	SNP_77981947**	470.35	20:C>T	G	C	A	G	C	G	T	G	G	A	C	G	G	C	A	A	C	A	A	C	G	G	C	T	G	C	A	A	C	G	G	C	G	G
QpcNAM.04	3D	SNP_10583358*	475.30	30:T>C	G	C	A	G	C	A	G	C	A	C	G	A	G	G	C	A	A	A	A	T	G	T	G	G	G	A	A	T	G	T	A	G	G	A
QpcNAM.05	4A	SNP_86989254*	282.94	8:G>C	G	C	A	G	C	T	C	G	C	C	G	T	C	C	G	C	G	A	G	C	A	C	C	A	C	C	C	T	G	G	C	C	G	C
QpcNAM.06	4C	SNP_82839974*	59.98	45:A>C	G	C	A	G	C	C	T	T	T	A	C	T	A	A	C	C	A	A	T	G	T	A	A	A	G	A	C	A	T	A	C	A	C	A
QpcNAM.07	5D	SNP_16624935*	488.76	27:A>C	G	C	A	G	A	C	C	G	C	C	G	C	G	T	A	G	C	A	C	C	C	G	C	G	C	G	A	G	G	C	C	G	A	G
QpcNAM.08	7D	SNP_3458380*	483.30	11:G>T	G	C	A	G	C	A	G	C	C	A	G	C	A	G	C	G	A	G	C	G	A	A	A	C	A	A	A	A	G	C	G	A	C	A

					Position																																			
QTL	Chr	Marker ID	Pos (Mbp)	SNP	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
QpcNAM.01	1C	SNP_5442448*	457.34	28:A>G	C	G	C	C	G	T	G	T	G	T	C	G	C	G	G	T	C	A	A	G	G	C	C	A	C	C	T	T	T	G	T	A	C	T
QpcNAM.02	1D	SNP_13743269*	298.81	38:C>T	T	G	T	C	A	G	T	G	T	C	A	G	G	C	T	C	A	G	G	T	T	A	T	C	A	G	T	T	T	G	G	G	A	G
QpcNAM.02	1D	SNP_5444542**	303.79	65:T>C	C	T	T	C	A	T	G	G	G	G	C	T	C	C	T	C	G	T	C	G	C	G	G	C	C	G	T	C	A	T	T	G	C	C
QpcNAM.03	2D	SNP_20626437**	174.04	19:A>T	G	G	G	C	A	T	A	C	G	A	G	T	C	A	G	G	T	G	G	T	C	A	G	C	T	C	G	G	G	A	G	A	T	T
QpcNAM.03	2D	SNP_20625033_1*	179.01	14:A>G	T	A	C	T	C	C	A	G	G	A	G	G	C	A	G	T	C	C	T	C	G	A	C	G	T	C	G	T	A	C	G	C	T	A
QpcNAM.03	2D	SNP_5440447**	183.99	35:C>G	C	C	G	C	T	G	C	T	C	G	A	G	A	G	G	C	T	A	C	G	C	G	G	T	A	G	C	G	C	C	T	A	A	C
QpcNAM.04	3D	SNP_77981947**	470.35	20:C>T	C	A	T	G	A	T	G	G	A	C	T	A	C	G	C	C	T	T	C	T	C	C	T	A	C	A	T	C	G	C	C	T	C	C
QpcNAM.04	3D	SNP_10583358*	475.30	30:T>C	T	C	C	G	A	G	A	T	C	G	G	A	A	G	A	G	C	G	G	T	T	C	A	G	C	A	G	G	A	A	T	G	C	C
QpcNAM.05	4A	SNP_86989254*	282.94	8:G>C	G	G	T	C	G	C	C	T	C	G	C	C	C	A	C	C	G	C	G	G	C	G	C	A	C	C	C	C	G	T	C	A	C	C
QpcNAM.06	4C	SNP_82839974*	59.98	45:A>C	C	C	T	T	T	T	G	T	G	A	A	C	T	T	T	G	T	T	T	G	T	G	C	A	C	C	C	A	A	T	T	G	G	A
QpcNAM.07	5D	SNP_16624935*	488.76	27:A>C	A	T	C	G	G	A	A	G	A	G	C	G	G	T	T	C	A	G	C	A	G	G	A	A	T	G	C	C	G	A	G	A	C	C
QpcNAM.08	7D	SNP_3458380*	483.30	11:G>T	T	G	A	C	A	C	A	T	C	A	T	T	G	A	T	G	C	T	C	C	T	C	C	T	T	C	C	G	A	T	C	C	A	C

