MAPPING THE *Ur-5* GENE CONFERRING RESISTANCE TO COMMON BEAN RUST

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MASTER OF SCIENCE

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ABSTRACT

Bean rust (*Uromyces appendiculatus* (Pers) Unger), is a highly virulent pathogen that negatively affects bean (*Phaseolus vulgaris* L.) seed yield. Over time, bean rust has the capacity to generate new races that overcome the existing resistance in cultivars. An alternative to control plant diseases is through breeding and pyramiding resistance genes. However, these genes need to be characterized first, to be used effectively. An F_2 population from the cross UI-114 /Mexico 309 (*Ur-5* gene) was evaluated with race 20-3 being prevalent in ND. A set of InDel markers were designed and tested for potential use in MAS. It was confirmed that the *Ur-5* is a single dominant gene and it was located between 547.7-1,396.4 Kb in the Pv04. The candidate genes found are mostly NB-ARC and LRR domains, and it included some genes models that encode cytochrome P450, and protein kinases that are also related to disease resistance.

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To my friends, I want to say that I really appreciate the moments we shared.

DEDICATION

To my dear parents Antonio Ixcotoyac and Geny Cabrera.

To my brother Antonio, and my sisters Priscila and Valeria.

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INTRODUCTION

There is great diversity of common beans *(Phaseolus vulgaris* L.) with different shapes, colors and sizes, classified as distinct market classes. Preference is mainly linked to cultural patterns of each location (Simoneti et al., 2008). Common bean in the U.S. market is primarily sold as dry beans, and is composed of many market classes with pinto, navy, black, great northern, kidney, and pink being the most important (Schumacher, 2017).

In 2019 the United States produced 0.94 million tonnes of dry bean (USDA-NASS, 2019). North Dakota ranked highest for production with 37%, followed by Minnesota with 20% and Michigan with 17%; together these states represent almost 75% of the total production at the national level.

Common bean productivity, as with other crops, is affected by biotic and abiotic factors. Genetic improvement toward the development varieties with superior seed yield and good agronomic characteristics, and breeders are also focusing their efforts on producing cultivars with resistance to pests and diseases, drought tolerance and with a better nutritional quality (Buddenhagen, 1983). Genetic resistance for diseases is widely used due to effectiveness, low-cost compared with chemical control, and reduced environmental impact. Therefore, it is of great importance for breeding programs to carry out genetic studies in order to identify genes that confer resistance to diseases (Walton, 1997).

Common bean rust (caused by the fungus *Uromyces appendiculatus* (Pers) Unger) is a very important disease in dry bean production, and it can cause significant seed yield losses. Severe outbreaks, particularly at early growth stages can resulting in seed yield losses of up to 100% of production (Stavely, 1991). Dry bean cultivars with genetic resistance to different rust races have been developed, but in many cases, the pathogen has overcome the genetic resistance.

U. appendiculatus (Pers) is a highly virulent pathogen given its capacity and rates for sexual recombination, mutation, and gene flow within a population (Araya et al., 2004). At least 10 rust resistance genes have been identified and named with the prefix *Ur*, and in most cases these genes show resistance against one or multiple *U. appendiculatus* races (Miklas et al, 2002).

North Dakota is the most important state for dry bean production in the United States. However, in recent years there has been an increase in common bean rust presence which could cause potential losses in production (Monclova-Santana et al., 2017). In 2008, a new rust race (20-3) virulent for the *Ur-3*, *Ur-6*, *Ur-7*, and the unknown gene from Montcalm was discovered in North Dakota (Markell et al., 2009). By 2016, the pathogen was found in 62% of dry bean fields surveyed, with an incidence ranging from 5 to 100%, despite the fact that some varieties with known rust resistance genes such as *Ur-3* were being grown (Pasche and Markell, 2017).

Pastor-Corrales et al. (2010a) determined that race 20-3 had overcome the resistance conferred by the *Ur-3* resistance gene previously known to be resistant to the rust races present in North Dakota. The *Ur-3* gene is probably the most common and deployed gene for rust resistance worldwide and it has been introgressed into many cultivars in North Dakota due to its broad resistance against many rust races, but unfortunately, race 20-3 overcome this resistance. Fortunately, genotypes that possesses either *Ur-5* or *Ur-11* genes (among few others), showed resistance against the race 20-3. These genes have been less exploited and are a good source for gene pyramiding to have a broader and long-term resistance in North Dakota. However, it is important when pyramiding genes to take into account the epistatic interaction between genes. For example, *Ur-11* is epistatic to *Ur-3, Ur-6* and *Ur-7* masking the effect of the other gene when they are combined in the same genotype (Hurtado-Gonzales et al., 2017). This study is focused on the *Ur-5* as an alternative that can used in gene pyramiding through the use of more

recent and efficient molecular tools such as InDel markers. The study also aims to provide more up-to-date information on the location and genetic bases of the *Ur-5.* Gene.

Given the economic importance of dry bean production in the state of North Dakota and the impact of common bean rust in recent years, the present study was proposed with the following objectives: a) to determine the inheritance of the *Ur-5* gene by evaluating the reaction of a F_2 population from the cross UI-114 x Mexico 309 segregating for the *Ur-5* resistance gene which confers resistance to the most prevalent rust race in North Dakota. b) to map the *Ur-5* gene and identify reliable molecular markers that could be used for marker-assisted selection, and 3) to identify potential functional candidate genes responsible for the resistance conferred by *Ur-5*. This information will aid dry bean breeding programs to develop new cultivars with a broader and long-term resistance against rust races.

LITERATURE REVIEW

Dry bean (*Phaseolus vulgaris* L.)

The genus *Phaseolus* belongs to the Leguminosae family, and is composed of more than 150 species, among which are annual and perennial plants that grow mostly in tropical regions. Within the genus *Phaseolus*, a large proportion of species are herbaceous plants, but some are a little woody at the base of the stem (Freytag and Debouck, 2002). In terms of use, this genus can include ornamental plants, and others have seeds and edible pods of fundamental importance in the diet worldwide (Nassar et al., 2010).

Among the *Phaseolus* genus there are five more advanced species in the domestication process that are of economic importance, including: tepary bean *(P. acutifolius),* runner bean *(P. coccineus),* lima bean *(P. lunatus),* year bean *(P. dumosus Syn. P. polyanthus),* and common bean *(P. vulgaris* L.*)* (Delgado-Salinas et al., 1999). *P. vulgaris* L. is the most important and most widely cultivated species. Its origin was in the Americas where wild species can be found from Mexico to Argentina, and its range of origin is indicative that this species is able to adapt to diverse environmental conditions (Becerra et al., 2011). A study conducted by Bitocchi et al. (2012) confirmed that *P. vulgaris* has a Mesoamerican rather than South American origin, located mainly in Mexico, and is proven to have a greater diversity to close wild relatives.

The common bean that we know today was domesticated both in Middle America and in Andean South America, giving rise to the concept of gene pools (Singh et al., 1991a). Considering the two common bean domestication centers, the germplasm can be grouped into six races, three races with origins in Middle America including: Durango, Jalisco, and Mesoamerica; and three races with origins in Andean South America, including: Chile, Nueva Granada, and Peru. It is important to mention that in the study done by Tobar et al. (2020), a new race called

Guatemala was confirmed as part of the Middle America gene pool, and is mostly composed of climbing beans. According to Singh et al. (1991b) there are several morphological and biochemical differences between the races of Middle America and Andean South America, such as allozyme profiles, shape of bracteole, and in most cases, the races of Middle America have a smaller seed size compared to the Andean South American races.

From a genetic improvement perspective, it is important to consider the center of origin of the crop because you can find greater genetic variability in wild relatives, in which is possible to identify genes that confer resistance against biotic and abiotic factors with the purpose of using this gene pool in breeding programs (Brozynska et al., 2016).

Nutritional value of dry bean

Common bean is the most important grain legume in the human diet worldwide, and it is estimated that it is a staple food for more than 300 million people in countries of Latin America and East/Sub Saharan Africa. Per capita consumption in East Africa is approximately 50 kg $year^{-1}$, while Latin America has a lower per capita consumption with a range of 10-18 kg $year^{-1}$ (Morales, 2003). In some of these countries, it is an indispensable food that provides most of the necessary requirements for protein, energy, and micronutrients, especially iron and zinc (Petry et al., 2015). Dry beans, in addition to providing protein and carbohydrates, are a source of dietary fiber and antioxidants (Rezande et al., 2018). Economics are another factor making dry beans an important food worldwide, as they are considered a low-cost source of vegetable protein. Dry beans are consumed both in developing and developed countries, and due to the diversity of species, they can be consumed in a variety of ways (Arenas, et al., 2013).

U.S. and North Dakota dry bean production

U.S. total production in 2019 was reported to be 0.94 million tonnes of dry bean obtained from 476,112 Ha. In the same year, the top five producing states were North Dakota (37%), Minnesota (20%), Michigan (17%), Nebraska (19%), and Idaho (5%), clearly showing that North Dakota is the most important state for dry bean production. In 2019, North Dakota reported a total production of 430,696 tonnes and a harvested area of 222,577 Ha. Pinto, black and navy bean were the most important market classes in North Dakota in 2019, representing about 94% of the total state production, with 250,096 tonnes, 95,536 tonnes and 58,856 tonnes respectively (USDA-NASS, 2020).

Common bean rust

Bean rust is caused by the pathogen (*Uromyces appendiculatus* (Pers) Unger (Syn. *U. phaseoli* (Reben) Wint). This disease has caused seed yield losses that can significantly affect the production, even up to more than 50% of crop seed yield (Harveson et al., 2013). However, early and severe infections are the most dangerous because it can cause seed yield losses up to 100% of production (Stavely, 1991). It is also important to emphasize that common bean rust is a highly variable and virulent pathogen, and this characteristic is influenced by the capacity for sexual recombination, mutation, gene flow and favorable environment conditions (Araya et al., 2004).

Common bean rust is characterized by reddish brown powdery-looking pustules and can be found on both sides of the same leaf (Figure 1). Symptoms also can appear on pods and the stem, and pustules are a source of inoculum that can be easily disseminated by any object with which they are in contact or by the wind. Larger pustules are usually surrounded by a yellow

halo, and when the disease attack is very severe the leaf may fall prematurely (Liberato and Sartorato, 2005).

Common bean rust is considered a disease with a macrocyclic life cycle due to the characteristics of the fungus, and this can complete the different cycle stages in the dry bean plant. The pathogen in its life cycle can produce different types of spores (Figure 1) which includes teliospores, basidospores, pycniospores, aeciospores and urediniospores. Teliospores are the type of spores which the pathogen has the ability to overwinter in crop residues. Then once optimal conditions occur, the teliospores germinate and produce basidiospores which are responsible for infecting the bean plant (host), and producing a structure called pycnia. The pycnia produces pycniospores which through cross fertilization produce an aecium, then this structure produces aeciospores which can also infect the plant, and later can form a structure called uredia. Once the uredia is formed, it has the capacity to produce urediniospores that can also infect the plant and increase the amount of uredia, and this allows the pathogen to re-infect the plant during the growth stage of the plant. As the uredia ages, these are transformed into teliospores with the characteristic of having a thicker wall, and this structure is the inoculum that overwintering for the following year (Stavely and Pastor-Corrales, 1989).

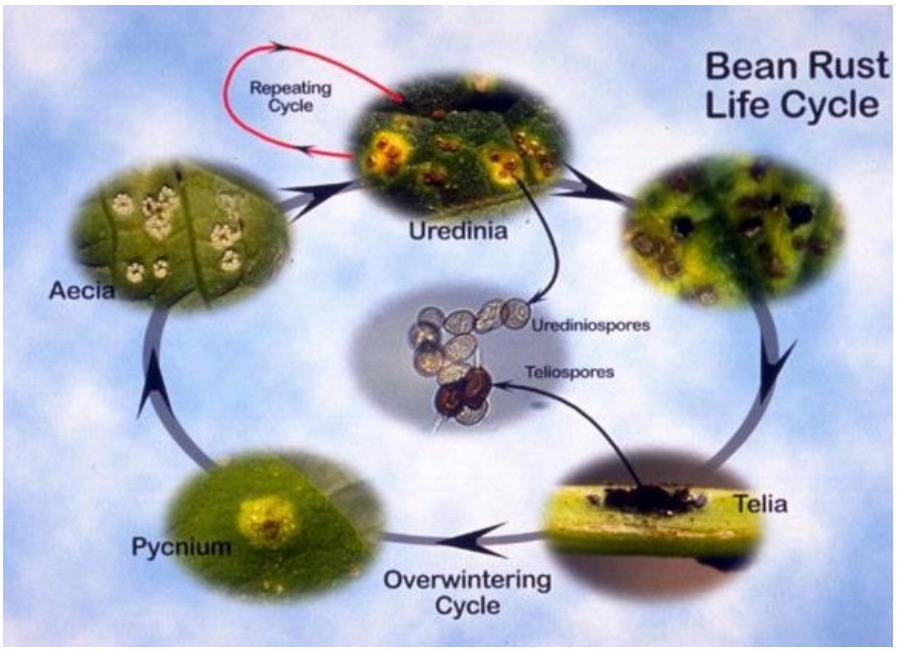

Figure 1. Life cycle of common bean rust.
Source: McMillan and Schwartz (https://www.ipmimages.org/detail.cfm?imgnum=5361050)

Race classification system and rust scale

Due to the high variability that *U. appendiculatus* possess, there was a need to group into races. A race is defined as a group of isolates that harbor the same virulent genes and those genes have the ability to interact with specific resistance genes in the host. In order to classify the races, a set of differential genotypes is required, in which these genotypes are known to have one or more specific resistance genes (Meyer et al., 2010).

In common bean, several differential cultivars have been identified, both include Andean and Mesoamerican origin, which have been used for the classification of *U. appendiculatus* races. However, the number of differential cultivars has changed over time, with the last modification in 2002 during the 3rd Bean Rust Workshop, designating a total of 12 differential cultivars (Table 1), 6 of Andean origin and 6 Mesoamerican (Steadman et al., 2002). This was done internationally standardize the classification of rust races. Each race is designated by two numbers separated by a hyphen, the first number consisting of the sum of the binary values

related to the susceptible Andean cultivars, and the second number is made up of the sum of the binary values of the susceptible Mesoamerican cultivars (Steadman et al., 2002).

Tale 1. Cultivars used as an international differential set for classification of *U. appendiculatus* races

Gene pool	Entry	Resistance gene	Binary value
Andean	Early Gallatin	*Ur-4*	1
	Redlands Pioneer	*Ur-13*	2
	Montcalm	Unknown	4
	PC-50	*Ur-9, Ur-12*	8
	Golden Gate Wax	*Ur-6*	16
	PI 260418	Unknown	32
Mesoamerican	Great Northern 1140	*Ur-7*	1
	Aurora	*Ur-3*	2
	Mexico 309	*Ur-5*	4
	Mexico 235	*Ur-3*$^{+}$	8
	CNC	Unknown	16
	PI 181996	*Ur-11*	32

Source: Steadman et al., 2002

Due to the great variability in proposed scales to measure pathogen damage, it was necessary to standardize the scales, adopting mainly the scale proposed by Stavely et al. (1983) and Mmbaga et al. (1996). This scale consists of 6 values based on the pustule diameter (Table 2). The uniformity in the use of the differentials, the scale to measure the pathogen damage, and the nomenclature to name the common bean rust races was necessary to create a system of international use, and to be able to interchange information between researchers.

Table 2. Description of *U. appendiculatus* grading scale

Infection type	Description	Phenotype
1	Immune, having no visible symptoms	Resistant
2	Necrotic spots, without sporulation, and <0.3 mm in diameter	Resistant
2,3	Reaction type 2 with few type 3	Resistant
3	Uredinia less than 0.3 mm diameter	Resistant
3,2	Reaction type 3 with few type 2	Resistant
3,4	Reaction type 3 with few type 4	Susceptible
4	Uredinia 0.3-0.49 mm diameter	Susceptible
4,5	Reaction type 4 with few type 5	Susceptible
5	Uredinia 0.5-0.8 mm diameter	Susceptible
5,4	Reaction type 5 with few type 4	Susceptible
5,6	Reaction type 5 with few type 6	Susceptible
6,5	Reaction type 6 with few type 5	Susceptible
6	Uredinia larger than 0.8 mm diameter	Susceptible

Stavely et al., 1983; Mmbaga et al., 1996

Common bean rust increase in North Dakota

During 2016, 40 dry bean commercial fields were surveyed in 11 counties in the North Dakota regions that are important in dry bean production to identify the presence of foliar diseases in dry beans (Pasche and Markell, 2017). Bean rust was found in 62% of the dry bean fields evaluated with a wide range of incidence of the disease ranging from 5% to 100%. Likewise, the authors mention that in the last 3 years before 2016, there has been an increase in both the severity and incidence of bean rust in the main dry bean producing areas.

Another study carried out by Monclova-Santana et al. (2017), aimed to determine the main races of common bean rust that were present in North Dakota during 2015 and 2016. The author reported that by 2015, 80% of the isolates belonged to race 20-3, a 10% attributed to race 28-3, and in the rest of the isolates races 20-2, 20-11, 21-3, 28-11 and 29-3 were detected. By 2016, approximately 60% of the isolates belonged to race 20-3, 20% to race 21-3, and the rest of isolates belonging to races 16-3, 24-3, 28-3, 29-3, 29-7, 29-31, 31-7. These results demonstrated

that some common bean rust races found are virulent for the *Ur-3, Ur-4*, *Ur-9, Ur-12,* and *Ur-13* resistance genes. It should be noted that the most predominant race in both years was 20-3 which was reported for the first time in North Dakota in 2008 (Markell et al., 2009).

Pastor-Corrales et al. (2010a), screened 70 dry bean genotypes from various market classes grown in the United States. The authors determined that 77% of the cultivars were susceptible, and 14% were resistant to the new rust races found in Michigan (22-3) and North Dakota (20-3). Several of the susceptible cultivars had the *Ur-3* resistance gene, while the resistant cultivars were determined to possess either the *Ur-5* or *Ur-11* resistance genes. Because common bean rust is a highly variable pathogen, the fact that breeding programs develop cultivars with rust resistance based on one gene puts the production of dry beans at risk, so it is recommended to include two or more genes with rust resistance in a cultivar to have a broader spectrum to different rust races (Pastor-Corrales et al., 2010b).

Genetic disease resistance

The resistance to common bean rust is a type of resistance mostly based on single and dominant genes (Kelly et al., 1996; Souza et al., 2007). Several genes have been identified that confer resistance against the common bean rust of both Mesoamerican and Andean origin. At least 10 genes have been identified, these being *Ur-3, Ur-4, Ur-5, Ur-6, Ur-7, Ur-9, Ur-11, Ur-12, Ur-13*, and *Ur-14*, and each gene has resistance against one or multiple *U. appendiculatus* races (Stavely, 1984; Stavely et al., 1989b; Kelly et al., 1996; Miklas et al, 2002).

The genetic resistance to diseases began to be discovered in the form of natural selection, in which surviving plants possessed genes that allowed them to stop the attack of pathogens. Then, the breeding programs took advantage of this to include in their objectives, the development of varieties with genetic resistance to pathogens (McDonald, 2004). The genes that

confer resistance against pathogens have the ability to recognize pathogen effector molecules when the pathogen attacks the host. After the recognition of effectors, it activates mechanisms in the plant that allow it to counterattack the pathogen and stop the symptoms and the progression of the disease (Gururani et al. al., 2012; Martin et al., 2003).

Maintaining resistance over time is difficult because of high pathogen variability. New races can emerge that overcome the resistance genes present in bean varieties or the resistance genes are not effective against them as it happened in Michigan in the year 2007 with the race 22-3, and then in North Dakota in 2008 when race 20-3 appeared. The resistance of the *Ur-3* gene was not effective against the race 20-3, and the *Ur-3* gene was present in many of the cultivars grown in these areas (Markell et al., 2009; Wright et al., 2008). The breakdowns of resistance against rust has consequently led to an increase in the presence of the disease, being detected in 62% of the fields sampled in North Dakota in 2016 (Pasche and Markell, 2017).

By 2001, at least 94 *U. appendiculatus* races have been identified and maintained by United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Bean Project at Beltsville, MD (Stavely, 1984; Stavely et al., 1989b). These races have been collected in the United States, and in some countries of Latin America, Africa, Europe and Asia (Pastor-Corrales, 2001).

Each *Ur-* gene confers resistance against either one or multiple *U. appendiculatus* races, so for example, the genes *Ur-3, Ur-4, Ur-5, Ur-6* and *Ur-11* are resistant to 55, 35, 73, 28 and 93 of the 94 races identified until 2001, being the *Ur-11* the gene with the widest spectrum of resistance, which has only been reported as susceptible to the Middle American race 108 (Pastor-Corrales, 2001). Therefore, in order to have a durable and effective resistance against this pathogen it is critical to not rely on just a single gene; gene pyramiding of multiple resistance

genes is necessary in case new races emerge that can overcome the effectiveness of any of the genes used in the pyramiding strategy (Stavely, 2000). For the particular case of North Dakota, it is recommended that the dry bean breeding program pyramid the *Ur-5* and *Ur-11* resistance genes because these have been shown to be effective for the control of the new rust races (Wright et al., 2009; Pastor-Corrales et al., 2010a; Monclova-Santana et al., 2017).