Chr: Chromosome Pos: Chromosome Position Oat_OT3098_v2 genome in Mega base pairs (Mbp)
* Significant SNP Marker
** Within the QTL window of 10Mbp of significant SNP Marker

Table S4 Infection type*on different parental donors in each linkeage group founded on this study.

	Linkeage Group				Linkeage Group		Linkeage Group	
Pathotype†	Ensiler	*Pc13*	*Pc46*	*Pc50*	*Pc36*	*Pc60*	*Pc38*	*Pc64*
133	NA	3+	3+	0;=	;N	;N	;1	;N
135	0;=	0;=	3+	0;=	;N	0;=	3+	;
155	;	;CN	3+	0;=	;C	0;-	;11-	;11-C
158	0;=	0;	3+	0;=	;	;	;1-	;11-N
159	0;	;11+	3+	0;=	;1N	;N-	12	;11-
164	0;	3+	3+	0;=	;CN	;N	;11+2N	;1N
191	0;=	3+	3+	0;=	;	;-	;11+	;11-C
221	0;=	0;B	3+	3+	;1	;N	;1-1=	;11+
223	0;=	0;=	3+	0;=	;1-	0;C-	;1	;1N
266	0;=	0;=	3+	0;=	;1	0;	;1	;1
271	0;-	0;=	3+	0;=	;	0;	;1+	;11-C
298	;-	3+	3+	0;=	;	0;-	3+	;
368	0;-	;CN	X- OR ;	0;=	;N	;CN	;CN	12N
391	22+	2N	;CN	0;=	;1=C	;N	;1	;12C
440	0;	0;	3+	0;=	;1=CN	;;-	3+	;11-C
499	0;=	22+N	;	0;=	;1-N	0;	;N-	XC
500	;	3+	;CN	0;=	;11+2C	;C-	;12	;N
501	0;	3+	;	0;=	;CN	3+	;	;N,;1-N
503	0;=	3+	;	0;=	;CN	3+	;	122+C
513	;N	3+	;N	0;=	;11+CN	;N	;	;
526	0;=	3+	;	0;=	;	3+	0;=	12CN
528	3+	3+	3+	0;=	;12	0;=	3+	;1-
529	0;=	0;C	12	0;=	;	0;=	0;	;1
530	;	3+	;N	0;=	11-N	;1N	3+	;N
536	1;=	3+	;N	0;=	;N	3+	;-N	12N
548	;	3+	;	0;=	;11+N	0;-	3+	;1-
549	;	3+	;N	0;=	3+	;N	3+	;N
550	0;-	3+	0;	0;=	;CN	3+	;	;12N
567	;	3+	;CN	0;-	;N	3+	;11-	;12N
573	0;	;CN	3+	3+	;11+N	;CN	;12C	;1-C
574	;	2CN	;	0;=	2C	;CN	;CN	122+3N
575	0;-	;12N	;	0;=	;1	0;=	;	;
579	;	3+	;	0;=	;12N	0;=	3+	;N
588	0;=	3CN	;CN	0;=	;11-N	;1CN	;C	;12C
593	;	3+	;CN	0;	;12N	0;N	3+	;N
609	;	3+	;	3+	12+	12-N	3+	;N
624	0;	3+	;	0;=	3+	;C	3+	;
628	;1=	3+	3+	0;=	;1-	33-C	;	3+
635	;	3+	3+	0;=	;CN	12N	;-	3+
636	;	3+	;	0;=	3+	;12	3+	;
638	;	3+	;	0;=	3+	;	3+	;1=

Continuation…**Table S4** Infection type* on different parental donors in each linkeage group founded on this study.