The *Ur-5* rust resistance gene

The *Ur-5* resistance gene was first reported by Stavely (1982). He identified the presence of a genetic block called B-190 that was composed of tightly linked monogenic factors, and composed of eight single dominant independent genes in the landrace Mexico 309 and named them as *UrA, UrB, UrC, UrD, UrE, UrF, UrG, UrH*. Stavely et al. (1989a) also reports the presence of the *Ur-5*, *Ur-6* and *Ur-7* genes in the genotype BelNeb-RR-1, a great northern improved germplasm line.

Then, in 1996, the systematization of symbols for the common bean rust resistance genes was carried out, and it was proposed to name the B-190 gene block as *Ur-5*, and the genes that make up the block were named *Ur-5A, Ur-5B, Ur-5C, Ur-5D, Ur-5E, Ur-5F, Ur-5G, Ur-5H* (Kelly et al., 1996). In 1999, Montrose pinto bean cultivar was released by Colorado Agriculture Experimental Station, which contains the *Ur-5* and *Ur-7* resistance genes (Brick et al., 2001). Miklas et al. (2002), determined that the *Ur-5* gene is located towards the end of the chromosome Pv04, and also reported that the *Co-9* gene for resistance to anthracnose (caused by the fungus *Colletotrichum lindemuthianum* Sacc. and Magn.) was in close proximity as well.

The first attempt to identify molecular markers linked to the *Ur-5* resistance gene for the purpose of being used in MAS (marker-assisted selection) was done by Haley et al., (1993), it was identified through the RAPD (random amplified polymorphic DNA) marker $OI19_{460}$.

Melotto and Kelly (1998), in their study they used SCAR (sequence characterized amplified region) marker, and they concluded that the marker SI19 was related to the *Ur-5* gene. Then Souza et al. (2007), conducted a study to validate if the SCAR marker SI19 was linked to the genotype Mexico 309 which is a source of the *Ur-5* gene. It was concluded that Mexico 309 contains the single dominant gene *Ur-5*, and the marker SI19 is at 3.31 cM of the gene and it is in the coupling phase.

Insertion and deletion (InDel) markers

Through time, genomic tools to perform marker assisted selection (MAS) have been evolving, making MAS more efficient and accurate. Recently, one the tools that has been exploited is insertion and deletion (InDel) markers because this tool has several advantages and positives aspects for geneticists (Păcurar et al., 2012; Moghaddam et al., 2014). A positive characteristic is that these markers represent an abundant source of genetic variation through the genome of plants and humans (Das et al., 2015; Mullaney et al., 2010). Another relevant characteristic of InDel markers is their codominant nature that make it easier to identify heterozygous individuals when both of the parental alleles are expressed at the same time (Das et al., 2015).

Recently, in addition to being used in MAS, the InDel markers have been used in several genetic approaches like mapping populations to generate fine mapping, QTL mapping and construction of phylogenetic trees in several crops like barley (*Hordeum vulgare* L.), chickpea (*Cicer arietinum* L.), Chinese kale (*Brassica oleracea* L. var. alboglabra Bailey) and dry beans *(Phaseolus vulgaris* L.), among other crops (Watt et al., 2020; Srivastava et al., 2016; Xu et al., 2019 and Moghaddam et al., 2014). Among the advantages well appreciated by breeders is the low cost and simplicity that these InDel markers represent because it doesn't require specialized

laboratory equipment and the genotyping results are easy to interpret reading the agarose gel electrophoresis (Hu et al., 2020).

Given the importance of dry bean production in the state of North Dakota and the increase of common bean rust in recent years, the *Ur-5* gene, in addition to the *Ur*-11 gene, are good alternatives to use in gene pyramiding to develop new cultivars with a broader and long-term resistance against rust races. Therefore, the present study was proposed with the following objectives:

1. To determine the inheritance of the *Ur-5* gene by evaluating the reaction of a F_2 population from the cross UI-114 x Mexico 309 segregating for the *Ur-5* resistance gene which confers resistance to the most prevalent rust race in North Dakota.
2. To map the *Ur-5* gene and identify reliable molecular markers that could be used for marker-assisted selection.
3. To identify potential functional candidate genes responsible for the resistance conferred by *Ur-5*.

MATERIAL AND METHODS

Phenotypic evaluation

Plant material

For this study, a bi-parental population was used both at the F_2 and $F_{2:3}$ generations, with 168 F_2 lines derived from the cross between UI-114/Mexico 309. Mexico 309 is a black bean, of Mesoamerican origin, considered as a differential cultivar for common bean rust and therefore, the original source of the *Ur-5* gene (Steadman et al., 2002). UI-114 is a pinto cultivar released by the University of Idaho in 1967, and it is considered the universal rust susceptible check because it lacks any known rust resistance genes (Brick et al., 2008). Initially, F_2 plants were inoculated and evaluated for resistance to *U. appendiculatus* race 20-3 in the greenhouse and kept for seed production. Subsequently, a progeny test was made using the $F_{2:3}$ seeds form each F_2 plant rated as resistant with the goal of identifying the homozygous dominant (RR) from the heterozygous (Rr) genotypes.

Inoculation of bean rust race 20-3

The inoculum of the rust race 20-3 was collected in a commercial field in North Dakota and was provided by Pulse Pathology program from Plant Pathology department of North Dakota State University. A single pustule was obtained from this isolate and multiplied for the evaluation. Inoculum preparation and inoculation was made following the procedure described by Acevedo et al. (2013). Briefly, the inoculum was prepared using 5 mg of urediniospore solution in 60 ml of tap water with 0.004% Tween-20 with a final concentration of 2.0×10^4 urediniospores/ml. The inoculum was applied using a hand sprayer (Figure 2) when the primary (cotyledonary) leaves fully expanded (6-7 days). After the inoculation process, the plants were transferred to a humidity chamber at 21 ± 1 °C for 24 h in dark conditions. The plants were later

transferred to the greenhouse at 20 ± 2 °C. After the inoculation process, it was very important in the first 3-4 days to avoid washing the spores from leaves.

Figure 2. Rust inoculation of the primary leaves using a hand sprayer.

The F_2 seeds were planted in 34 fl oz plastic pots with peat moss as substrate. F_2 plants were inoculated using the methodology described above. F_2 plants were maintained in the green house at 22 ± 2 ºC during approximately 3-4 months to produce $F_{2:3}$ seed.

$F_{2:3}$ progeny test

In order to confirm which of the F_2 plants had either a homozygous dominant genotype for resistant (RR) or heterozygous resistant (Rr), F_3 seed produced by the F_2 plants was harvested and only resistant F_2 lines were inoculated again. A total of 16 seeds from each resistant F_2 plant were used for this progeny test. Planting and inoculation were made following the methods described above. Within each tray, 16 F_3 seeds of 5 F_2 plants were planted along with 5 plants of UI-114 and Mexico 309 as the susceptible and resistant checks, respectively.

Phenotypic evaluation of the disease reaction

Evaluation of the rust reaction in the F_2 and F_3 plants was done visually and using a zoom lupe 10x (PEAK®) to measure the diameter of the rust pustules 14-16 days after inoculation. The disease reaction was classified according to the standard 1 to 6 scale. Plants scored as 1, 2 or 3

were considered resistant, and plants that had a score of 4, 5 or 6 were rated as susceptible (Stavely et al., 1983; Mmbaga et al., 1996). If a plant showed more than one rust pustule size, it was scored by representing first the most prevalent reaction, followed by the second, with both scores separated by coma. Based on the disease reaction and using the expected phenotypic and genotypic segregation ratios for a single dominant gene (3:1) or (1:2:1) Chi-square test (χ^2) was performed at $P \leq 0.05$ using 1 or 2 df, respectively.

Genotypic evaluation

DNA extraction

DNA of the parental cultivars and the F_2 plants were obtained, by collecting a small amount of tissue (0.5 g) from the first trifoliate leaf. The plant tissue was harvested and placed in 96 well high polypropylene plates, and immediately frozen using liquid nitrogen, then the plates containing the tissue were maintained at freezing temperature under -24 °C.

The DNA extraction was done using the Mag-Bind® Plant DNA Plus kit and the DNA extraction was done following the supplier's protocol (Omega Bio-tek, 2019). Then the DNA concentration in each sample was quantified using the NanoDrop from Thermo SCIENTIFIC, diluted to ~10 ng/µl for all samples for a working stock plate. DNA was storage at -20°C until the day it was used.

Insertion and deletion (InDel) markers

The location of the marker SI19 was used to locate the *Ur-5* on the short arm of the chromosome Pv04 (Miklas et al. 2002) and based on the locations primers were designed. InDel markers within the chromosome Pv04 of the bean genome were designed using the sequence files of the 2 parents involved in this study (Mexico 309 and UI-114), which were provided by the bean genomics laboratory of NDSU (unpublished). For the sequencing, high-molecular

weight DNA of each parental was used, then it was fragmented. Each fragment was labeled and used to create a DNA sequencing library using Illumina DNA sequencing technology. The DNA fragment barcoding was done using the 10X Chromium System (http://10xgenomics.com). The assembly was performed using the Supernova software from 10X Genomics, and these procedures were performed by the Hudson Alpha Institute for Biotechnology, Huntsville, Alabama.

Both sequences were aligned using the software IGV version 2.8.6. Based on the reported location of the *Ur-5* gene in the beginning of the chromosome Pv04, it was stablished an interval between 89,886 to 2,563,208 bp, primer sites were visually identified as deletions or insertions based on the differences between the two sequences. Forward primer was created on the left side and reverse primer on the right side. The criteria used for selecting the primers were: melting temperature 60 ± 0.4 °C, GC content 35-50% and minimum length 14 bp and maximum length 35 bp. Primers were validated first in silico through the Oligo Analyzer tool in the Integrated DNA Technologies website https://www.idtdna.com/calc/analyzer. After synthesis, the primers were validated on the two parental DNA (Mexico 309 vs UI-114). Primer pairs producing clear polymorphism were subsequently tested on the F_2 population.

Polymerase chain reaction (PCR) and electrophoresis

For the preparation of the master mix, a total volume of 18 ul was prepared using 12.4 ul dH_2O, 2 ul of 10x PCR buffer, 1.0 ul dNTPs (10 mM), 1.0 ul forward primer (5uM), 1.0 reverse primer (5 uM), 0.6 ul Taq-polymerase enzyme and it 2 ul of DNA (10 ng/ul). The thermocycler was set up with the following conditions: 95 °C during 3 min; 45 cycles of 95 °C during 20 sec, 58°C during 30 sec, and 72 °C during 1 min, followed by a final cycle at 72 °C during 10 min and held at 10 °C.

The PCR product was run on a 3% agarose gel prepared with 9 g general purpose agarose MIDSCI, 285 ml distilled water, 15 ml 20x TBE (Tris-borate-EDTA) electrophoresis buffer and 6 µl ethidium bromide (10 mg/ml). For each sample, 3 µl of loading dye 10x was added, and 16 µl of each sample was placed in each of the wells in the gel. Each gel (size 14x23 cm) included ladder used as a molecular-weight size reference and it was also included parental DNA. The voltage conditions used in the electrophoresis were 140 V, for 1.5 h using an electrophoresis equipment from VWR® Scientific Products.

After the electrophoresis step, an image of the gel was capture with UV transillumination and the imagine was analyzed using the software AlphaImager HP system from Proteinsimple™. Each gel image was visually scored based on the size of amplicons present. When the sample showed a single amplicon (homozygous) it was assigned a 1 for UI114 or 2 for Mexico 309; however, if the sample showed both amplicons (heterozygous), it was assigned a value of 3.

Genetic mapping and candidate genes

The construction of the linkage map was done using the MapDisto version 2.1.7. with the genetic information obtained from the selected markers that were run on the F_2 population and the phenotypic data. MapDisto parameters were set for an F_2 population, 184 individuals as the population size, and the number of markers were 11 including the phenotype data. The following values were used as data codes: 1=UI-114 homozygous, 2=Mexico 309 homozygous, 3=heterozygous, and the missing data was represented by a period. For the linkage groups, a Min LOD score of 3.0 and dmax of 0.3 were used as threshold values. In order to calculate the genetic distances (cM) based on the recombination values, the Kosambi function was set in MapDisto (Lorieux, 2012). Segregation ratios for each InDel markers were calculated in the F_2

population using Chi-square test (P<0.05) in order to identify markers that would show a segregation distortion. All the functions in MapDisto were executed manually.

To identify potential candidate genes related to the resistance conferred by the *Ur-5* gene, the version 2.1 of the bean genome annotation of *P. Vulgaris* was used (G19833-Chaucha Chuga): https://phytozome-next.jgi.doe.gov/info/Pvulgaris_v2_1 (Schmutz et al., 2014). The candidate genes were identified within the genomic region delimited by the two closest flanking markers to the *Ur-5* gene.

RESULTS

F_2 Phenotypic evaluation and F_3 progeny test

In this study, 188 F_2 plants originated from the population generated by the cross between UI114 x Mex-309 plus the parental genotypes were inoculated with the rust race 20-3, but only 168 F_2 plants showed disease reaction (Table A1). In the remaining 20 plants, germination was delayed and the primary leaf was not sufficiently developed at the time of inoculation. The susceptible parent UI-114 had a rust reaction values of 4,5 with pustule diameters from 0.3-0.5 and 0.5-0.8 mm, respectively. The resistant parent, Mexico 309, showed a rust score of 2 and 3 that correspond to the formation of necrotic spots (hypersensitive response) and tiny pustules with a diameter less than a 0.3 mm (Figure 3).

Figure 3. Rust reaction in the resistant parent Mexico 309 (left) and the susceptible parent UI-114 (right) to *U. appendiculatus* race 20-3.

Based on the known information about the *Ur-5* rust resistance gene being a single dominant gene (Stavely, 1984), a Chi-square test (χ^2) was made for the F_2 population using the

expected 3:1 phenotypic segregation ratio (resistant:susceptible). Based on the data obtained in the F_2 population (Table 3), the phenotypic segregation ratio in this study (2.4:1) fits in the expected ratio of 3:1, confirming that the *Ur-5* rust resistant gene present in the cultivar Mexico-309 is a single and dominant gene as confirmed by the Chi-square test.

Table 3. Phenotypic distribution of response of F_2 population to infection by race 20-3 of *U. appendiculatus* and Chi-square test based on expected 3:1 ratio

F_2 Phenotype	Expected plants	Observed plants	χ^2 calculated	*P* value*
Resistant	126	119		
Susceptible	42	49	1.556	0.21
Total F_2 plants	168	168		

*The values for the significance of the χ^2 test was at $P \leq 0.05$ and 1 df.

To identify the genotype of the 119 plants that showed a resistant reaction (RR or Rr) in the F_2 phenotypic evaluation (Table 3), a progeny test was made using 16 F_3 seeds per line to classify each F_2 individual as homozygous resistant (RR) or heterozygous resistant (Rr). After having done the progeny test through the disease screening on the F_3 plants (Table A2.), it was expected a genotypic segregation ratio of 1:2:1 (RR:Rr:rr) on the F_2 population based on the F_3 progeny test. Based on the χ^2 test (Table 4), the genotypic segregation ratio obtained in this population (1:1.77:1.14) fits in the expected ratio as confirmed by the Chi-square test.

Table 4. Genotypic distribution of response of F_2 population to infection by race 20-3 of *U. appendiculatus* and Chi–square test based on expected 1:2:1 ratio

F_2 Genotype£	Expected plants	Observed plants	χ^2 calculated	*P* value*
RR	42	43		
Rr	84	76	1.556	0.38
rr	42	49		
Total	168	168		

£RR= Homozygous resistant, Rr= Heterozygous resistant, rr= Homozygous susceptible.
*The values for the significance of the χ^2 test was at $P \leq 0.05$ and 2 df.

Polymorphic InDel markers and recombination analysis

After a total of 72 InDel markers were designed across the chromosome Pv04, a primer test was made using the parental DNA (Mexico-309 and UI-114) to confirm which were polymorphic. The primer test was done based on the amplicons shown on the agarose gel that represent each of the alleles belonging to each parent. The primers selected were those that clearly showed a codominant nature and allowed to distinguish between the homozygous susceptible (rr), homozygous resistant (RR) and heterozygous resistant (Rr) individuals (Table 5). From the total of 72 InDel designed, 10 were polymorphic and, covered the region between 168,371 bp to 1,396,952 bp on PV04 based on v2.1 of the *P. vulgaris* genome reference (G19833-Chaucha Chuga) in the chromosome. Then, the 10 polymorphic InDel markers were run on the F_2 population DNA to map the genetic region associated to the resistant *Ur-5* gene.

Table 5. Polymorphic InDel markers used in the F2 population of UI-114/Mexico 309

InDel Name	Orientation*	v2.1 location	Primer Sequence (5′–3′)	GC (%)	Melt T (°C)
Pv04_168371	F	168371	CAAGAGAGAGTCAGGAGTGACATGCTTC	50.0	60.1
Pv04_168371	R	168129	GCCGCGTTTGTGTTATCACGC	50.0	59.9
Pv04_248402	F	248402	GAATAAGTCTTGGAAGTCACACCTCAACTGG	45.2	60.2
Pv04_248402	R	248877	ACTTTGTGCAGATTTTGGGAAGGCTC	46.2	59.9
Pv04_265669	F	265669	GTCACTCATGGACTGACCTTTCCAGAAT	46.4	60.0
Pv04_265669	R	266094	GCAAGTTTACGGTGGACTGGTTTACATG	46.4	59.8
Pv04_266202	F	266202	ACAAGGTCCCTGACTCCGAAGAAATC	50.0	60.2
Pv04_266202	R	266454	GTTACTGCAGAGACAACGCTATTGAACTCTC	45.2	60.1
Pv04_318718	F	318718	AGACACCGACAACACCATAAACGTATAAGAGA	40.6	60.3
Pv04_318718	R	318925	CAATCTTGAAAATATTGTGGCAAAGGCACATGT	36.4	60.1
Pv04_374755	F	374755	TTTGTCTTCTAGGCCTCCATGGATGG	50.0	60.2
Pv04_374755	R	375209	CGCAATAACGACGATTCTCTAACCGTC	46.4	60.0
Pv04_461378	F	461378	ACCATGGACTACTAGGTTGCATGATGTTG	44.8	60.1
Pv04_461378	R	461898	ATCATTGAATGCATAAGCCATTTGCAGCT	37.9	59.7
Pv04_547688	F	547688	CAGTCTGTTGAGAGAGTTCCATGAAGAGAAGA	48.0	60.0
Pv04_547688	R	547928	TAAAATATATCCCACACCAAAGTCATCAGCAGC	39.4	60.0
Pv04_1276903	F	1276903	TTTCCTTTGTGTATATTGGGTGCAGTCCA	41.4	60.0
Pv04_1276903	R	1277345	TGAAGAACCAACAACGGCAGTTTCC	48.0	60.0
Pv04_1396431	F	1396431	GCATACCCAAATGGTTCAAGACACAAGG	46.4	60.0
Pv04_1396431	R	1396952	TCGGAAATCCATTGCAGCACTAGAAGA	44.4	59.9

*F=Forward, R=Reverse

The 10 InDel markers showed a codominant segregation mode and based on the χ^2 test all of them had a segregation ratio that fit in the expected F_2 genotypic ratio of 1:2:1 (Table 6).

Table 6. Chi–square test for the genotypic segregation of InDel markers located in the Chromosome Pv04 in an F_2 population (UI-114/Mexico 309) based on 1:2:1 ratio

ID	InDel marker	Number of F_2 plants[£]			$\chi^2_{1:2:1}$	*P* value*
		RR	Rr	rr		
1	Pv04_168371	50	43	82	1.25	0.53
2	Pv04_248402	47	42	82	0.58	0.75
3	Pv04_265669	48	44	84	0.55	0.76
4	Pv04_266202	50	44	88	0.59	0.74
5	Pv04_318718	46	42	88	0.18	0.91
6	Pv04_374755	47	42	90	0.28	0.87
7	Pv04_461378	51	42	86	1.18	0.55
8	Pv04_547688	51	43	88	0.90	0.64
9	Pv04_1276903	50	42	89	0.76	0.68
10	Pv04_1396431	49	41	88	0.74	0.69

[£]RR= Homozygous resistant, Rr= Heterozygous resistant, rr= Homozygous susceptible.
*The value for the significance of the χ^2 test at $P \leq 0.05$ was 5.99 and 2 df.

Based on the gel scores, 8 F_2 individuals were identified in the population (Figure 4) that showed recombination events in the region delimited by markers Pv04_266202 and Pv04_11396431. The physical locations in the Pv04 of the markers Pv04_266202 and Pv04_1396431 are 266,202 bp and 1,396,431 bp, respectively, which delimited a recombinant region of 1.13 Mb based on the *P. vulgaris* reference genome v2.1 (Schmutz et al., 2014). The region containing the *Ur-5* gene was narrowed down between the markers Pv04_547688 and Pv04_139643 are 547,688 and 1,396,643 bp, which span a reduced region of 849 Kb.