Pathotype†	Linkeage Group				Linkeage Group		Linkeage Group	
	Ensiler	*Pc13*	*Pc46*	*Pc50*	*Pc36*	*Pc60*	*Pc38*	*Pc64*
642	;	3+	;	0;=	3+	;12	3+	;
653	0;=	3+	3+	0;=	;11+	2+CN	0;	3+
655	0;-	3+	0;-	0;=	3+	3+	;	;12N
673	3+	12N	3+	;N	;C	3+	;11+	3+
684	;	3+	;N	0;=	3+	;1-N	3+	;N

*Data obtained from Plant Breeding Institute, University of Sydney – Camden campus, NSW, Australia. IT: according described by Nazareno *et al*. (2018), incompatible reactions include the following: 0, no urediniospores; fleck (;), presence of flecks; 1, few small pustules; 2, small pustules, presence of green islands; all of these may be accompanied by necrosis (N) and/or chlorosis (C). Compatible reactions include the following: 3, large pustules surrounded by chlorotic halos; 4, large pustules, often coalescing. The signal + and – represents symptoms. NA: No data available. The presence of two numbers indicates an intermediary class.
†Pathotypes were from a wide collection of single pustule isolates from the Cereal Rust Collection in the Plant Breeding Institute, University of Sydney – Camden campus, NSW, Australia.

5. FINAL CONSIDERATIONS

The resistance to Crown Rust can be attributed to either a single gene or a combination of complex genes. On Brazil, the experiment on Field, due the complexity of the races, just complex resistances are still useful. Using a population with introgressed *A. strigosa* resistance in *A. sativa* was characterized by the genetic inheritance of crown rust resistance. The population '07BT333' x 'UFRGS 970461' shows a complex resistance. Evaluating 332 F_2 lines from this population, at least 4 genes were segregating on this population. The parental '07BT333' shows an immune phenotype which was assigned to two major genes ('R1' and 'R2'). The third one ('R3') was consider a minor gene and its origin could not be elucidated. Also, we reported the presence of a Resistance Suppressor gene ('S') partially dominant. When resistance results from a combination of genes, it becomes stronger as the pathogen faces more difficulty in overcoming it. However, it also becomes challenging to pass on this resistance through generations during the breeding process. Wild species like *A. strigosa* have demonstrated being a valuable source of genes that could significantly contribute to oat breeding.

Genomic regions associated with Crown Rust resistance were also identified in Oats. Using Nest Association Mapping Approach, in a population composed by 14 'donors' and Swan as a 'founder' was genotyped by DartSeq. 15 940 High- quality SNPs were used to identify QTLs associated with Crown Rust resistance. The Qui-square results showed that some populations had a single dominant gene-controlling resistance, while others had more complex resistance. Eight QTLs were identified on different chromosomes, and previously mapped genes were confirmed through QTL mapping using IBD-method. The detected QTLs explained a wide range of phenotypic variation (R^2 of 2% - 21%). Flanking SNPs were reported for each QTL. Several genes were shown be linked or allelic as in case of *Pc13, Pc46* and *Pc50*, Pc38 and Pc64, Pc36 and Pc60. Some were considered potential QTL gene candidates as Pc36 and Pc60 on the Qpc.NAM.01, Pc45 on Qpc.NAM.03, *Pc13*, *Pc46*

and *Pc50* on Qpc.NAM.04, *Pc70* on Qpc.NAM.07 and *Pc38* and *Pc64* on Qpc.NAM.08. The location of previous mapped genes were also confirmed as Pc38 on chromosome 7D, Pc45 on 2D, Pc46 and Pc50 on 3D. Location analysis revealed numerous genes in the region of the SNP markers related to disease resistance such as LRR and CYP450.

6. SUPPLEMENTAR MATTERIAL

Literature Review

Table 1: Oat crown rust resistance genes.