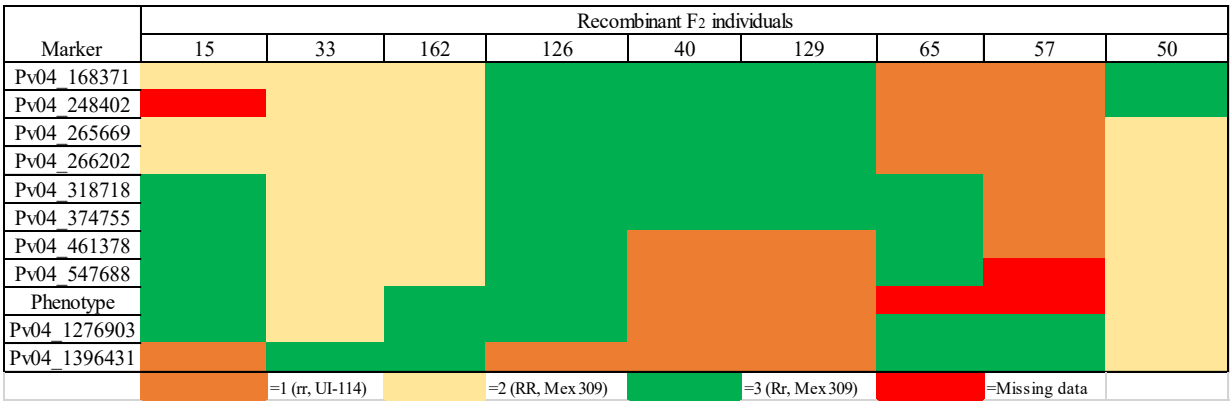

Figure 4. Graphical representation of the recombinant individuals in the F_2 population with the polymorphic InDel markers.

Based on recombination frequency, a genetic map was drawn using the genetic distances (cM) of each InDel marker (Figure 5).

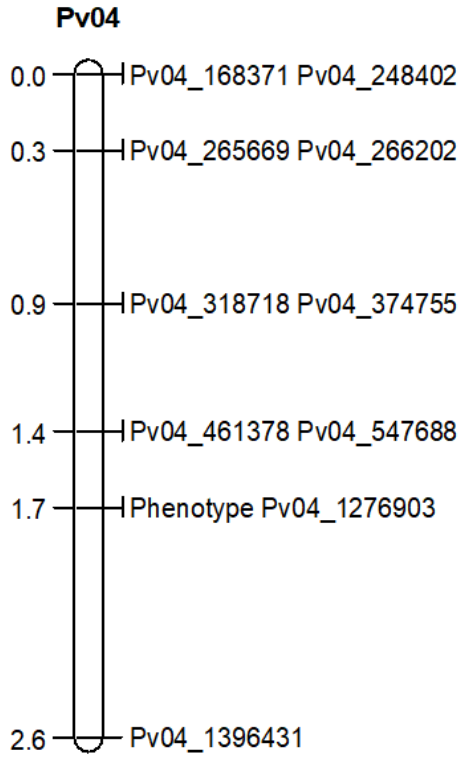

Figure 5. Genetic map of the *Ur-5* gene based on 10 InDel markers in the Pv04. Genetic distance (cM) is shown on the left, and InDel markers are shown on the right.

Candidate genes

The identified recombinant region (1.13 Mb) contains approximately 90 annotated gene models based on the bean genome v2.1. Almost 50% of these genes are related to disease resistance proteins containing the NB-ARC and LRR domains. This region also includes some genes models that encode cytochrome P450, and protein kinases, which are also been reported as related with plant disease resistance (Table A3). Then the region harboring the *Ur-5* gene was narrowed down between 516,385 (Pv04_547688) and 1,396,643 (Pv04_1396431) bp, which span a region of 849 Kb, and the candidate genes are mostly NB-ARC, LRR and protein kinases domains (Table 7).

Table 7. Candidate gene models related to plant disease resistance in the delimited region of the *Ur-5* gene located in Pv04

Start position(bp)	End position(bp)	Gene name v2.1	Best hit Arabidopsis	Gene function
567896	570454	Phvul.004G007750	AT3G14470.1	NB-ARC domain-containing disease resistance protein
618293	622960	Phvul.004G007900	AT3G14470.1	NB-ARC domain-containing disease resistance protein
634170	640604	Phvul.004G008001	AT3G14470.1	NB-ARC domain-containing disease resistance protein
657081	659750	Phvul.004G008101	AT3G14470.1	NB-ARC domain-containing disease resistance protein
671773	674107	Phvul.004G008200	AT3G14470.1	NB-ARC domain-containing disease resistance protein
677817	680151	Phvul.004G008250	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
706950	711423	Phvul.004G008351	AT3G14470.1	NB-ARC domain-containing disease resistance protein
732245	733453	Phvul.004G008400	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
733519	734080	Phvul.004G008450	AT3G14470.1	NB-ARC domain-containing disease resistance protein
746809	753397	Phvul.004G008560	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
762295	765654	Phvul.004G008620	AT3G14470.1	NB-ARC domain-containing disease resistance protein
768791	773819	Phvul.004G008680	AT3G14470.1	NB-ARC domain-containing disease resistance protein
796775	801277	Phvul.004G008740	AT3G14470.1	NB-ARC domain-containing disease resistance protein
819458	826737	Phvul.004G008921	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
833532	836825	Phvul.004G008981	AT3G14470.1	NB-ARC domain-containing disease resistance protein
838678	843077	Phvul.004G009041	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
847290	848413	Phvul.004G009101	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
863222	865511	Phvul.004G009221	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
879439	884653	Phvul.004G009281	AT3G14470.1	NB-ARC domain-containing disease resistance protein
906948	907805	Phvul.004G009461	AT3G14470.1	NB-ARC domain-containing disease resistance protein
909231	916054	Phvul.004G009521	AT3G14470.1	NB-ARC domain-containing disease resistance protein
920780	922280	Phvul.004G009581	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
939934	943174	Phvul.004G009821	AT3G14470.1	NB-ARC domain-containing disease resistance protein

Source: v2.1 of the *P. vulgaris* genome, available at https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris

Table 7. Candidate gene models related to plant disease resistance in the delimited region of the *Ur-5* gene located in Pv04 (continued)

Start position(bp)	End position(bp)	Gene name v2.1	Best hit Arabidopsis	Gene function
961070	964494	Phvul.004G009909	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
964943	966979	Phvul.004G009918	AT3G14470.1	NB-ARC domain-containing disease resistance protein
967700	972035	Phvul.004G009927	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
989558	993520	Phvul.004G009936	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1043536	1044075	Phvul.004G009100	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1063726	1067743	Phvul.004G009136	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1103140	1118146	Phvul.004G009154	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1129267	1132629	Phvul.004G008900	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1137597	1139195	Phvul.004G008909	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1146369	1149704	Phvul.004G008918	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
1156680	1160125	Phvul.004G009300	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1172345	1176922	Phvul.004G009500	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1191387	1192524	Phvul.004G009518	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
1203535	1207848	Phvul.004G009527	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1228652	1233028	Phvul.004G009800	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1278226	1282234	Phvul.004G010400	AT5G40440.1	mitogen-activated protein kinase kinase 3
1385799	1398207	Phvul.004G012600	AT5G14720.1	Protein kinase superfamily protein

Source: v2.1 of the *P. vulgaris* genome, available at https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris

DISCUSSION

F_2 Phenotypic evaluation and F_3 progeny test

After the disease screening of 168 F_2 plants using the rust race 20-3, the χ^2 test shown that the inheritance segregation ratio of the resistance/susceptible response in the F_2 and F_3 populations fits in the expected 3:1 (resistant:susceptible) phenotypic ratio and the expected 1:2:1 (RR:Rr:rr) genotypic ratio for a single dominant gene, confirming previous results.

Stavely (1984), concluded that the rust resistance was controlled by a monogenic dominant gene; he used a F_2 population of 119 plants and he used the line B-190 as the source of the *Ur-5*. Souza et al. (2007), used 170 F_2 plants and the genotype Mexico 309 as a source of *Ur-5* gene, and confirmed that Mexico 309 had the single and dominant *Ur-5* gene. Hurtado-Gonzales et al. (2019) in their study used 378 F_2 plants derived from the cross between Mexico 309 and Early Gallatin. They used Mexico 309 as a source of *Ur-5* gene and they found that the observed ratio in their experiment fits in the expected ratio 3:1 ($\chi2 = 0.794$, *P* value=0.373) confirming that the resistance in Mexico 309 is conferred by a single and dominant gene as it was verified in this study.

Polymorphic InDel markers

A total of 72 InDel were designed and 10 were polymorphic between the susceptible parent UI114 and the resistant parent Mexico 309. The 10 InDel markers in this study showed a codominant segregation that clearly allowed distinguishing between the resistant and susceptible alleles (RR, rr). According to the χ^2 test the segregation ratio fits well in the 1:2:1 expected ratio at the F_2 generation and none of the markers showed segregation distortion.

These results agree with other studies which also used InDel markers for mapping genes and they were able to confirm the expected codominant segregation ratio of 1:2:1 (Chongjing, et

al., 2020). Ferdous et al. (2019) in their study they develop InDel markers to map a single and dominant gene responsible for resistance to blackleg disease in cabbage (*Leptosphaeria maculans* (anamorph: *Phoma lingam*)), and they found codominant InDel markers that distinguish between the homozygous and heterozygous genotypes. This codominant characteristic is mentioned by Das et al. (2015), they explained that the codominant amplification in InDel markers make it easier for genotyping compared to other types of markers with different modes of inheritance.

New InDel markers have been designed that are convenient and easy to use for genotyping compared to other types of molecular markers previously reported. The RAPD marker SI19 linked to *Ur-5* gene that was validated by Souza et al. (2007) using the Mexico 309, he concluded that the marker was at 3.31 cM of the locus.

A BLAST search was performed to identify the physical location of the SI19 marker, and it was at 2,035,398 bp on Pv04 of the G19833 bean reference genome assembly (v2.1) which is outside the region that was delimited in this study. A drawback of the SI19 marker is that it only amplifies a single polymorphic band (dominant), identifying only the genotypes that have the *Ur-5* gene, while the InDel designed in this study make it possible to distinguish between homozygous and heterozygous genotypes (co-dominant). The InDel marker Pv04_1276903 could be used as potential marker in MAS, because this marker is codominant and it showed clear separation between the amplicons. In addition, this proposed marker is tightly linked to the *Ur-5* gene (0.0 cM). However, it will be necessary to validate this marker in other genotypes that contains the *Ur-5* gene.

Recombinant individuals and mapping the *Ur-5* locus

The recombinant region was flanked by the markers Pv04_266202 and M1396431 located on the bean reference genome v2.1 at 266,202 bp and 1,396,431 bp, respectively, which delimited a recombinant region of 1.13 Mb in the chromosome Pv04. Of the total 179 F_2 individuals genotyped, only 8 individuals showed recombination events in the region flanked by the markers Pv04_266202 and Pv04_1396431. The region that contains the *Ur-5* gene was narrowed down between the markers Pv04_547688 and Pv04_139643, that are located at 547,688 and 1,396,643 bp, which span a region of 849 Kb.

Montejo (2017), as a result of GWAS found significant peaks related to bean rust race 20-3 in the Pv04 at 379,943 and 379,989 bp (bean reference genome v2), and these peaks are in the recombinant region identified in the present study. Monclova-Santana (2019) performed a GWAS analysis using advanced breeding lines and the Middle America diversity panel, and it was detected significant SNPs in chromosome Pv01, Pv03, Pv04, Pv06, Pv08, and Pv10 related to resistance to race 20-3. In the Pv04, she found significant peaks at 521,536 and 554,762 bp that could be related to the *Ur-5* gene, and these peaks are within the region of 849 kb where it was targeted the *Ur-5* gene in the present study.

Valentini et al., (2015), studied the interaction between the *Ur-4* and *Ur-5* genes, and they used 182 F_2 plants derived from a cross between Mexico 309 (*Ur-5*) and Early Gallatin (*Ur-4*) which is a similar population size compared to the present study, but they used a different parental in the cross. They reported as part of their results, that the SSR markers BARCPVSSR04582 and BARCPVSSR04600 were linked to the *Ur-5* resistant gene at 0.0 cM in the chromosome Pv04 which suggested that they were not able to find recombinant individuals for that region in their F_2 population.

The results from this study agree with the study done by Hurtado-Gonzales et al. (2019), where they used 378 F_2 plants from a cross between Mexico 309 and Early Gallatin, which is a large population size compared with the present study. They defined as flanking markers the SSR marker BARCPVSSR04569 and the KASP marker SS64, and they found only six individuals that showed recombination in a recombinant region of 1.02 Mb in the Pv04 which is bigger in size compared to the region found in this study (849 kb). Then, they genotyped the F_2 population with 10 more markers located in the recombinant region and they found very few recombinant events among the markers, and they selected as the best marker, the KASP SS183 located at 576,802 bp on Pv04, position based on v1.0 of the bean genome.

Results from this study indicate that the *Ur-5* gene is located between 547.7-1,396.4 Kb in the chromosome Pv04 based on the bean genome v2.1, which span a region of 849 Kb. However, it would be necessary more research to narrow down even more the region. The results reported agree with other studies that concluded that the *Ur-5* gene is located in the short arm of the chromosome Pv04 in a genomic region that present very low recombination frequency (Miklas et al., 2002; Valentini et al., 2015; Hurtado-Gonzales et al., 2019).

Candidate genes

Almost 50% of the candidate genes identified in the 849 Kb region containing the *Ur-5* gen in the Pv04 are related to plant disease resistance proteins belonging to the NB-ARC and LRR domains (Schmutz et al., 2014). This suggest the presence of an important gene cluster for disease resistance. The delimited region also includes some genes models that encode protein kinases that are also related to plant disease resistance.

The immune system in plants can be activated by the recognition of PTI (pathogen-associated molecular pattern (PAMP)-triggered immunity) being this the first mechanism of

plant defense (Ausubel, 2005). As a second mechanism of defense is ETI (effector-triggered immunity) (Tsuda et al., 2009). NB-ARC and LRR proteins are encoded by several resistance genes and these have an important function in the recognition of the pathogen effectors and trigger immunity responses (Dangl and Jones, 2001). When the ETI is activated in the plant, as a mechanism of defense against the pathogen, the plant activate programmed cell death (PCD) usually known as hypersensitive response (HR), to stop the pathogen infection (Hurley et al., 2014) This is especially important in the case of the observed reaction of the *Ur-5* gene to race 20-3, which is a typical HR response to the pathogen.

Some of the candidate genes identified during the present studies agree with the candidate genes identified by Montejo (2017) who reported the genes models Phvul.004G005800 and Phvul.004G005900 that encode cytochrome P450. The genes models in the region that showed significant peaks related to bean rust race 20-3 in the Pv04 are present in the recombinant region identified in the present study. Monclova-Santana (2019) reported that downstream of the significant markers related to resistance against *U. appendiculatus* race 20-3 located in the Pv04, these gene models that encode NB-ARC domains which are important in disease resistance, which is very similar to the results presented in this study.

Schmutz et al. (2014), mention that in the Pv04 are located clusters of resistance genes and some of them at the end of the chromosome Pv04, and many of these resistance genes encode nucleotide binding (NB) and leucine rich repeated (LRR) domains that are proteins directly related to plant defense mechanism against pathogens. Miklas et al. (2002) reported that towards the end of the Pv04 complex resistance genes clusters exist such as the *Ur-5*, which is composed of a block of genes and due to the complexity of these clusters, it spans wide genomic regions.

It is important to mention that the chromosome Pv04 contained other rust resistance genes that are located in the telomeric regions like the *Ur-14* (Valentini et al., 2017). Pv04 also harbors other clusters of genes related to disease resistance genes like anthracnose loci *Co-3*, *Co-9* (Miklas et al., 2002; Geffroy et al., 2008; Murube et al. 2019). angular leaf spot loci *Phg-3* (Valentini et al., 2017) and the HB4.2 QTL for resistance to race 6 of *Pseudomonas syringae pv. phaseolicola (Psph)*, casual pathogen of halo blight (Tock et al., 2017).

Banoo et al. (2020) found significant SNPs related to anthracnose race 3, 87 and 503 in the Pv04 in a region between 1.3-2.72 Mb in the bean genome v2.0. They found in the region genes models that encode LRR and NB-ARC proteins related to plant disease resistance. The region identified is very close to the region that we found in the present study related to the *Ur-5* rust resistance gene. Geffroy et al. (1999) mentioned that one of the explanations of these complex cluster of genes that confer resistance against rust and other diseases could be that these genes were derived from the same ancestral gene or cluster of genes.

The present study confirms the mode of inheritance of the *Ur-5* rust resistance gene. The *Ur-5* gene provides a wide range of resistance against many rust races that have been identified, including the race 20-3, which is currently the most prevalent race in North Dakota. The wide range of resistance makes it as a good source to be used in gene pyramiding along with genes such as *Ur-11* for example. New InDel markers have been designed that are convenient and easy to use compared to other types of markers previously reported. It is recommended the marker Pv04_1276903 to be used potentially in MAS because this marker is codominant, and it makes possible to distinguish between homozygous and heterozygous genotypes, which was not possible with the previously reported markers. Even more, these new markers are based on physical positions (bp) rather than on estimated recombination frequency (cM). The proposed

markers are tightly linked to the *Ur-5* gene; however, it would be necessary to validate the utility of this markers in other genotypes that contains the *Ur-5* gene. A narrower region from 547,688 to 1,396,431 bp which span 849 Kb, appears to harbor the *Ur-5* gene in the beginning of the chromosome Pv04. Potential candidate genes encoding NB-ARC, LRR and protein kinases involved in the resistance have also been identified. However, it would be necessary for more research to specifically pinpoint the region coding for *Ur-5*, even using additional tools such as transcriptomics and gene cloning. This information will contribute to the understanding of the molecular bases of the *Ur-5* gene and it will aid dry bean breeding programs to develop new cultivars with a broader and long-term resistance against rust races.

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APPENDIX

Table A1. Disease reaction of the F_2 population (UI114 x mex-309) using standard scale (1-6) for rust evaluation

ID	Code	Infection Type	
1	1.1-2	3	R
2	1.1-3	5	S
3	1.1-4	5	S
4	1.1-5	3	R
5	1.1-6	3	R
6	1.1-7	5,6	S
7	1.1-8	3	R
8	1.1-9	2	R
9	1.1-10	3	R
10	1.1-12	3	R
11	1.1-14	5	S
12	1.2-2	3	R
13	1.2-3	3	R
14	1.2-4	2,3	R
15	1.2-5	5	S
16	1.2-6	3	R
17	1.2-7	5	S
18	1.2-8	3	R
19	1.2-9	5	S
20	1.2-11	3	R
21	1.2-12	3	R
22	1.2-13	5	S
23	1.2-14	3	R
24	1.3-1	3	R
25	1.3-2	2,3	R
26	1.3-3	3	R
27	1.3-4	2	R
28	1.3-5	5	S
29	1.3-6	3	R

Table A1. Disease reaction of the F_2 population (UI114 x mex-309) using standard scale (1-6) for rust evaluation (continued)

ID	Code	Infection Type	
30	1.3-7	5	S
31	1.3-8	3	R
32	1.3-9	5	S
33	1.3-10	3	R
34	1.3-11	3	R
35	1.3-13	5	S
36	1.3-14	3	R
37	1.4-1	3	R
38	1.4-3	3	R
39	1.4-4	3	R
40	1.4-5	3	R
41	1.4-6	3	R
42	1.4-7	2,3	R
43	1.4-9	2,3	R
44	1.4-10	3	R
45	1.4-11	5	S
46	1.4-12	3	R
47	7.1-1	3	R
48	7.1-2	3	R
49	7.1-3	3	R
50	7.1-4	3	R
51	7.1-5	4	S
52	7.1-6	3	R
53	7.1-8	2,3	R
54	7.1-9	2	R
55	7.1-10	3	R
56	7.1-11	3	R
57	7.1-12	3,2	R
58	7.1-13	2	R
59	7.1-14	3	R
60	7.2-1	3	R
61	7.2-2	3,2	R
62	7.2-5	5	S
63	7.2-7	3	R

Table A1. Disease reaction of the F_2 population (UI114 x mex-309) using standard scale (1-6) for rust evaluation (continued)