Gene	Original source	Avena species	Gene	Original source	Avena species	Gene	Original source	Avena species
Pc1	Red Rustproof	*A. byzantina*	*Pc8*	Santa Fe	*A. byzantina*	*Pc19*	CI 3815	*A. strigosa*
Pc2	Victoria	*A. byzantina*	*Pc 9*	Ukraine	*A. sativa*	*Pc20*	CI 7233	*A. abyssinica*
Pc2b	Anthony/Bond/Boone		*Pc9c*	Santa Fe	*A. byzantina*	*Pc21*	Santa Fe	*A. byzantina*
Pc3	Bond	*A. byzantina*	*Pc10*	Klein 69B	*A. byzantina*	*Pc22*	Ceirch du Bach	*A. sativa*
Pc3c	Ukraine	*A. sativa*	*Pc11*	Victoria	*A. byzantina*	*Pc23*	C.D 3820	*A. strigosa*
Pc4	Bond	*A. byzantina*	*Pc12*	Victoria	*A. byzantina*	*Pc24*	Garry	*A. sativa*
Pc4c	Ukraine	*A. sativa*	*Pc13*	Clinton	*A. sativa*	*Pc25*	Garry	*A. sativa*
Pc5	Landhafer	*A. byzantina*	*Pc14*	Ascencao	*A. byzantina*	*Pc26*	Garry	*A. sativa*
Pc6	Santa Fe	*A. byzantina*	*Pc15*	Saia	*A. strigosa*	*Pc27*	Garry	*A. sativa*
Pc6c	Ukraine	*A. sativa*	*Pc16*	Saia	*A. strigosa*	*Pc28*	Garry	*A. sativa*
Pc6d	Trispernia	*A. sativa*	*Pc17*	Saia	*A. strigosa*	*Pc29*	Glabrota	*A. glabrota*
Pc7	Santa Fe	*A. byzantina*	*Pc18*	Glabrota	*A. glabrota*	*Pc30*	CI 3815	*A. strigosa*

Continuation…Table 1: Oat crown rust resistance genes.

Gene	Original source	Avena species	Gene	Original source	Avena species	Gene	Original source	Avena species
Pc31	CI 4746	*A. strigosa*	*Pc46*	F-290	*A. sterilis*	*Pc61*	PI 287211	*A. sterilis*
Pc32	CeirchLlwyd	*A. strigosa*	*Pc47*	CI 8081A	*A. sterilis*	*Pc62*	CAV 4274	*A. sterilis*
Pc33	CeirchLlwyd	*A. strigosa*	*Pc48*	F-158	*A. sterilis*	*Pc63*	CAV 4540	*A. sterilis*
Pc34	D-60	*A. sterilis*	*Pc49*	F-158	*A. sterilis*	*Pc64*	CAV 4248	*A. sterilis*
Pc35	D-137	*A. sterilis*	*Pc50*	CW 486	*A. sterilis*	*Pc65*	CAV 4248	*A. sterilis*
Pc36	CI 8081	*A. sterilis*	*Pc51*	Wahl No.8	*A. sterilis*	*Pc66*	CAV 4248	*A. sterilis*
Pc37	CD 7994	*A. strigosa*	*Pc52*	Wahl No.2	*A. sterilis*	*Pc67*	CAV 4656	*A. sterilis*
Pc38	CW 491-4	*A. sterilis*	*Pc53*	6-112-1-15	*A. sterilis*	*Pc68*	CAV 4904	*A. sterilis*
Pc39	F-366	*A. sterilis*	*Pc54*	CAV 1832	*A. sterilis*	*Pc69*	CAV 1387	*A. sterilis*
Pc40	F-83	*A. sterilis*	*Pc55*	CAV 4963	*A. sterilis*	*Pc70*	PI 318282	*A. sterilis*
Pc41	F-83	*A. sterilis*	*Pc56*	CAV 1964	*A. sterilis*	*Pc71*	IA B437	*A. sterilis*
Pc42	F-83	*A. sterilis*	*Pc57*	CI 8295	*A. sterilis*	*Pc72*	PI 298129	*A. sterilis*
Pc43	F-83	*A. sterilis*	*Pc58*	PI 295919	*A. sterilis*	*Pc73*	PI 309560	*A. sterilis*
Pc44	Kyto	*A. sativa*	*Pc59*	PI 296244	*A. sterilis*	*Pc74*	PI 309560	*A. sterilis*
Pc45	F-169	*A. sterilis*	*Pc60*	PI 287211	*A. sterilis*	*Pc75*	IB 2402	*A. sterilis*
Pc76	IB 2465	*A. sterilis*	*Pc84*	CI 3815	*A. strigosa*	*Pc92*	Obee/Midsouth	*A. strigosa*
Pc77	IB 2433	*A. sterilis*	*Pc85*	CI 3815	*A. strigosa*	*Pc93*	CI 8330	
Pc78	IB 1454	*A. trichophylla*	*Pc86*	CI 3815	*A. strigosa*	*Pc94*	RL 1697	*A. strigosa*
Pc79	IB 1454	*A. trichophylla*	*Pc87*	CI 3815	*A. strigosa*	*Pc95*	Wisc X 1588-2	*A. sativa*
Pc80	IB 3432	*A. sterilis*	*Pc88*	CI 3815	*A. strigosa*	*Pc96*	RL 1730	*A. sativa*
Pc81	CI 3815	*A. strigosa*	*Pc89*	CI 3815	*A. strigosa*	*Pc97*	CAV 1180	*A. sterilis*
Pc82	CI 3815	*A. strigosa*	*Pc90*	CI 3815	*A. strigosa*	*Pc98*	CAV 1979	*A. sterilis*
Pc83	CI 3815	*A. strigosa*	*Pc91*	CW 57	*A. longiglumis*			

Adapted from GNANESH, B. N. *et al*. (2014)

Table 2: Australian Differential Collection of Crown Rust Genes.

Genotype	**Gene**		**Genotype**	**Gene**
H458	*Pch548*		Warrego	Unknown
Wix4361-9	*Pcwix1, Wix2*		Bettong	Unknown
Amagalon	*Pc91*		Barcoo	Unknown
Culgoa	Unknown		Landhafer	*Pc5, Pc4*
Cleanleaf	*Pc38,39,52*		Santa Fe	*Pc6,7,8,21*
Pc68	*Pc68*		Ukraine	*Pc3c,4c,6c,9*
Tam.0301	*Pc58*		Trispernia	*Pc6d*
Tam.0.312	*Pc59*		Bondvic	*Pc3,4*
Pc61	*Pc61*		Pc45	*Pc45*
Pc39	*Pc39*		Pc48	*Pc48*
Pc38	*Pc38*		Nugene	*Pc48,68*
Swan	*Pc1*		Gwydir	Unknown
Pc36	*Pc36*		Volta	*Pc50,68*
Pc46	*Pc46*		*Pc 92*	*Pc 92*
Pc50	*Pc50*		*Pc 94*	*Pc 94*
Pc51	*Pc51*		Saia	*Pc15,16,17*
Pc52	*Pc52*		Ensiler	Unknown
Pc55	*Pc55*		Genie	*Pc48,56*
Pc56	*Pc56*		Drover	*Pc91*
Pc63	*Pc63*		Aladdin	Unknown
Pc64	*Pc64*		Coker 227	*Pcb 60-2*
Pc71	*Pc71*		Coker234	*Pcb 62*
X716	Unknown			

7. VITA

Jessica Argenta is the daughter of Ines Maria Rissardo and Augusto Argenta. She was born on August 4th, 1993, in Marau, Rio Grande do Sul state, Brazil. Studied elementary and high school in her hometown. Moved to Sertão, a town also in Rio Grande do Sul state, Brazil, in February 2011, where attended a technical course in Agriculture at the Federal Institute of Rio Grande do Sul. In February of 2012 started, in the same institution, her Bachelor of Science degree in Agronomy. The Technical course graduation was in June 16th 2012 and Bachelor graduation on March 3rd, 2017. Jessica has been working with plant breeding since the first semester of his bachelor's degree. She was awarded with 4 fellowships to work as an undergraduate Junior Scientist. In 2017, moved to Porto Alegre, Rio Grande do Sul and started the Master of Science graduate course in Plant Breeding at the Federal University of Rio Grande do Sul, being awarded with a scholarship by CNPq foundation. Earned a Master of Science degree on July 19th, 2019, and started the doctoral graduate course in Plant Breeding also at the Federal University of Rio Grande do Sul, being awarded with a scholarship from CAPES. As a doctoral candidate Jessica received a scholarship, from CAPES, to pursued them last two semester of her doctoral course at the Plant Breeding Institute (PBI), University of Sydney, as a Researcher Visitor, from September 2022 to July 2023, where she developed a significant part of her thesis.