ID	Code	Infection Type	
64	7.2-8	5	S
65	7.2-9	4	S
66	7.2-10	5	S
67	7.2-11	2,3	R
68	7.2-12	5	S
69	7.3-1	3	R
70	7.3-2	3	R
71	7.3-3	3	R
72	7.3-4	3	R
73	7.3-5	5	S
74	7.3-6	1	R
75	7.3-7	2	R
76	7.3-8	4,5	S
77	7.3-9	3	R
78	7.3-10	2	R
79	7.3-11	3	R
80	7.3-12	2	R
81	7.3-13	2,3	R
82	7.3-14	5,6	S
83	7.4-1	3	R
84	7.4-2	3,2	R
85	7.4-3	3	R
86	7.4-4	3	R
87	7.4-5	3	R
88	7.4-6	3	R
89	7.4-7	3	R
90	7.4-8	3	R
91	7.4-9	3,2	R
92	7.4-10	5	S
93	7.4-11	3	R
94	7.4-12	2	R
95	7.4-13	5	S
96	7.4-14	2,3	R
97	7.5-1	5	S

Table A1. Disease reaction of the F_2 population (UI114 x mex-309) using standard scale (1-6) for rust evaluation (continued)

ID	Code	Infection Type	
98	7.5-3	2,3	R
99	7.5-4	3	R
100	7.5-5	3	R
101	7.5-6	5	S
102	7.5-7	5	S
103	7.5-9	2,3	R
104	7.5-10	2,3	R
105	7.5-11	2	R
106	7.5-12	3	R
107	7.5-13	3	R
108	7.5-14	3	R
109	7.6-1	3,4	S
110	7.6-2	3	R
111	7.6-3	2	R
112	7.6-4	2	R
113	7.6-6	3	R
114	7.6-8	2,3	R
115	7.6-9	3	R
116	7.6-10	2	R
117	7.6-11	4	S
118	7.6-12	3	R
119	7.6-13	5	S
120	7.6-14	2,3	R
121	7.7-1	5	S
122	7.7-2	3	R
123	7.7-4	5	S
124	7.7-5	5	S
125	7.7-6	3	R
126	7.7-7	5	S
127	7.7-8	5	S
128	7.7-10	3	R
129	7.7-11	2,3	R
130	7.7-12	3,2	R
131	8.2-1	3	R

Table A1. Disease reaction of the F_2 population (UI114 x mex-309) using standard scale (1-6) for rust evaluation (continued)

ID	Code	Infection Type	
132	8.2-2	5	S
133	8.2-3	3	R
134	8.2-4	3	R
135	8.2-5	5	S
136	8.2-6	4	S
137	8.2-7	3	R
138	8.2-8	5,6	S
139	8.2-10	3	R
140	8.2-11	2	R
141	8.2-13	5	S
142	8.2-14	3,2	R
143	9.2-1	5	S
144	9.2-2	3	R
145	9.2-3	2	R
146	9.2-4	2	R
147	9.2-6	2,3	R
148	9.2-5	4	S
149	9.2-7	2,3	R
150	9.2-8	5	S
151	9.2-9	3	R
152	9.2-10	2	R
153	9.2-11	4	S
154	9.2-12	5	S
155	9.3-1	3	R
156	9.3-2	5,4	S
157	9.3-3	3	R
158	9.3-4	2,3	R
159	9.3-5	3	R
160	9.3-6	2,3	R
161	9.3-7	5	S
162	9.3-8	4	S
163	9.3-9	2,3	R
164	9.3-10	3	R

Table A1. Disease reaction of the F_2 population (UI114 x mex-309) using standard scale (1-6) for rust evaluation (continued)

ID	Code	Infection Type	
165	9.3-11	2,3	R
166	9.3-12	5	S
167	9.3-13	2	R
168	9.3-15	2	R

Table A2. Disease reaction of the F_3 seeds using standard scale (1-6) for rust evaluation

Code	Infection type															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1.1-2	X*	4	4,5	4	4,5	4	3,2	3	5,4	3	5	3	3	3	5	3
1.1-5	3,2	2,3	3	3	3	3	2,3	2,3	3	3	3	3	3	3,4	3	3
1.1-6	3	5	3	3	3	3	3	3	3	3	3,4	5	5,4	3	5	3,2
1.1-8	3,2	4	4	2,3	4	3	2,3	3	5	3	5,6	3	X	5	5,4	3
1.1-9	2,3	3,2	3,2	3	3	3	3	3	3	3	3	3	3	3	3	3
1.1-10	3	X	3	3	3	3	3	3	3	3	3	3	3	3	3	3
1.1-12	3	3	3	2	2	3	3	3	3	2,3	3	3	2,3	3	3	2,3
1.2-2	3	3	3	2,3	3	3	3	3	3	3	3	2,3	3	3	3	3
1.2-3	4	3	4	3	3	2,3	3	3	4	3	5,4	4	4	3	5,4	3
1.2-4	3	3	4	3	2	3	4	3,2	3	5	3	2,3	5	4,5	2	2,3
1.2-6	3	4	X	3	3,2	3	X	2,3	4	3	2,3	3	3	2,3	3	5
1.2-8	3,2	3,2	3	3,2	3,2	2,3	3,2	3	3	2,3	3	2,3	3	3	3	3
1.2-11	3	3,2	4	5	3	X	3,2	3,2	3	5	3	3	2,3	3	2,3	3
1.2-12	3,2	3	3,2	3	4	4	3,2	3	5,6	3	5	2	3	3	2,3	3
1.2-14	3,2	3	3,2	3	3,2	3	3	3	3	3	3	3	2,3	3	3	3
1.3-1	3	3,2	4,5	4	3	3	3	3	3	3	5,4	3	3	3	3,2	3
1.3-2	2,3	2,3	2,3	3,2	2,3	2	3	X	2,3	3	2,3	3,2	3	4	2,3	3
1.3-3	2,3	3	2,3	3	3	2,3	3	2,3	3	3	3	3	3	3	3	3
1.3-4	X	3	3	2,3	3	3	3	3	3	3	3	3	3	3	3	3
1.3-6	3	4,5	2,3	3	3	3	4,5	2,3	3	5	3	5	3	X	3	5
1.3-8	3	3	5	2,3	3	3	2,3	5	3	5	3	5	3	3	3	3
1.3-10	X	3,2	4	5,4	2,3	3	5	4,5	3	5,6	3	3	3	3	3	2,3
1.3-11	3	3	5,4	3	2,3	3	3,2	4	3	5	3	3	3	2,3	2,3	2,3
1.3-14	3	3	3	4	3	3,2	2,3	3	2,3	2,3	3,2	3	3	5	2,3	2,3
1.4-1	3	X	4	4	3	3	4	4	4	3	5	3	X	5	3	4
1.4-3	2	3	3	3	3	3	2,3	X	2,3	3	3	3	2,3	2,3	3	3
1.4-4	4	3	2	3	3,2	3	4	3	3	3	5,4	3	2,3	3	4,5	3
1.4-5	2,3	3	2,3	2	3	3	3	2,3	3	3	2,3	3	3	3	3	3
1.4-6	3	3	3	3,2	3	3	3	3	4	4,5	3	5	5	3	3	3
1.4-7	3,2	3	3	4	3	3	3	3	3,2	3	3	5	3	3	3	3
1.4-9	3	3	3	4	3	3	4	3	5	X	5	5	3	3	2,3	3
1.4-10	3	3	3	2,3	2,3	3	4	3	3	2,3	3	3	3	3	4,5	3

*X: Missing data

Table A2. Disease reaction of the F_3 seeds using standard scale (1-6) for rust evaluation (continued)

Code	Infection type															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
7.1-1	3	3	3,4	4	3,4	3	4	5	3	3,4	5,6	3	5	3,4	3	3
7.1-2	3	3	2,3	3	3	3	3	3	3	2,3	3	3	X*	2,3	3	2,3
7.1-3	3	3	4	3	3,2	2,3	4	3	4,5	3	3	3	5	3	3	3
7.1-4	2	X	3	3	3	2,3	3	3	3	3	3	2,3	2,3	3	5	3
7.1-6	4	3	3	2,3	4	3,2	3	3	3	3	3	4,5	3	4,5	3	5
7.1-8	3	3	3,2	2,3	2,3	2,3	X	5	5	3	3	5	5	3	5,4	3
7.1-9	2,3	2,3	2,3	2,3	3	2,3	2,3	2,3	2,3	2,3	2,3	3	3	2,3	2,3	2,3
7.1-10	3	3	3	3	3	5,6	3	3	3	3	3	5,6	5	3	3	X
7.1-11	X	4	3	3	2	3	2,3	3	3	4	3	4	3	3	5	3
7.1-12	3	3	4	5	3	3	3	3	5	5,4	3	3	3	5	3	3
7.1-13	4,5	3	4	3	3	3	3	3	3	3	3	3	4	3	3	5,4
7.1-14	3	3	3	X	3	4	3	3,2	3	3	3	3	2,3	3	3	4
7.2-1	5,4	3	2	3,2	3,2	2,3	3	4,5	5	3	2,3	3	3	3	3	3
7.2-2	3	3	3	3,2	3	3	3,2	3	3	3	3	3	3	3	3	3
7.2-7	3,2	3	3,2	3	2,3	3	2,3	3	3	2,3	3	3	3	3	3	3
7.2-11	X	2,3	4,5	2,3	5	3	5	5,4	3	5	3	3	3	3	3	5
7.3-1	3	3	3	3,2	3	3	3,2	3	3	3	3	3	3	3	3	3
7.3-2	2,3	2,3	3	3	X	X	3	4,5	X	3	X	3	3	3	3	3
7.3-3	2,3	3	2	3	2,3	3	3,2	2,3	3	3	3	3	3	3	2,3	2,3
7.3-4	3	3,2	3,2	X	4,5	X	3	4,5	3	3	3	X	3	3	3	2,3
7.3-6	3	2,3	2,3	3,2	X	2	3,2	2,3	2,3	3	3	2,3	2,3	3	3	2,3
7.3-7	3	3,2	3	3	3	3	3,2	X	3	3	3	3	2,3	3	3	3
7.3-9	3	4	3	4	X	4	3	3	3	X	3	X	3,4	X	3	3
7.3-10	3	2,3	3,2	5	2,3	X	5	2,3	3,2	5	5	3	3	3	2,3	3
7.3-11	3	3	3,2	3	3	3	X	2	3	3	3	3	3	3	3	3
7.3-12	3	3	3,2	3,2	3,2	3	2	3,2	3	3	X	X	3	3	X	X
7.3-13	3	3	3	X	3	3	4	3	3	3	3	X	3	X	3	3
7.4-1	5	3	2,3	2,3	3	3	4	3	5	3	3	5,6	3	3	5	3
7.4-2	3	5	3	3	4	3	X	3	3	3	3	3	3	2,3	3	3
7.4-3	3	3	3	3	2,3	2	X	3	3	5	3	3	3	5	2,3	5
7.4-4	3	3	3	2	2,3	2	3	X	3	3	3	3	3	3	3,2	3

*X: Missing data

Table A2. Disease reaction of the F_3 seeds using standard scale (1-6) for rust evaluation (continued)

Code	Infection type															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
7.4-6	3,2	2,3	3	3	3	X*	3	5	X	3,2	5	3	3	3,2	5,4	5
7.4-7	3,2	3,2	3	3	2,3	2,3	3	3	3	3	2,3	3	3,2	3	3	3
7.4-8	3	3,4	5	3,4	5	3,4	3	3	5	3	3,2	3	3	3	3	5
7.4-9	4,5	4,5	4,5	3	4,5	2,3	2,3	4	3	5	5	3	3	5	3	5,4
7.4-11	3	3	3,2	3	3	3	3	3	3	3	3	X	3	2,3	3	3
7.4-12	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
7.4-14	3	3	3	3	3	3	3	3	X	3	3	3	2,3	3	3	2,3
7.5-3	3	3	3	3	X	2,3	X	3	2	3	X	3	3	2	3	3
7.5-4	2	2,3	3	3	3	3	4	3	3,2	5,4	3	3,2	2,3	4	3,2	X
7.5-5	2,3	4,5	3	4,5	3	2	4,5	2,3	2,3	2,3	3	3	2,3	5	3	3
7.5-9	4,5	2,3	4	3	2,3	2	4,5	2,3	4	3	3	3	5	X	3	3
7.5-10	3	3	3	2	3	2	4	3	3	2,3	3,2	4,5	3	3	5	3
7.5-11	2,3	3	2,3	3	3	3	3	X	3	3	3	3	2,3	3	3	3
7.5-12	3	3	3	2,3	5	3	4,5	3	4,5	3	5	5	3	3	5	3
7.5-13	4	4,5	4,5	4,5	4,5	3,4	3	3	3	3	3	3	3	3	3	4,5
7.5-14	3	3	3	3	2,3	2,3	2,3	2,3	3	3	X	3	3	X	2	3
7.6-2	3	2,3	3	3	3	3	3	2,3	3	3	3	3	3	3	2,3	X
7.6-3	3	4	3	3	3	3	3	3	X	5	4	3	5	3	2	X
7.6-4	3	2,3	3	3	3	2,3	3	3	3	2,3	3	2,3	2,3	X	3	3
7.6-6	3	2,3	3	3	3	X	3	3	X	3	3	3	X	2,3	3	3
7.6-8	3	3	3	3	3	X	3	2	2,3	2,3	2,3	3	2,3	3	3	2,3
7.6-9	4	4	3	3	3	3	3	3	3	3	3	3	3	3	3	2,3
7.6-10	3	3	3	3	4	2	4	3	X	3	3	3	3	3	5	3
7.6-12	3	4	2,3	2,3	2,3	4	3	3	3	5	3	3	3	5	5	3
7.6-14	3	3	3	2,3	3	3	3	2,3	2	3	3	3	X	X	X	X
7.7-2	3	2,3	2,3	2,3	2,3	2,3	2,3	4	3	3	3	3	2,3	3	3	3
7.7-6	3	3	3	3	2,3	3	3	3	3	3	3	3	3	3	2,3	3
7.7-10	3	2,3	4,5	4,5	3,4	3	3	X	3	5	3,4	3	5	3	3	5
7.7-11	3	2,3	3	2,3	2,3	3	3	3,4	3	3	3,4	3	4	3	3	2,3
7.7-12	3	2,3	3	2,3	3	3	4	2,3	3	3	3	3	2,3	5	5	5

*X: Missing data

Table A2. Disease reaction of the F_3 seeds using standard scale (1-6) for rust evaluation (continued)

Code	Infection type															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
8.2-3	3,4	2,3	2,3	4	3	4	3	4	3	2,3	X*	4,5	2,3	5	2,3	5
8.2-4	3	4	3	3,2	3	3	4	3	4	4,5	5	2,3	3	3	3	X
8.2-7	2,3	3	3	3	3	3	3	2,3	2	3	3	3	3	3	3	3
8.2-10	2,3	3	3	3	3	2,3	3	3	3	2,3	3	3	2,3	3	2,3	3
8.2-11	3	3	3	3	3	4	4	3	3	5	5	3	2,3	3	2,3	3
8.2-14	3	2,3	X	3	4	4,5	4	3	2,3	4	3	5,4	2,3	3	2,3	4
9.2-2	3	3	3	2,3	2,3	4	4	4	4	2,3	2,3	3,2	3,2	4	3	X
9.2-3	3	4	3	2,3	2,3	3,4	3	3	3	3	3	3	5,4	2,3	3	3
9.2-4	2,3	3,4	4	3	2,3	3,2	3	3	3	5	3	5,6	3	2,3	3	2,3
9.2-6	3	3	3	3	3	3	3	3	3	5,4	5	3	3	4	3	X
9.2-7	3	3	4,5	2,3	3	3	5	5	2,3	5,4	5	5	3	4	5	3
9.2-9	3	2,3	3	3	3	3	3,2	3	3	3	3	3	3	3	3	3
9.2-10	3	3	3	3	3	3	2,3	3	3	3	3	3	3	X	2,3	X
9.3-1	3	3	3	2,3	2	4	3	4	3	3	3	3	5	3	3	5
9.3-3	2,3	3	4	4	2,3	2	4	2,3	2,3	2,3	4,5	2,3	3	2,3	X	3
9.3-4	3	3	3	3	3	3	3	2,3	3	2,3	3	3	3	3	3	3
9.3-5	3	3	2,3	5	3	3	4	3,4	3	3	X	3	3	3	5	3
9.3-6	2,3	2,3	2,3	3,2	4,5	5	3	4	3	X	5	5	3	3,2	5	3
9.3-9	3	4	4	4,5	3	2,3	3	3	5	3	5,4	5	2,3	3	5	X
9.3-10	4	3,4	4	X	2,3	3,2	4	2,3	3	2,3	3	2,3	2	3	3	5
9.3-11	2	X	3	2,3	3	3	3	2,3	3	X	3	2	3	3	3	3
9.3-13	3	3	3	2	3	3	3	2,3	3	3	3	2,3	3,2	2,3	3	3
9.3-8(15)	2	4	4	3	3	3	4	3	4	3,4	4	5	3	3	3	3

*X: Missing data

Table A3. Gene models related to plant disease resistance in the recombinant region related to *Ur-5* gene located in Pv04

Start position(bp)	End position(bp)	Gene name v2.1	Best hit Arabidopsis	Gene function
368,531	370,087	Phvul.004G005700	AT4G39490.1	cytochrome P450, family 96, subfamily A, polypeptide 10
372,078	374,079	Phvul.004G005800	AT4G39490.1	cytochrome P450, family 96, subfamily A, polypeptide 10
380,970	382,499	Phvul.004G005900	AT4G39490.1	cytochrome P450, family 96, subfamily A, polypeptide 10
386,618	394,940	Phvul.004G006000	AT4G39490.1	cytochrome P450, family 96, subfamily A, polypeptide 10
394,161	395,837	Phvul.004G006050	AT4G39490.1	cytochrome P450, family 96, subfamily A, polypeptide 10
401,756	403,445	Phvul.004G006100	AT4G39490.1	cytochrome P450, family 96, subfamily A, polypeptide 10
431,954	433,623	Phvul.004G006300	AT2G23180.1	cytochrome P450, family 96, subfamily A, polypeptide 1
567896	570454	Phvul.004G007750	AT3G14470.1	NB-ARC domain-containing disease resistance protein
618293	622960	Phvul.004G007900	AT3G14470.1	NB-ARC domain-containing disease resistance protein
634170	640604	Phvul.004G008001	AT3G14470.1	NB-ARC domain-containing disease resistance protein
657081	659750	Phvul.004G008101	AT3G14470.1	NB-ARC domain-containing disease resistance protein
671773	674107	Phvul.004G008200	AT3G14470.1	NB-ARC domain-containing disease resistance protein
677817	680151	Phvul.004G008250	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
706950	711423	Phvul.004G008351	AT3G14470.1	NB-ARC domain-containing disease resistance protein
732245	733453	Phvul.004G008400	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
733519	734080	Phvul.004G008450	AT3G14470.1	NB-ARC domain-containing disease resistance protein
746809	753397	Phvul.004G008560	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
762295	765654	Phvul.004G008620	AT3G14470.1	NB-ARC domain-containing disease resistance protein
768791	773819	Phvul.004G008680	AT3G14470.1	NB-ARC domain-containing disease resistance protein
796775	801277	Phvul.004G008740	AT3G14470.1	NB-ARC domain-containing disease resistance protein
819458	826737	Phvul.004G008921	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
833532	836825	Phvul.004G008981	AT3G14470.1	NB-ARC domain-containing disease resistance protein
838678	843077	Phvul.004G009041	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein

Source: v2.1 of the *P. vulgaris* genome, available at https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris

Table A3. Gene models related to plant disease resistance in the recombinant region related to *Ur-5* gene located in Pv04 (continued)

Start position(bp)	End position(bp)	Gene name v2.1	Best hit Arabidopsis	Gene function
847290	848413	Phvul.004G009101	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
863222	865511	Phvul.004G009221	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
879439	884653	Phvul.004G009281	AT3G14470.1	NB-ARC domain-containing disease resistance protein
906948	907805	Phvul.004G009461	AT3G14470.1	NB-ARC domain-containing disease resistance protein
909231	916054	Phvul.004G009521	AT3G14470.1	NB-ARC domain-containing disease resistance protein
920780	922280	Phvul.004G009581	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
939934	943174	Phvul.004G009821	AT3G14470.1	NB-ARC domain-containing disease resistance protein
961070	964494	Phvul.004G009909	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
964943	966979	Phvul.004G009918	AT3G14470.1	NB-ARC domain-containing disease resistance protein
967700	972035	Phvul.004G009927	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
989558	993520	Phvul.004G009936	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1043536	1044075	Phvul.004G009100	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1063726	1067743	Phvul.004G009136	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1103140	1118146	Phvul.004G009154	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1129267	1132629	Phvul.004G008900	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1137597	1139195	Phvul.004G008909	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1146369	1149704	Phvul.004G008918	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
1156680	1160125	Phvul.004G009300	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1191387	1192524	Phvul.004G009518	AT3G14460.1	LRR and NB-ARC domains-containing disease resistance protein
1203535	1207848	Phvul.004G009527	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1228652	1233028	Phvul.004G009800	AT3G14470.1	NB-ARC domain-containing disease resistance protein
1278226	1282234	Phvul.004G010400	AT5G40440.1	mitogen-activated protein kinase kinase 3
1385799	1398207	Phvul.004G012600	AT5G14720.1	Protein kinase superfamily protein

Source: v2.1 of the *P. vulgaris* genome, available at https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris