MAPPING *Ur-6*, A BEAN RUST RESISTANCE GENE IN COMMON BEAN

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MASTER OF SCIENCE

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ABSTRACT

Bean rust, caused by the fungus *Uromyces appendiculatus* (Pers.:Pers) Unger, is a disease of common bean (*Phaseolus vulgaris*) prevalent in the Americas and Africa. The most cost-effective countermeasure to bean rust is genetic resistance. While 17 dominant rust resistance genes (named with *Ur-* symbol) have been identified in common bean, not all of these genes have been genetically fine-mapped. To expand our knowledge of rust resistance genes in common bean, *Ur-6* was mapped in the common bean genome. A GWAS analyses suggested that *Ur-6* is present on chromosome Pv07 of *P. vulgaris*. Two InDel markers tightly linked to *Ur-6* were developed by F_2 bi-parental mapping and may prove effective for marker-assisted selection in bean breeding programs in the future. Further, 25 candidate genes were identified and are the potential focus of future gene validation research.

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TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGEMENTS iv

LIST OF TABLES vii

LIST OF FIGURES viii

LIST OF APPENDIX TABLES ix

INTRODUCTION 1

LITERATURE REVIEW 3

Importance of Common Bean in World Agriculture 3

Common Bean 4

Bean Rust 7

Molecular Interactions Between Common Bean and Bean Rust 8

Genetic Diversity of Bean Rust 18

Genome Wide Association Studies in Common Bean 21

MATERIALS AND METHODS 24

Phenotypic Analysis of Middle American Diversity Panel 24

Genome-wide Association Study 25

InDel Marker Development 26

Construction of Genetic Linkage Map 30

Candidate Gene Identification 30

RESULTS 32

MDP Screening and GWAS 32

F_2 Screening and Genetic Linkage Analysis 34

Candidate Genes 39

DISCUSSION 42

Mapping the *Ur-6* locus 42
Putative Candidate Genes 45
Identification of *Ur*-6 InDel Marker 48
CONCLUSIONS 50
REFERENCES 51
APPENDIX 60

LIST OF TABLES

Table		Page
1.	Rust resistance genes described in common bean (*Phaseolus Vulgaris*)	18
2.	Modified binary nomenclature system for Standard Bean Rust Differential Set	21
3.	Bean rust grading scale used to screen the MDP and F_2 population for response to bean rust race 47.	25
4.	Start and end position of primers for the InDel markers used to map *Ur-6* in the common bean genome.	28
5.	Details of the InDel markers used to map *Ur-6* in the common bean genome.	29
6.	InDel haplotypes of race 47 resistant (G19833, GGW, Olathe) and susceptible (UI 111, UI 114) genotypes across the Pv07 region surrounding the mapped *Ur-6* locus. *Ur-6* cosegregates with marker Pv07_6963716.	38
7.	Observed differences in the translated amino acid sequences for the candidate genes between the G19833 and UI 111 reference genomes.	40
8.	Predicted gene functions for candidate genes.	41

LIST OF FIGURES

Figure — Page

1. Position of InDel markers on Pv07 used to map the position of the *Ur-6* gene in common bean A) Position of InDel markers on Pv07 used to map the position of the *Ur-6* gene in common bean. The InDel markers are so close together when viewing the entire region that they are merged together in this view. B) Position of InDel markers within relevant Pv07 locus identified by GWAS. 30

2. GWAS analysis of common bean Middle American Diversity response to bean rust race 47 infection. This analysis was carried out with 239 MDP genotypes and 152,470 SNPs with MAF ≥ 5%. The green lines represent the cutoffs for the top 0.1% and top 0.01% of SNPs, respectively. 33

3. GWAS analysis of common bean Middle American Diversity response to bean rust race 47 infection, with genotypes containing *Ur-11* or *Ur-6+* removed. This analysis was carried out with 189 MDP genotypes and 152,470 SNPs with MAF ≥ 5%. The green lines represent the cutoffs for the top 0.1% and top 0.01% of SNPs, respectively. 33

4. Polymorphic InDels identified between UI 114 and GGW A) Polymorphic InDels identified between UI 114 and GGW and then verified by PCR and agarose gel electrophoresis. B) Segregation pattern of marker Pv07_6966113 within the F_2 population. Lanes marked "S" and "R" contain genomic DNA from plants graded susceptible or resistant to race 47, respectively. The two lanes with red text and an asterisk contain genomic DNA from susceptible plants but have a heterozygous banding pattern for the marker, indicating recombination has occurred between the genetic marker and *Ur-6*. 34

5. Genetic linkage map generated in MapDisto. Marker Pv07_6714298 cosegregates and marker Pv07_6963716 is closely linked with the *Ur-6* locus. 36

6. Graphical phenotyping of informative F_2 individuals. For the marker data, H = Heterozygous, B = UI 114/Susceptible, and D = Resistant. Individuals P2-B5 and P3-D2 are observed to possess crossover breakpoints on either side of the mapped. 37

LIST OF APPENDIX TABLES

Table Page

A1. Average bean rust race 47 reaction scores for MDP genotypes. 60

INTRODUCTION

Common bean (*Phaseolus vulgaris*) is one of the most important crops around the world, providing many vitamins and minerals missing from other common human crops in addition to beans being comprised of 19-31% protein (Bressani 1983; Ma and Bliss 1978). Because their nutritional benefits, common bean has become quite the cash crop as well, with farmers in Latin America able to generate hundreds of dollars per hectare annually with local cultivars and genetically improved varieties generating even greater profit margins (Osorno and McClean 2014).

Bean rust, caused by *Uromyces appendiculatus* (Pers.:Pers) Unger, is a disease of common bean prevalent in the Americas and Africa. In North America, practices of utilizing fungicide to combat this disease limit yield losses to 40-50%, in contrast with countries in South America and Africa where farmers without access to fungicide have reported that bean rust has caused from 75% to almost complete yield loss (Liebenberg and Pretorius 2010). Due to the cost associated with fungicide application, the most cost-effective countermeasure to bean rust is genetic resistance. While 17 dominant rust resistance genes (named with *Ur-* symbol) have been identified in common bean, only a small set of these genes have been mapped to the extent that effective genetic markers are available for selection in bean breeding programs.

Presently, the most commonly utilized bean rust resistance genes are *Ur-3* and *Ur-11*, due to the broad-spectrum resistance they provide against many races of bean rust (Hurtado-Gonzales et al. 2017). While these genes provide resistance to many races of bean rust, they are not infallible and other resistance genes are necessary for providing resistance to other races of bean rust. Further, a wider variety of bean rust resistance genes is needed to generate cultivars with different sets of rust resistance genes, enabling farmers to respond to the changing

prevalence of different races of bean rust in different regions. While more narrow-spectrum bean rust resistance genes have been identified and characterized, less research interest has been placed upon them, leading to a deficit in genomic information about these genes and a want for tools enabling the incorporation of these genes into breeding programs.

The goal of this research is to identify the location of the rust resistance gene *Ur-6* within the common bean genome, and to generate a genetic marker linked to the gene to be utilized in marker-assisted selection within common bean breeding programs. To expand our knowledge of rust resistance genes in common bean, *Ur-6* was mapped in the common bean genome. To accomplish this, genetic analyses using the combination of a Genome-Wide Association Study (GWAS) with the Middle American Diversity Panel (MDP) and bi-parental F_2 population for *Ur-6* with InDel markers were carried out to map *Ur-6* and identify a set of potential candidate genes.

LITERATURE REVIEW

Importance of Common Bean in World Agriculture

Beans are a crucial source of dietary proteins, vitamins, and minerals for many countries in Latin America as well as eastern and southern Africa, where other crops provide primarily carbohydrate-based nutrition. Beans are comprised of roughly 19-31% protein, the largest constituent being the storage protein phaseolin (Ma and Bliss 1978; Bressani 1983). Phaseolin, like other legume seed proteins, contains low levels of sulfur amino acids and high levels of lysine (Evans and Bandemer 1967). This amino acid composition complements that of cereal crops common in Africa and Latin America, allowing for a 2:1 ratio of cereals to beans consumed to produce a balanced diet (Bressani 1983). However, seed yields are generally low in Latin America and Africa relative to other locations in the world (Vandemark et al. 2015). Introducing varieties resistant to diseases may improve the nutrition and health of hundreds of millions of people in the world, particularly those living in developing countries, by relieving food insecurity due to low yields.

The vitamin biotin has a multifaceted role in the human body as a cofactor for many metabolic enzymes, amino acid catabolism, oxaloacetate synthesis, and an epigenetic regulator of gene expression (Knowles 1989; Lietzan and St Maurice 2014; Mock 2017). Despite the importance of biotin to many key cellular processes required by most organisms, biotin biosynthesis is only found in plants and some microbial organisms. Developed nations typically utilize chemically synthesized biotin to supplement food and drinks consumed by humans or incorporate the biotin into different cosmetic products. In nations where processed foods with biotin supplementation are uncommon, beans can be an important source of biotin for human cellular processes (Broughton et al. 2003).

Despite most indicators that the general human health has recently improved in developing nations, micronutrient deficiencies have increased in prevalence. This is likely due to the seed coat being removed from the cereal grain, the most micronutrient-rich part of the grain. However, beans are typically consumed whole, making them a crucial source of micronutrients in the diets of people in developing nations. Beans supply iron, zinc, phosphorus, magnesium, manganese, copper, and calcium, minerals, that when deficient, can stunt the growth of children (Broughton et al. 2003). However, efforts are currently underway to generate varieties of beans that are biofortified with greater levels of nutrients lacking from the diets of people in developing nations (Miller and Welch 2013).

In addition to the many nutritional benefits that common beans provide, they also are a financial boon to farmers. In Latin America local cultivars can generate hundreds of dollars per hectare annually, with genetically improved varieties generating even greater profit margins (Osorno and McClean 2014). In the United States, dry beans are a very prominent and profitable crop, annually ranking in the top 16 crops produced and having a farmgate value exceeding $1 billion each year (nass.usda.gov). Within the United States, North Dakota is the largest producer, containing 252,929 hectares of dry beans across 1,682 farms (USDA-ERS, 2016). Further, beans are able to fix atmospheric nitrogen into bioavailable forms (Rondon et al. 2007). This enables farmers to either intercrop or rotate crops and reduce the amount of fertilizer needed to be applied to fields which in turn decreases overall production costs.

Common Bean

The last whole-genome duplication (WGD) event to occur in the evolutionary history of legumes occurred ~56.5 million years ago, prior to the divergence of *P. vulgaris* and *G. max*

(soybean) that occurred ~19.2 million years ago (Lavin et al. 2005; Schmutz et al. 2010). The wild ancestor of *P. vulgaris* originated in Mesoamerica (Bitocchi et al. 2012).

The *Phaseolus* genus contains approximately 70 species, five of which have been domesticated: *P. vulgaris*, *P. coccineus*, *P. dumosus (*syn. *P. polyanthus)*, *P. acudefetifolius*, and *P. lunatus*. Two of these species, *P. vulgaris* and *P. lunatus*, have wild forms distributed throughout Mesoamerica and South America, and both species underwent two unique and isolated domestication events (Bitocchi et al. 2017). The *Phaseolus* genus diversified within the past four to six million years, prior to the tectonic events of the late Miocene and early Pilocene that created a land bridge between the North and South American continents (Delgado-Salinas et al. 2006; O'Dea et al. 2016). Of the five domesticated *Phaseolus* species, all except *P. lunatus* belong to one of the eight clades comprising the genus. The other domesticated species fall under the Vulgaris group, which has been dated to be approximately four million years ago (Delgado-Salinas et al. 2006). Within the Vulgaris group, *P. vulgaris*, *P. coccineus*, and *P. dumosus* are particularly closely related. These species are capable of being interbred, a process that does not occur in nature due to differences in life cycles and mating mechanisms (Mendel 1866; Debouck 1999).

Prior to the domestication events of *P. vulgaris*, the wild population split into two distinct gene pools, Middle American and Andean, which diverged from a common ancestor approximately 111,000 years ago (Mamidi et al. 2013). Immediately after this divergence, both gene pools underwent a bottleneck lasting approximately 40,000 years. A combination of the Middle American gene pool having nearly twice the bottleneck population size as the Andean gene pool and asymmetric gene flow between the two pools, with greater flow from the Andean gene pool into the Middle American gene pool, resulted in reduced diversity in the modern

Andean gene pool relative to the Middle American gene pool (Rossi et al. 2009; Bitocchi et al. 2012; Mamidi et al. 2013).

The Middle American gene pool is known to have been domesticated in Mexico, as were many other new world crop species, but there is still debate in which region of Mexico the domestication event occurred. In 2009 it was purported that *P. vulgaris* was domesticated in the Rio Lerma–Rio Grande de Santiago basin of western-central Mexico (Kwak et al. 2009). This region is separated from the river basin, where maize was domesticated, by a few hundred kilometers of mountainous terrain. This led (Kwak et al. 2009) to propose that both species were domesticated separately and later united into the traditional Milpa intercropping agricultural practices of Mesoamerican culture. However, a separate group of researchers proposed that the Middle American gene pool was domesticated in the Oaxaca Valley of south Mexico, a region overlapping with the known location of maize domestication, rather than the Lerma river basin (Bitocchi et al. 2013). The region of overlap between the area of maize and bean domestication contains a portion of the Lerma river, one of the rivers proposed to spread the Mesoamerican Milpa intercropping system, allowing the Milpa system to develop naturally and spread readily (Zizumbo-Villarreal and Colunga-GarcíaMarín 2010).

The Andean gene pool pre-domestication bottleneck, and the subsequent loss of genetic diversity, has impaired many efforts to identify the location of domestication. However, analysis of SNP data has helped narrow the domestication location for the Andean gene pool to a region that spans southern Bolivia and northern Argentina (Rodriguez et al. 2016). This finding is in agreement with previous archeological evidence that placed the Andean bean domestication occurred in northern Argentina (Tarragó 1980).

Bean Rust

Biotic stresses significantly impact crop yield in modern agriculture. The ability of plant pathogens to evolve and overcome host resistances and varying environmental factors makes biotic stresses a persistence problem for maximizing yield. Of the different plant diseases, the largest group of pathogens are rust fungi (*Pucciniales*) with over eight thousand identified species (Aime et al. 2014). These fungi pose a serious danger to crops world-wide, including wheat, soybean, coffee, trees, and common bean (Lorrain et al. 2019).

Of the fungal diseases that afflict common bean, a major disease is bean rust, caused by the pathogen *Uromyces appendiculatus* (Pers.:Pers.) Unger. *U. appendiculatus* is an obligate parasite with an autoecious macrocyclic life cycle that completes its entire life cycle on *P. vulgaris* (McMillan et al. 2003). The life cycle of *U. appendiculatus* has multiple stages, with the most commonly observed stage being the uredinial stage, where powdery dark cinnamon-brown urediniospores are produced. Urediniospores are dikaryotic and are capable of producing more urediniospores when infecting a new host plant which further continues its life cycle.

Toward the end of the common bean life cycle, *U. appendiculatus* produces dark diploid teliospores that lay dormant in the soil during unfavorable conditions, such as winter. When teliospores germinate, they enter the basidial stage and produce haploid basidiospores, which can travel long distances. As a result, basidiospores typically infect the same plant on which they are were produced. Germinating basidiospores enter the pycnidial stage and haploid pycniospores are produced. Each pycnia, the cup-like structures that produces pycniospores, produces one of two different mating groups of pycniospores. When a pycniospore is transferred, typically by insects, to a pycnium of the opposite mating type, pycniospores of opposite types, figuratively either male (+, spermatia) or female (-, receptive hyphae), fuse to create a single dikaryotic cell

from the original two haploid cells (Ordonez and Groth 2009; Horton et al. 2005). Once the fusion has occurred, the fungus proceeds to the aerial stage, where aeciospores are produced. These spores then infect new host plants and generate uredia pustules, starting the *U. appendiculatus* life cycle anew.

Molecular Interactions Between Common Bean and Bean Rust

P. vulgaris possesses many different methods to resist infection by different pathogens. The majority of pathogens are avirulent due to pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), which is activated in response to the recognition of a broad range of molecular structures or signals common among different subsets of pathogens. *U. appendiculatus,* having evolved to utilize *P. vulgaris* as a host, and presumably secretes effectors to silence PTI. The secreted effectors can be recognized by resistance (R) gene products, which may or may not be present in any given *P. vulgaris* genotype. The recognition of the *U. appendiculatus* effector by a host R gene induces effector-triggered immunity (ETI), which causes a rapid molecular response to the pathogen. As the effector is produced by a gene that causes the *U. appendiculatus* spore to be recognized, the gene is considered to be an avirulence (avr) gene (Zhang et al. 2018).

The constant struggle between host and pathogen has led to coevolution and ever-changing allele frequencies across populations of both *P. vulgaris* resistance genes and *U. appendiculatus* avr genes. This has led to the occurrence of a gene-for-gene relationship between host and pathogen, where each effector/avr gene in *U. appendiculatus* has a corresponding R gene in *P. vulgaris* (Flor 1971).

R genes are typically dominant and confer partial or full resistance to fungus, virus, nematode, and oomycete pathogens (Acevedo et al. 2013). Naturally occurring R genes have

been utilized in selective crop breeding since early human history. The first R gene to be successfully cloned was *Hm1* from maize, where it confers race-specific resistance to *Cochliobolus carbonum* (Johal and Briggs 1992). Following this initial success, R genes from different species were cloned with increasing frequency, leading to hundreds of published examples.

Two R gene mechanisms of resistance involve pathogen perception and loss of susceptibility. Further dividing these approaches reveals nine different mechanisms (Kourelis and van der Hoorn 2018). Extracellular pathogen perception by receptor-like proteins/kinases (RLPs/RLKs) occurs directly (mechanism 1) or indirectly (mechanism 2). Intracellular pathogen perception typically utilizes nucleotide-binding oligomerization domain-like receptors, typically called NOD-like receptors (NLRs). NLRs can perceive pathogen effectors directly (mechanism 3) or indirectly (mechanism 4), as well as through the use of integrated domains (mechanism 5). Pathogens attempting to alter host gene expression with transcription-activator like effectors can be tricked into activating host immune response genes (mechanism 6). Loss of susceptibility has been found to occur actively through the degradation or detoxification of pathogen components (mechanism 7), passively through the loss or modifications of pathogen effector targets (mechanism 8), or host-reprograming to impede pathogen growth of development (mechanism 9).

Direct extracellular pathogen perception (mechanism 1) by RLPs and RLKs is commonly considered to involve the recognition of PAMPs (Restrepo-Montoya et al. 2020). Due to this, RLPs and RLKs are not typically thought of as R genes, despite the fact that their transfer to other plant species has conferred broad-range bacterial pathogen resistance (Lacombe et al. 2010). However, some effectors are directly perceived by RLPs and RLKs, such as the fungal

effector ethylene-inducing xylanase (EIX) by the tomato RLP LeEix2. When overexpressed in EIX-nonresponsive tobacco plants, LeEix2 was found to bind EIX and induce a hypersensitive immune response (Ron and Avni 2004).

Indirect extracellular pathogen perception (mechanism 2) occurs through the recognition of modified host factors. Modifications to RLPs and RLKs, rather than to factors secreted by the pathogen, provides greater flexibility with regards to pathogen perception. For example, the tomato R gene product Cf-2 is an RLP that is dependent on the Cys protease Rcr3. Rcr3 is the target of the fungal Avr2 effector from *Cladosporium fulvum* and the unrelated nematode effector GrVap1 from *Globodera rostochiensis* (Luderer et al. 2002; Lozano-Torres et al. 2012). These effectors both act as protease inhibitors and modify Rcr3; the inhibited Rcr3 is then recognized by Cf-2, which triggers an immune signaling cascade. In the absence of Cf-2, tomatoes with Rcr3 have increased susceptibility, solidifying the definition of *Cf-2* as an R gene (Lozano-Torres et al. 2012).

Intracellular effector perception utilizes NLRs, a group of proteins with multiple domains characterized by a C-terminal leucine-rich repeat (LRR) domain, a nucleotide-binding (NB-ARC) domain, and an N-terminal protein-binding domain (Chakraborty et al. 2018). In plants the N-terminal domain are either a coiled-coil (CC) or TOLL/interleukin-1 (TIR) domain; TIR domains are not found in monocots, but both CC and TIR domains are components of NLRs of dicots, mosses, and liverworts (Takken and Goverse 2012). The NB-ARC domain serves as a molecular switch for the activation and deactivation of NLRs. An inactive NLR has ADP bound to the NB-ARC domain, producing a condensed protein conformation that prevents the function of the other NLR domains. The binding of ATP to the NB-ARC domain triggers a

conformational change that opens up the LRR and CC/TIR domains for interactions with other molecules (Sukarta et al. 2016).

The direct perception of intracellular effectors (mechanism 3) may occur through the binding of an effector molecule to the LRR of a plant NLR (Krasileva et al. 2010). These observations supported the concept that the alteration of the variable residues in an NLR LRR domain serve to alter disease resistance (Sela et al. 2012). Changes to residues in other domains of the NLR altered effector perception, leading to the development of an equilibrium-based model where the binding of an effector to the LRR stabilizes the "on" state of the ATP-bound NB-ARC domain (Bernoux et al. 2016).

The indirect perception of intracellular effectors (mechanism 4) is a mechanism of pathogen perception that contains two major models of action for R proteins: the Guard Model and the Decoy Model. The Guard Model was originally proposed to explain how the tomato proteins Pto and Prf detect the *Pseudomonas syringae* effector AvrPto. This model was later generalized to other host/pathogen interaction and proposes that R proteins act by monitoring proteins targeted by pathogen effectors (Van Der Biezen and Jones 1998; Dangl and Jones 2001). Due to this monitoring, the R protein is considered a "guard" protein, whereas the effector target is considered a "guardee" due to it being guarded by the R protein. In the absence of a functional guard R protein, the modification of the host guardee protein by the pathogen effector serves to increase the pathogen's virulence.

The Decoy Model functions in a similar manner. The key difference for this model is that the effector target monitored by the R protein does not increase pathogen fitness in the absence of the R protein (van der Hoorn and Kamoun 2008). Typically, the decoy mimics other effector targets. This mimicry either arose from a gene duplication event or through the independent

evolution of a target mimic. As the decoy serves to alert the R protein to the presence of pathogen effectors but does not prevent the effector target that it mimics from being modified, the decoy can be likened to a molecular tripwire alarm system.

While the Guard and Decoy Models are capable of describing many known examples of indirect intracellular effector detection, perception can also occur due to NLR monitoring of cellular homeostasis. For example, the Arabidopsis NLR SUMM2 monitors the phosphorylation status of the receptor-like cellular kinase CRCK3, a target of the MEKK1-MKK1/2-MPK4 immune signaling cascade (Zhang et al. 2017). SUMM2 identifies the accumulation of unphosphorylated CRCK3 in the cell, enabling it to monitor the presence of an effector that may interfere with the upstream signaling cascade. Aside from serving as a protein being monitored by SUMM2, CRCK3 has no known cellular function, a fact determined by studying CRCK3 knockouts which do not exhibit any defects in the PAMP-triggered immunity signaled by the MEKK1-MKK1/2-MPK4 pathway. However, SUMM2 is not conserved among higher plants species while CRCK3 is conserved, suggesting that there is a molecular function for CRCK3 outside the SUMM2 effector monitoring system (Zhang et al. 2017). As SUMM2 does not bind to the target of an effector, this example does not fit into the current accepted definitions of the Guard and Decoy Models. It is possible that the Guard and Decoy model could be expanded in the future to accommodate an entirely new model that accounts for SUMM2's monitoring of multiple different proteins in a signaling cascade by proxy.

Mechanism 5 is based on plant NLRs that contain integrated domains (NLR-IDs) that are targeted by effectors (Sarris et al. 2016). One NLR-ID is the Arabidopsis protein RRS1-R that contains a WRKY transcription factor domain. This R protein confers resistance to multiple pathogens, though it was originally identified for conferring resistance to *Ralstonia*

solanacearum, a bacteria that express PopP2, an acetyltransferase that targets WRKY transcription factors (Le Roux et al. 2015). NLR-IDs have the potential to be developed as a biotechnological tool, with the replacement of the bait domain with other domains of interest possibly serving to generate immunity to certain pathogens.

Xanthomonas species sometimes utilize transcription activator-like effectors (TALEs) to alter host gene expression. Executor genes are R genes that are transcriptionally activated by *Xanthomonas* TALEs and confer immunity (mechanism 6). The original target of the *Xanthomonas* TALEs are genes that would serve as susceptibility factors, but executor genes have a decoy promoter sequence to act as a trap for these pathogens (van der Hoorn and Kamoun 2008). The first executor gene cloned was the rice gene *Xa27*, which confers resistance to *Xanthomonas oryzae* pv. *oryzae* (Gu et al. 2005).

In addition to identifying pathogen effectors, some R genes reduce or eliminate host susceptibility to a pathogen. The active loss of susceptibility (mechanism 7) is utilized by R genes that encode proteins that interrupt key pathogen molecular virulence processes. The first R gene ever cloned, the maize gene *HM1*, falls under this mechanism as it inactivates a toxin expressed by *C. carbonum* race 1 (Johal and Briggs 1992). This mechanism is particularly prevalent as a means to combat viral infection since viruses utilizing a much smaller number of proteins and molecular processes than other types of pathogens. For example, tomatoes possess R genes that, when expressed, can bind to viral replication proteins, inactivating them, or silencing viral genes by methylation with RNAi (Ishibashi et al. 2014; Verlaan et al. 2013; Butterbach et al. 2014).

While most of the R genes discussed are dominant, the passive loss of susceptibility through the loss of its interaction with pathogen effectors (mechanism 8) is typically associated

with recessive R genes. This mechanism is commonly implemented against viral pathogens, with half of all known anti-viral R genes using this mechanism (Truniger and Aranda 2009). Most recessive R genes act against potyviruses, where recessive mutant translation initiation factors are unable to bind to the cap of viral transcripts (Kang et al. 2005; Truniger and Aranda 2009). Recessive R genes are also used against bacterial pathogens, where one third of the rice R genes that confer resistance against *X. oryzae* pv. *oryzae* express recessive gene action (Liu et al. 2011). Recessive R genes are implemented in this host-pathogen relation due to the pathogen's use of TALEs. Recessive mutations in the promoters of host susceptibility genes or in the host transcription factor utilized by TALEs interfere with bacterial virulence (Chu et al. 2006; Iyer-Pascuzzi et al. 2008; Yuan et al. 2016).

Mutating components of molecular pathways is another passive method of losing susceptibility to pathogens (mechanism 9). This method is typically recessive but may also be manifested through dominant negative alleles. The most notable example of this mechanism is the loss-of-function recessive allele *mlo*. The dominant wild-type allele, *MLO*, is a G protein-coupled receptor in both monocot and dicot plants that negatively regulates programmed cell death responses to both biotic and abiotic stress stimuli (Hückelhoven and Panstruga 2011; Piffanelli et al. 2002). In barley and Arabidopsis, *MLO* is co-expressed with three genes, *PEN1*, *PEN2*, and *PEN3* that confer resistance to powdery mildew. In this system, the dominant allele negatively regulates these resistance genes (Humphry et al. 2010). While the recessive *mlo* allele causes spontaneous cell death and leaf lesions, this allele also allows for the expression of the *PEN1-3* genes, rendering the plant immune to powdery mildews (Hückelhoven and Panstruga 2011; Buschges et al. 1997). R genes utilizing this mechanism typically alter an inefficient

immune response, allowing for a faster and/or stronger immune response than is observed in wild-type alleles (Kourelis and van der Hoorn 2018).

While it is advantageous for a plant to be resistant to a pathogen, the disease resistance comes with a costs such as yield penalties or decreased efficiency (Brown 2002). For example, the barley *mlo* R gene reduces yields by approximately 4.2% (Ning and Wang 2018). The cost associated with R genes is highly variable, and may be due to the R gene itself or through tight linkage to yield-reducing genes (Ning et al. 2017). Due to this decreased yield, the resistant genotypes are considered undesirable for agricultural cultivation. Research efforts are focusing on identifying resistance genes with negligible or no yield penalty (Xu et al. 2017). In rice, a resistance gene was cloned that simultaneously provided race-specific resistance against *X. oryzae* pv. *oryzae*, no effect on yield, and improvement in other agronomic traits (Hu et al. 2017).

To minimize the detrimental R gene effects, epigenetic regulation is frequently found to tightly control R gene expression, such as with *SNC1* and *RPP4* that are upregulated by H3K4 trimethylation, or the upregulation of a plant R gene that is mediated by the trimethylation of H3K36 (Xia et al. 2013; Richard et al. 2018). Presently, no histone post-translational modifications have been found to negatively regulate NLR genes in plants, likely due to experimental biases (Richard et al. 2018). However, plant NLR gene regulation has been found to be mediated by cytosine methylation. DNA methylation conferred by siRNA suppressed the basal expression of R genes, while both genome-wide and directed DNA demethylation in response to bacterial inoculation serve to rapidly induce the expression of immune genes, including R genes (Yu et al. 2013; Deleris et al. 2016; Dowen et al. 2012).

R genes are typically found in repeat-rich regions of a plant genome. It is likely that these repeated sequences facilitate the generation of new R genes, a process that is based on unequal crossing-over and gene conversion (Richter et al. 1995). This mechanism of generating new R genes leads to the clustering of resistance genes at a few genomic loci, rather than being dispersed throughout the genome (Meziadi et al. 2017). This process allows for selection pressures to act upon newly generated R genes and the extinction of non-functional or deleterious R genes (Michelmore and Meyers 1998; McClean and Raatz 2017). This process is particularly effective at generating new NLR genes, as slight changes to binding specificity or alterations of a domain can enable the detection of different pathogen effectors. This concept is supported by the discovery that NLR genes are the fastest evolving gene family in flowering plants (Lu et al. 2016). In addition to unequal crossing over and gene conversion, the presence of resistance gene clusters in repeat-rich region allows for retrotransposons to alter NLRs and transport them to different regions of the genome, a phenomenon that has been found to have occurred in hot pepper plants (Kim et al. 2017).

Common bean has a large number of disease resistance gene clusters and one of the largest single clusters (McClean and Raatz 2017). Of the 376 NLR genes identified in common bean, 35 are clustered on the distal end of the short arm of Pv04, 58 are clustered on the distal end of short arm of Pv10, and 60 are clustered on the distal end of long arm of Pv11 (Meziadi et al. 2016). These large clusters are all present within the subtelomeric regions of their respective chromosomes, likely due to the greater degree of plasticity in subtelomeric regions along with a greater degree of silencing occurring, which would mitigate the growth impairment from the NLR genes (Yi and Richards 2007; Chen et al. 2018).

Due to different selection pressures, different genotypes of *P. vulgaris* possess different R genes. Common bean R genes that confer resistance to rust are defined by the prefix *Ur-* (Kelly et al. 1996). The distribution of these genes is divided between the Middle American and the Andean gene pools (Hurtado-Gonzales et al. 2017). Currently there are 17 different rust resistance genes that have been described (Table 1), several of which have been lost because the stocks are no longer available. The number of *Ur* genes may increase as the interactions between the host and pathogen are further elucidated (de Souza et al. 2011; Hurtado-Gonzales et al. 2017).

The identification of specific rust resistance genes and the races of bean rust to which they confer resistance generates an opportunity to develop bean cultivars resistant to multiple races of rust through a method known as resistance gene pyramiding. This approach is greatly enhanced by the discovery of tightly linked genetic markers for different resistance genes, which enable marker-assisted selection to speed up the screening of hybrid plants produced as part of a resistance gene pyramiding breeding program (Hurtado-Gonzales et al. 2017).

Table 1. Rust resistance genes described in common bean (*Phaseolus Vulgaris*)

Gene	Gene Pool	Isolate Cultivar	Location	Notes
Ur-1	MA	B1627 (Gallaroy Genotype I)		Obsolete resistance gene, no seed stocks for lines with this gene exist.
Ur-2	MA	B2090 (Gallaroy Genotype II)		Obsolete resistance gene, no seed stocks for lines with this gene exist.
Ur-2^2	MA	AxS 37		Closely linked with *Ur-2*, important rust resistance source in Brazil.
Ur-3	MA	Aurora	Pv11	Tightly linked gene cluster or single dominant gene
Ur-3$^+$	MA	Mexico 235 and Ecuador 299	Pv11	Contains additional race specificity beyond that *Ur-3*, but confers varying degrees of resistance to different rust races
Ur-4	A	Early Gallatin	Pv06	
Ur-5	MA	Mexico 309	Pv04	Block of tightly linked dominant genes
Ur-6	A	Golden Gate Wax	Pv11	
Ur-6+	A/MA	Pinto Olathe	Pv11	Contains additional race specificity beyond that of *Ur-6*; Andean gene in a Middle American background
Ur-7	MA	Great Northern 1140	Pv11	
Ur-8	A	U.S. #3		Obsolete resistance gene
Ur-9	A	PC-50	Pv01	
Ur-10	A	Resisto		Confers a resistance reaction of slow rusting; has not been tagged nor mapped
Ur-11	NA	PI181996	Pv11	Block of tightly linked dominant genes; linked with *Ur-3*
Ur-12	A	PC-50	Pv7	Conditions adult plant resistance and is expressed at the fourth trifoliate stage
Ur-13	A	Redlands Pioneer	Pv8	Despite Redlands Pioneer being an Andean differential cultivar, *Ur-13* appears to be of Middle American origin
Ur-14	MA	Ouro Negro	Pv4	

Adapted from (de Souza et al. 2011) and other literature.

Genetic Diversity of Bean Rust

U. appendiculatus is a highly variable fungal pathogen, where 373 different rust pathotypes from over 2,000 isolates collected from the Dominican Republic, Honduras, Puerto Rico, and USA have been described (Mmbaga et al. 1996). (Within the literature, the terms

"pathotype" and "race" are frequently used synonymously; for the purpose of this paper, I will solely be referring to rust isolates with differing virulence-related genes, resulting in differing virulence patterns on the same bean cultivars, as races.) Further research into the diversity of rust virulence in Honduras revealed even greater diversity than previously reported. A total of 91 races were characterized from 385 Honduran rust isolates (approximately one race for every four isolates) instead of 373 races from 2,000 isolates (approximately one race for every five to six isolates) (Acevedo et al. 2013). The great degree of rust diversity was made possible by the detailed rust susceptibility grading scale originally proposed by Stavely (Stavely 1984) that scores subtle differences in resistance/susceptibility phenotypes.

The high degree of pathogenic variation makes the naming of different races a complicated process that changed multiple times. Bean rust nomenclature systems utilize differential sets of common bean genotypes. The degree of virulence for a rust isolate on each of the cultivars in a set is used to assign a name to the rust isolate being tested. One of the earliest differential sets utilized six different cultivars [US#3, California Small White, Pinto, Kentucky Wonder (KW) 765, KW 780, and KW 814] to differentiate six already named races of bean rust (then named races 1, 2, 6, 11, 12, and 17) (Zaumeyer and Harter 1941). Zaumeyer went on to use this differential set as a working tool, rather than a standard, going on to identify a new race of bean rust using a differential set of eight different cultivars (US#3, Bountiful, KW 643, Pinto, KW 765, KW 780, KW 814, and Golden Gate Wax) (Zaumeyer 1960). The first standardization of a basic differential set occurred at the first International Rust Workshop held in Puerto Rico in 1983, where a set of 20 genotypes was selected Shortly after the workshop, it was discovered that the 20th cultivar (Mountaineer White Half Runner) reacted identically to KW 780, causing future researchers to exclude it from the differential set (Stavely 1984; Stavely 1989).

The standard differential set was updated at the 3rd International Rust Workshop held in South Africa in 2002 and consists of 12 different cultivars, six from the Middle American gene pool and six from the Andean gene pool (Steadman et al. 2002). This new set has the advantage of providing a greater representation to the Andean gene pool and the resistance genes present therein, along with providing the resistant gene(s) present in many of the cultivars. At the time of its publication, it was noted that this set was intended to be used "until a set of isolines with single rust resistance genes is developed," due to the inclusion of cultivars that had multiple resistance genes or unknown resistance gene(s) (Steadman et al. 2002). Due to the usage of a grading scale, rather than a binary resistant/susceptible score, the usage of a relatively small number of cultivars in a differential set has the capability of differentiating many different races of bean rust. While the present standard is widely utilized, older differential sets are likely to be also evaluated for the purpose of comparing newly isolated races, and to provide a finer definition of its racial virulence capabilities.

With the introduction of the new standard differential set at the 3rd International Rust Workshop, it was also agreed upon that the rust races be organized under a binary nomenclature system. A new bean rust isolate is defined by summing the value assigned to each resistant genotype of the differential set (Habgood 1970). To differentiate between the Andean and Middle American gene pools, the values are calculated separately and the final numerical designation reported as two numbers separated by a hyphen (Table 2) (Steadman et al. 2002).

Table 2. Modified binary nomenclature system for Standard Bean Rust Differential Set

Gene Pool	Cultivar	Binary value	Decenary Value	Resistance gene(s)
Andean	Early Gallatain	2^0	1	*Ur-4*
	Redlands Pioneer	2^1	2	*Ur-13*
	Montcalm	2^2	4	*Unknown*
	PC-50	2^3	8	*Ur-9, Ur-12*
	Golden Gate wax	2^4	16	*Ur-6*
	PI 260418	2^5	32	*Unknown*
Middle American	GN 1140	2^0	1	*Ur-7*
	Aurora	2^1	2	*Ur-3*
	Mexico 309	2^2	4	*Ur-5*
	Mexico 235	2^3	8	*Ur-3+*
	CNC	2^4	16	*Unknown*
	PI 181996	2^5	32	*Ur-11*

Adapted from (Steadman et al. 2002)

Genome Wide Association Studies in Common Bean

The ability to select for desired traits allows farmers to increase yield while decreasing overall cost and work required. Marker assisted selection (MAS) allows the relatively rapid selection of genotypes with desired traits, rather than screening over multiple many generations of advanced within a field. MAS in *P. vulgaris* is more effective at selecting for disease resistance than insect resistance, possibly due to the fact that insect resistance is often polygenic, making the identification of relevant genetic markers more difficult (Miklas et al. 2006).

Advances in genomic technology have allowed for different types of genetic markers to be utilized, including SNPs, InDels, RFLPs, AFLPs, SSRs (microsatellites), and CNVs (Ariani et al. 2016; Schmutz et al. 2014). More recently genome wide association studies have placed a greater reliance on SNP markers (Oladzad et al. 2019a). This movement towards SNPs as the

standard genetic marker is likely due to their ability to utilize next-generation sequencing technology to map genes of interest with much more precision than with older technologies (Hurtado-Gonzales et al. 2017; Osorno and McClean 2014). SNPs have been utilized to select for putative pleiotropic genetic factors that were elucidated by multi-trait mixed model analysis (Oladzad et al. 2019a).

Advances in genetic markers and the release of the *P. vulgaris* reference genome (537.2 Mb assembled of the total 587 Mb genome, 99.1% sequence anchoring to 11 pseudo-chromosomes) has helped characterize the evolutionary history of common bean, including the identification of two separate domestication events for Middle American and Andean gene pools (Schmutz et al. 2014). Genetic markers form the basis for most gene discovery research and are used to identify quantitative trait loci (QTL) with large effects from bi-parental populations. (Singh and Singh 2015). These studies utilize linkage disequilibrium (LD) to determine the degree that genetic markers are linked with traits of interest, identifying the general location of the gene(s) controlling the traits of interest. In contrast, genome wide association studies (GWASs) are capable of identifying loci with much smaller effects on a given trait, due to its usage of multiple genotypes with different recombination histories (Oladzad et al. 2019a). GWAS in *P. vulgaris* utilizing the Middle American Diversity Panel (MDP; n≈300), developed by the USDA funded BeanCAP project (Moghaddam et al. 2016), have identified genomic loci and candidate genes associated with important agronomic traits (Moghaddam et al. 2016), nutritional content (Moghaddam et al. 2017; McClean et al. 2017), increased leaf and seed size (Schmutz et al. 2014), abiotic stress tolerance (Soltani et al. 2017; Oladzad et al. 2019a), and resistance to different types of pathogens (Oladzad et al. 2019b; Jain et al. 2019). The Andean Diversity Panel (ADP; n≈350) (Cichy et al. 2015) was used to map loci associated with cooking

time (Cichy et al. 2015), nutritional content (Katuuramu et al. 2018), symbiotic nitrogen fixation (Kamfwa et al. 2015a), abiotic stress tolerance (Soltani et al. 2018), agronomic traits (Kamfwa et al. 2015b), and resistance to different types of pathogens (Zuiderveen et al. 2016; Tock et al. 2017). GWAS utilizes LD, knowledge of the population structure, and different statistical models to identify the genomic loci associated with trait(s) of interest (Sul et al. 2018). Further, GWAS require the generation of large SNP datasets, which is commonly done with NextGen sequencing technology, and a reference genome (Oladzad et al. 2019b). As sequencing technology progresses, so too does the detail and definition of reference genomes, allowing for more QTL to be mapped with greater precision.

MATERIALS AND METHODS

Phenotypic Analysis of Middle American Diversity Panel

The Middle American Diversity Panel (MDP) (n = 260) was screened for resistance or susceptibility to bean rust race 47 (race 15-3). Spores of bean rust race 47 isolate were developed by (Stavely 1984) and provided by Talo Pastor-Corrales (USDA-ARS, Beltsville, MD USA). Golden Gate Wax (*Ur-6*) and UI 114 (bean rust race 47 susceptible) were used as internal controls to confirm successful rust inoculation. All MDP and control plants were grown in ~100 cm^2 square pots containing one plant per pot, with five plants prepared for each genotype screened. The primary (unifoliate) leaves of the bean plants were inoculated 8-12 days after seeding, when the primary leaves were ~35-65% expanded. Rust inocula were prepared using 0.1 mL of frozen race 47 *U. appendiculatus* urediniospores suspended in 100 mL of room temperature distilled water and 0.01% TWEEN 20. After inoculation, the plants were transferred to a dark humidity chamber (20 ± 1°C; relative humidity ≥ 95%) for 18 hours. After this post-inoculation period, the plants were transferred to a growth chamber, where visible rust symptoms were observable on susceptible plants at ~12 days after inoculation. MDP plants were scored according to the current standard bean rust grading scale (Table 3; Stavely *et al.* 1989), which is based on the size of pustules or necrotic flecks. To ensure accuracy of pustule and necrotic fleck grading, a measuring loupe (Peak Optics; La Quinta CA USA) was used in grading bean rust reaction phenotypes. The most prevalent infection response score for all plants of each genotype was chosen to represent the phenotype. This protocol was also carried out on the landrace G19833, the genotype of one of the common bean reference genomes.

Table 3. Bean rust grading scale used to screen the MDP and F_2 population for response to bean rust race 47.

Grade	Definition	Resistance/Susceptible
1	Immune - No visible Symptoms	Resistant
2	Necrotic spots < 0.3 mm in diameter	Resistant
2+	Necrotic spots 0.3 - 1.0 mm in diameter	Resistant
2++	Necrotic spots 1.0 - 3.0 mm in diameter	Resistant
2+++	Necrotic spots > 0.3 mm in diameter	Resistant
3	Uredia spores < 0.3 mm in diameter	Resistant
4	Uredia spores 0.3 - 0.5 mm in diameter	Susceptible
5	Uredia spores 0.5 - 0.8 mm in diameter	Susceptible
6	Uredia spores > 0.8 mm in diameter	Susceptible

Adapted from (Stavely 1989; Ballantyne 1974)

Genome-wide Association Study

The GWAS used 128,199 SNPs, with a minor allele frequency (MAF) ≥ 5%, from those SNPs identified from a large collection of Middle American genotypes (Oladzad et al. 2019a). Raw data (scores 1-6) from graded MDP genotypes were converted into a binary classification, where genotypes with average rust race 47 inoculation scores ≤ 3.0 were considered resistant and genotypes with average scores > 3.0 were considered susceptible. Genotypes known to possess *Ur-11* were excluded from the GWAS analyses because that gene is epistatic to *Ur-6*.

SNP association analysis was performed in GAPIT (Lipka et al. 2012), with population structure estimated by principal component analysis (PCA), with two PCAs accounting for ~25% of the variation being included in the model as a fixed effect. GAPIT's EMMA algorithm was used to measure relatedness, which was considered a random effect. This algorithm is based upon the linear mixed model equation $y = X\beta + Zu + e$ where y is a vector of the disease response phenotype, X is a matrix of fixed effects, β is a vector of the coefficients of the fixed

effects, u is the random effect of the mixed model where Var(u) = $\sigma^2_g K$, Z is the corresponding design matrix for u, and e is an n × n matrix of residual effects such that Var(e) = $\sigma^2_e I$ (Kang et al. 2008; Bandillo et al. 2015). The R package "gap" was used to generate Manhattan and QQ plots.

InDel Marker Development

10X libraries for Golden Gate Wax (GGW) and UI 114 were constructed, fragment sequenced (2 x 150 bp), and scaffolds assembled at HudsonAlpha Institute of Biotechnology (Huntsville, Alabama USA). GGW and UI 114 scaffolds were aligned to the common bean reference genome v2.1 (Chaucha Chuga = G19833) using the National Library of Medicine's BLASTn (Altschul et al. 1990). Scaffolds of the two genotypes that mapped to the targeted region defined by the GWAS SNP peak were aligned to the G19833 reference genome in Integrative Genomics Viewer (IGV) v2.5.2. Primer pairs were designed for InDels polymorphic between UI 114 and GGW in the target region (Table 4).

201 F_2 plants derived from the cross UI 114 × Golden Gate Wax were grown and inoculated with rust race 47, following the same methods implemented for the MDP plants, and phenotypically scored. DNA was extracted from each F_2 plant using the following procedure. Early trifoliate leaves were collected and placed in 96 well sitting in liquid nitrogen. Following the snap freezing during tissue collection, the plates were desiccated by a rotary evaporator. Following desiccation, DNA was extracted and purified using the Mag-Bind® Plant DNA Plus 96 Kit from Omega Bio-tek (Norcross, GA USA). The purified DNA samples were quantified using a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific; Waltham, MA USA) and adjusted to a standard concentration of 20 ng/μL.

The DNA from each F_2 plant and MDP genotype was amplified with primer pairs for InDel locations using the following procedure. Polymerase chain reactions (PCR) were carried out in 96 well plates, with each well containing 20 µL of reagent solution, which was comprised of 12.4 µL of purified water, 2 µL of 10× PCR buffer, 1 µL of 10mM dNTP, 1 µL of each primer at 5 µM (2 µL total), and 0.6 µL of Taq polymerase. Samples were incubated on a thermal cycler at 95 °C for 30 sec; 36 cycles of 95 °C for 30 sec, the specific primer pair's annealing temperature (see Table 5) for 20 sec, and 72 °C for 90 sec; followed by 72 °C for 5 min and a 4 °C hold. PCR products were then visualized on a 3% agarose gel containing ethidium bromide.

Table 4. Start and end position of primers for the InDel markers used to map *Ur-6* in the common bean genome.

InDel Marker	Start Position (bp)	End Position (bp)
Pv07_5005158	5,005,158	5,005,481
Pv07_5793966	5,793,966	5,794,521
Pv07_5818590	5,818,590	5,818,782
Pv07_6183030	6,183,030	6,183,216
Pv07_6186490	6,186,490	6,186,690
Pv07_6644510	6,644,510	6,644,831
Pv07_6664900	6,664,900	6,665,026
Pv07_6688717	6,688,717	6,689,165
Pv07_6714298	6,714,298	6,714,609
Pv07_6963716	6,963,716	6,963,821
Pv07_6966113a	6,966,113	6,966,212
Pv07_6966113b	6,966,113	6,966,258
Pv07_7217801	7,217,801	7,217,932
Pv07_7390824	7,390,824	7,391,202
Pv07_7619298	7,619,298	7,619,706
Pv07_7930515	7,930,515	7,930,611
Pv07-8381613	8,381,613	8,381,758
Pv07_8630989	8,630,989	8,631,297
Pv07_9778066	9,778,066	9,778,498
Pv07_10826153	10,826,153	10,826,415

Table 5. Details of the InDel markers used to map *Ur-6* in the common bean genome.

InDel Marker	PCR Annealing Temperature (°C)	Forward Primer Sequence	Reverse Primer Sequence
Pv07_5005158	65	GGT GGA TTG CAC TGT ATG AAT GAT GAT ATG CT	GAT AGC TCA GTT GGG AGA GCG TCA
Pv07_5793966	65	GTC ACG CAT TAT GTG ATC TCT AAG GAC ACA AG	GTG ATC ACC TTC CAA GGG CAC C
Pv07_5818590	65	CCA AGT ACA AAC AGC TCT CAA GCA ACG	GCT CCT AAT GGT TAC CCA CTA AAA TCT TCA AGC
Pv07_6183030	65	CCC AGT CAT GCA AAA GTT GCA AAC GA	CTC AAG CCA AGG TCT CTC CCA TCT
Pv07_6186490	65	GAA CCT TTC ATT GTT TGG CCC TTG TAA AGG	GTT CTT TGC GTG CTT AAG TGC CTT AAC TG
Pv07_6644510	65	CTA GCT CGT TCG ATA TAT CAG GCC GTA C	CTT TTC ACT TCT CAT TAT CCC CCT GTT GTA GT
Pv07_6664900	60	CAG TTT GAC GCC ATC TCT TCC CAT G	CTG AAG AGA TGC CGA CAA GAT AGC CA
Pv07_6688717	60	CCG ATT GCT CAG CTA TCC TGC CTA A	GAA GAG ACA TCA GTT TGA ACC ACA CTA TGA GG
Pv07_6714298	60	GCA TCT ATC CCT ATC CAC GGG AGA AC	GTG TCA CAT CCT CAC CAA ATA TCA CGC
Pv07_6963716	55	TGT CTA GTG GCC CTG AAA GGA GTC	GGA TCC TCC ACA TGT GGT GTA GAA CT
Pv07_6966113a	55	AGA TGT GGA AAC AAT AGT GTG TCC TCT TTT TCT TG	CTG CCT ATG TCA TTT GGG AAA CCG C
Pv07_6966113b	65	CTG CCT ATG TCA TTT GGG AAA CCG C	GTT TGA TAC TGA CCT GTG TCT TGA ACA ATT TGT GA
Pv07_7217801	65	TGA TTT GAG TGA ACC CTC ATT CGA TAC TGT TTG	CAA GGT TCG ACT ATT CGA GTT ACC AGT GG
Pv07_7390824	65	GGG AGA CAC TTG AAT GTT CAT TGA AGT CAA ACT	GGG GCA TGT TTA TGT GCA TGC TGA C
Pv07_7619298	65	GCC TCT CCA TTC TAA TCT GAG GCA TAC TC	GGA TGA CTG ACT GAC TGA CTG TCC TTT
Pv07_7930515	65	CAA GGC ATT GGG GTC TCT ACT CTG C	GTG TGT CTC TCT CCT AGG GTT TGG G
Pv07-8381613	65	GCC CGC TAG AAC AGA CAC TAC TCC	GAA CAG ACT TGT ACA GTA ACG ATA GAC GAG GC
Pv07_8630989	62	GAG GGT AGA TTG CAC ACA CCT GTA TGA C	CAG ATT TAT ACT GTT TGG TTC TAG AAT CTA GAC CCA GC
Pv07_9778066	60	GGT TAC ATT TTA TCT CAC CGT CCC CAT TTT CC	CAC ACC TTG TGT GCA TCC ACG TAT C
Pv07_10826153	60	GCT TCT AAG TAG TTT CAA GTC TCA CTA AGT GTA GAT GC	CCT CCA CAA TCT TCT CAG CAT ACT TGA CC

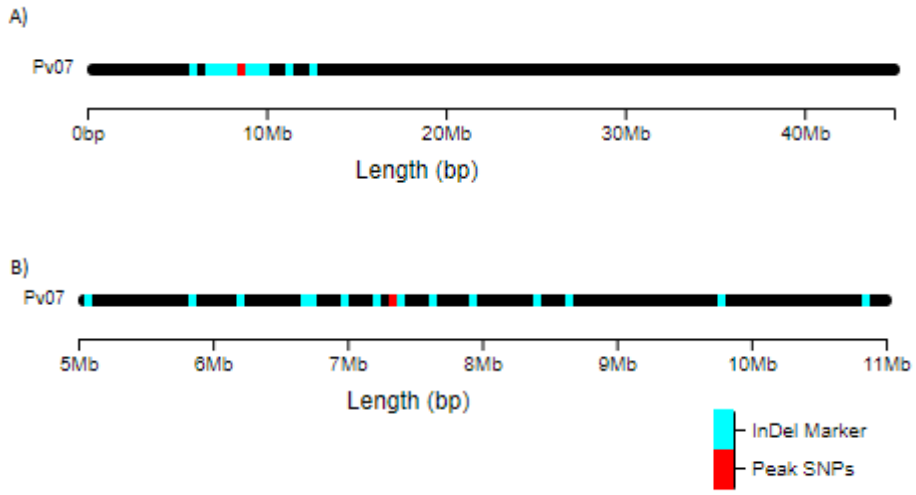

Figure 1. Position of InDel markers on Pv07 used to map the position of the *Ur-6* gene in common bean A) Position of InDel markers on Pv07 used to map the position of the *Ur-6* gene in common bean. The InDel markers are so close together when viewing the entire region that they are merged together in this view. B) Position of InDel markers within relevant Pv07 locus identified by GWAS.

Construction of Genetic Linkage Map

A genetic map consisting of the InDel markers and the race 47 phenotypic response in the F_2 population (197 plants) was calculated using the software MapDisto 2.1.7 (Heffelfinger et al. 2017). Default settings of the regression mapping algorithm (filtered loci with >50% missing data, SARF used to find best order for loci, inversion checking, and the sum of adjacent LOD scores to locally improve the linkage map) were used to define linkage order and distances, with a minimum likelihood of odds (LOD) ≥ 3.0 used to test linkages. The identification of the best loci order by SARF confirmed the physical order of the InDel markers.

Candidate Gene Identification

Candidate genes were identified from the interval defined by the physical positions of the two InDel flanking the *Ur-6* locus in the F_2 genetic map. The putative functions of genes in this region, for both reference genotypes (G19833 and UI 111), were recorded along with potential

gene functions based on Pfam and PANTHER domains identified in Phytozome v13 and evaluated for relevance to plant pathogen defense (Goodstein et al. 2012; Schmutz et al. 2014; McClean 2020).

RESULTS

MDP Screening and GWAS

A total of 53 MDP genotypes were resistant to bean rust race 47 infection. Those genotypes are: 115M (Black Rhino), A285, A801, ABC-Weihing, ABCP-15, AC Ole, AC Pintoba, Agassiz, Apache, Arthur, BelNeb-RR-2, Beryl R, Black Magic, Burke, CDC Pinnacle, Coyne, Desert Rose, Domino, Fargo, Focus, GN9-1, I9365-25, ICB-10, ICB-12, Jackpot, Kimberly, Kodiak, Max, ND041062-1, NDZ06249, NE2-09-1, NE2-09-4, NE2-09-8, Neptune, Pink Floyd, PT7-2, PT9-17, Puebla 152, Quincy, Rojo Chiquito, S08418, SDPI-1, SEA 10, Shoshone, Swan Valley, TARS09-RR004, TARS-VCI-4B, Topaz, USPT-CBB-1, USPT-WM-1, USWA-50, Weihing, and Win Mor. The landrace G19833, the *P. Vulgaris* reference genome genotype, was also found to be resistant to rust race 47.

The GWAS was carried out with SNP data from 239 MDP genotypes. To prevent any interference from genotypes that displayed more intermediate phenotypes, only genotypes that were extremely resistant or extremely susceptible were included in the analysis (53 resistant & 86 susceptible). The phenotypic data for the GWAS was treated as binary data (resistant or susceptible). Race 47 resistance was mapped to the proximal end of Pv07 (Figure 2), with the peak SNP ($-\log_{10}(p) = 10.43$) located at 7,287,411 bp.

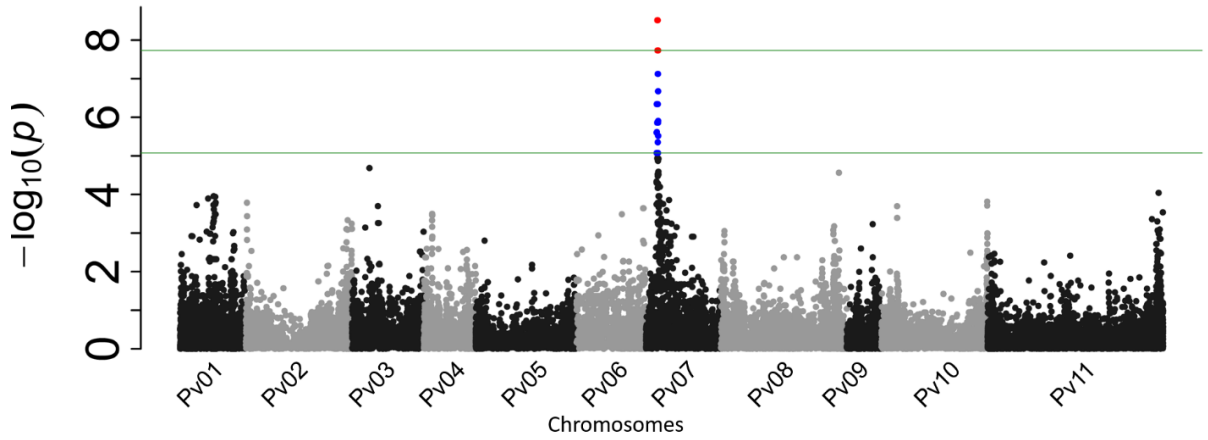

Figure 2. GWAS analysis of common bean Middle American Diversity response to bean rust race 47 infection. This analysis was carried out with 239 MDP genotypes and 152,470 SNPs with MAF ≥ 5%. The green lines represent the cutoffs for the top 0.1% and top 0.01% of SNPs, respectively.

Another GWAS was carried out with SNP data generated with MDP genotypes whose genotype did not match the expected phenotype. These lines may contain another gene(s) (*Ur-11*, *Ur-6+*, or others) that provides race 47 resistance/susceptibility independent of *Ur-6*. The pathogen response phenotype mapped to the proximal end of Pv07 (Figure 3), with the peak SNP ($-\log_{10}(p) = 17.55$) located at 7,275,718 bp.

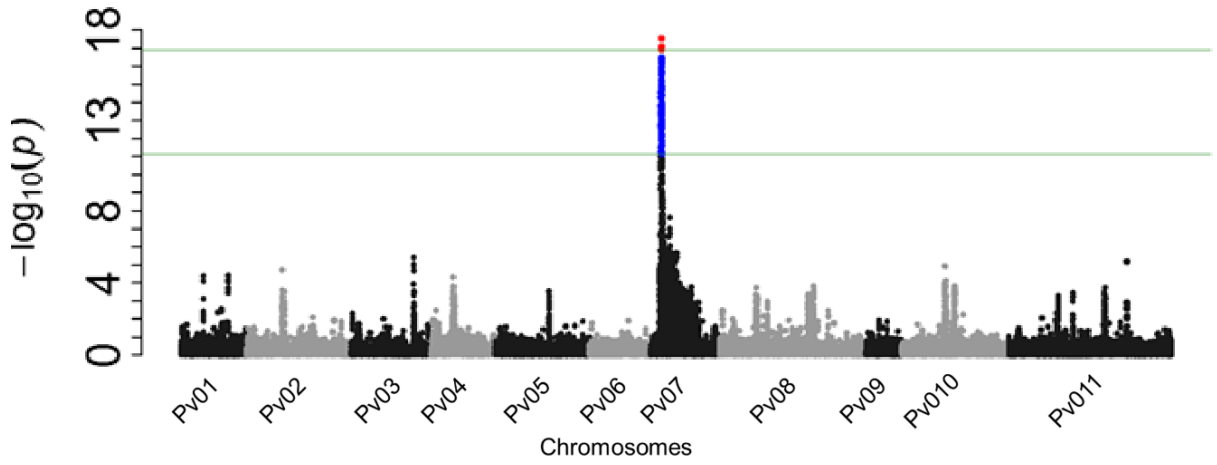

Figure 3. GWAS analysis of common bean Middle American Diversity response to bean rust race 47 infection, with genotypes containing *Ur-11* or *Ur-6+* removed. This analysis was carried out with 189 MDP genotypes and 152,470 SNPs with MAF ≥ 5%. The green lines represent the cutoffs for the top 0.1% and top 0.01% of SNPs, respectively.

F_2 Screening and Genetic Linkage Analysis

A total of 197 F_2 plants generated from the GGW × UI 114 cross were evaluated for their reaction to race 47 of *U. appendiculatus*. Of these F_2 plants, 131 were resistant and 60 were susceptible. This number fits the ratio of 3 resistant to 1 susceptible ($X^2 = 4.19$; *P* value = 0.0407).

The genomic locus identified by the MDP GWAS was targeted for InDel marker development. DNA was extracted for InDel screening from each F_2 plant. 20 markers polymorphic between the parents GGW and UI 114 were used to map the *Ur-6* locus in the F_2 population of 59 total InDel markers developed.

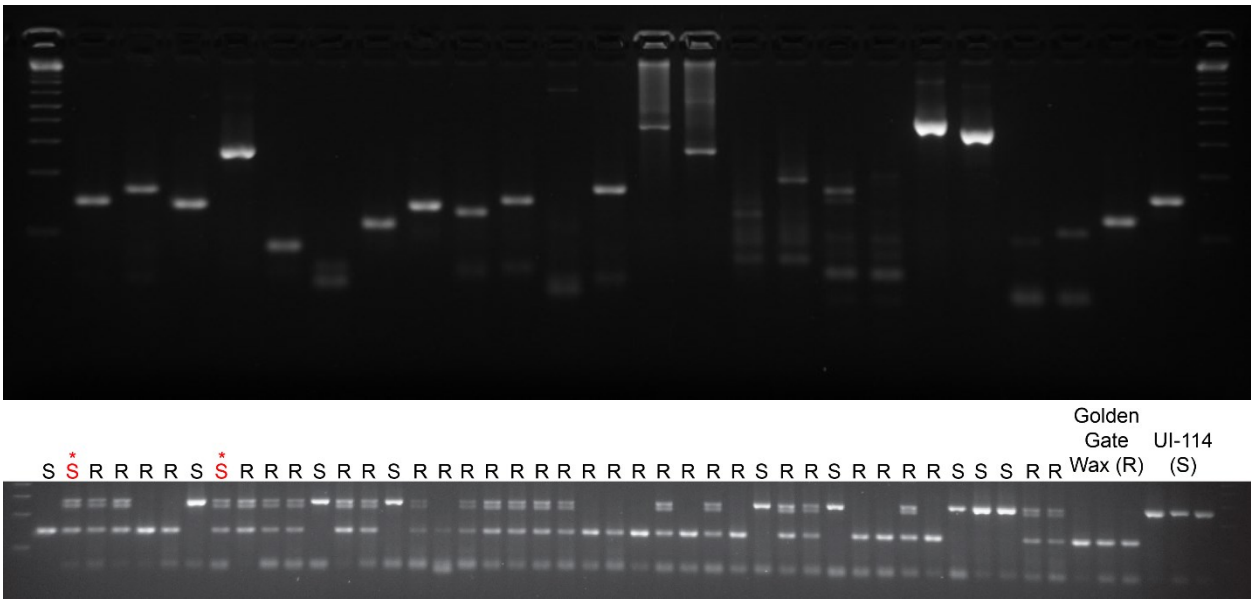

Figure 4. Polymorphic InDels identified between UI 114 and GGW A) Polymorphic InDels identified between UI 114 and GGW and then verified by PCR and agarose gel electrophoresis. B) Segregation pattern of marker Pv07_6966113 within the F_2 population. Lanes marked "S" and "R" contain genomic DNA from plants graded susceptible or resistant to race 47, respectively. The two lanes with red text and an asterisk contain genomic DNA from susceptible plants but have a heterozygous banding pattern for the marker, indicating recombination has occurred between the genetic marker and *Ur-6*.

Linkage analysis of the 20 InDel markers positioned the *Ur-6* locus between Pv07_6714298 (6,714,298 bp) and Pv07_6966113 (6,966,113 bp). Further, the linkage analysis has revealed one marker (Pv07_6963716) cosegregates with the *Ur-6* locus (Figure 5).

A graphical genotype map (Figure 6) was employed to visually analyze the marker data. Aberrant data, for improved analysis with genetic linkage software, such as very closely linked double crossovers or individual with genotypes scores that disagreed significantly from the other marker data for the individual, were converted to missing data. Using this cleaned data, the *Ur-6* locus was positioned between the indel markers Pv07_6714298 and Pv07_6963716.

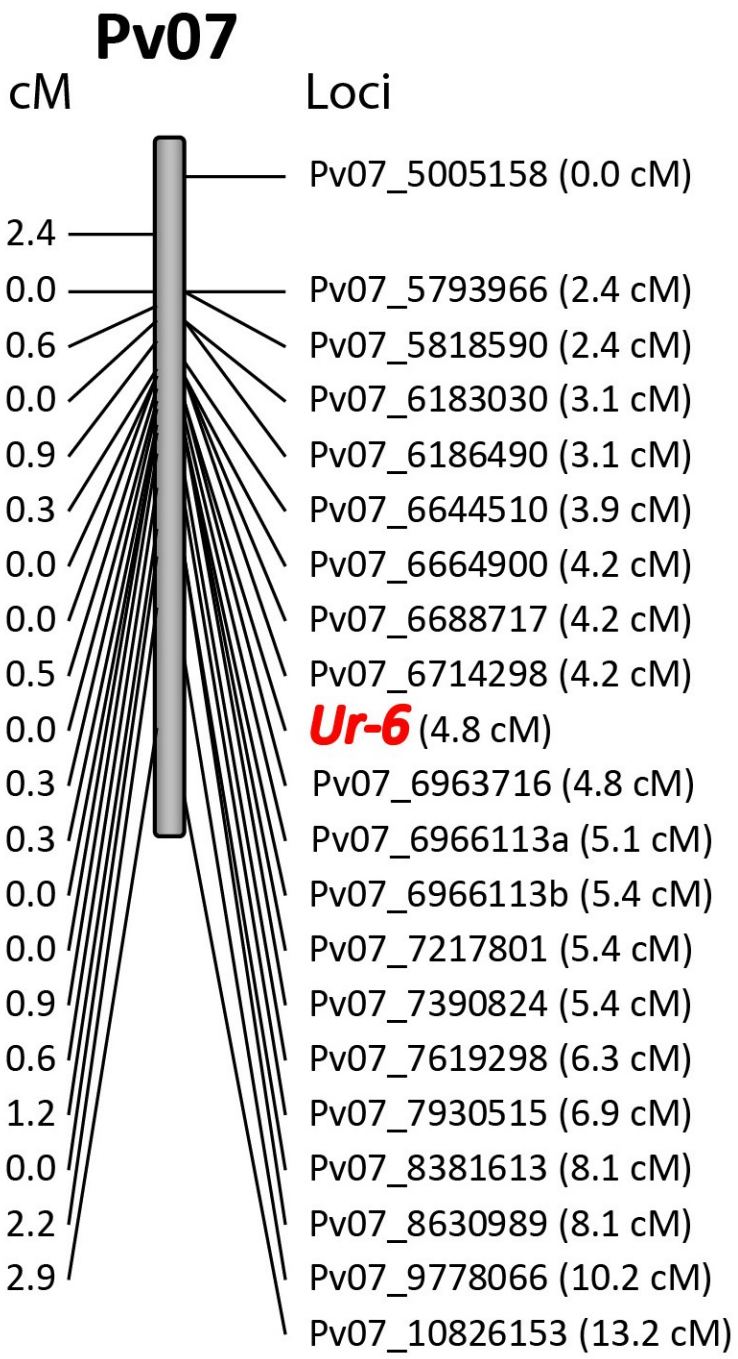

Figure 5. Genetic linkage map generated in MapDisto. Marker Pv07_6714298 cosegregates and marker Pv07_6963716 is closely linked with the *Ur-6* locus.

Marker\Individual	P1-A10	P1-A11	P1-B8	P1-C11	P1-D11	P1-E10	P1-E12	P1-H2	P2-B5	P2-B9	P2-D4	P3-A7	P3-A10	P3-B6	P3-B7	P3-B9	P3-D2	P3-D11	P3-E9	P3-E10	P3-F3	P3-G9
Pv07_5005158	B	H	B	B	H	H	B	H	B	B	B	B	B	B	B	B	H	H	H	B	B	H
Pv07_5793966	B	H	B	-	B	-	B	-	B	B	B	B	-	B	B	B	H	B	B	B	B	H
Pv07_5818590	B	H	B	B	B	-	B	H	B	B	B	B	B	B	B	B	H	B	-	B	B	H
Pv07_6183030	H	H	B	B	B	H	B	H	B	B	B	B	B	-	B	B	H	B	B	B	B	H
Pv07_6186490	H	H	B	B	B	H	B	H	B	B	B	B	B	B	B	B	H	B	B	B	B	H
Pv07_6644510	H	H	H	B	B	H	B	B	B	B	B	B	B	B	B	B	H	B	B	B	B	H
Pv07_6664900	H	H	H	B	B	H	B	B	B	B	B	B	B	B	B	B	H	B	B	B	B	H
Pv07_6688717	H	H	H	B	B	H	B	B	B	B	B	B	B	B	B	B	H	B	B	B	B	H
Pv07_6714298	H	H	H	B	B	H	B	B	B	B	B	B	B	B	B	B	H	B	B	B	B	H
Race 47 Response	D	D	D	B	B	D	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	D
Pv07_6963716	H	H	H	B	B	H	B	B	-	-	-	B	B	B	B	B	B	B	B	B	B	H
Pv07_6966113a	H	H	H	B	B	H	B	B	H	B	B	B	B	B	B	B	B	B	B	B	B	H
Pv07_6966113b	H	H	H	B	B	H	B	B	H	B	B	B	B	B	B	B	B	B	B	B	B	H
Pv07_7217801	H	H	H	B	B	H	-	B	H	B	B	B	B	B	B	B	B	B	B	B	B	H
Pv07_7390824	H	H	H	B	B	H	B	-	H	B	B	B	B	B	B	B	B	B	B	B	B	H
Pv07_7619298	H	B	H	B	B	H	B	B	H	H	B	B	B	B	B	B	B	B	B	B	B	H
Pv07_7930515	H	B	H	B	B	H	B	B	H	H	B	B	B	B	B	B	B	B	B	B	B	B
Pv07-8381613	H	B	H	B	B	B	B	B	H	H	B	B	B	B	B	B	B	B	B	B	H	B
Pv07_8630989	H	B	H	-	B	B	B	B	H	H	B	B	B	-	-	B	B	B	B	B	H	B
Pv07_9778066	H	B	H	H	H	B	H	B	H	H	B	H	H	B	B	B	B	B	B	B	H	B
Pv07_10826153	H	B	H	H	H	B	H	B	H	H	H	H	H	H	H	H	B	B	B	H	H	-

Figure 6. Graphical phenotyping of informative F_2 individuals. For the marker data, H = Heterozygous, B = UI 114/Susceptible, and D = Resistant. Individuals P2-B5 and P3-D2 are observed to possess crossover breakpoints on either side of the mapped.

To assess the broad applicability of the developed InDel markers and to analyze the haplotypes present in this region in key MDP genotypes, marker data was compared for the two reference genomes, Olathe, and the parents of the bi-parental population (Table 6).

Table 6. InDel haplotypes of race 47 resistant (G19833, GGW, Olathe) and susceptible (UI 111, UI 114) genotypes across the Pv07 region surrounding the mapped *Ur-6* locus. *Ur-6* cosegregates with marker Pv07_6963716.

	Genotype				
Indel marker	G19833	GGW	UI 114	UI 111	Olathe
Pv07_5005158	Ins	Ins	Del	Del	Del
Pv07_5793966	Ins	Ins	Del	Del	Del
Pv07_5818590	Ins	Ins	Del	Del	Del
Pv07_6183030	Del	Del	Ins	Ins	Ins
Pv07_6186490	Del	Del	Ins	Ins	Ins
Pv07_6644510	Ins	Ins	Del	Del	Del
Pv07_6664900	Del	Del	Ins	Ins	Ins
Pv07_6688717	Del	Del	Ins	Ins	Ins
Pv07_6714298	Ins	Ins	Del	Del	Del
Pv07_6963716	Del	Del	Ins	Ins	Ins
Pv07_6966113	Del	Del	Ins	Ins	Ins
Pv07_7217801	Del	Del	Ins	Ins	Ins
Pv07_7390824	Ins	Ins	Del	Del	Del
Pv07_7619298	Ins	Ins	Del	Del	Del
Pv07_7930515	Del	Del	Ins	Ins	Ins
Pv07_8381613	Del	Del	Ins	Ins	Ins
Pv07_8630989	Del	Ins	Del	Del	Del
Pv07_9778066	Ins	Del	Ins	Ins	Ins
Pv07_10826153	Ins	Ins	Del	Del	Del

Candidate Genes

Using the region delineated by indel markers Pv07_6714298 and Pv07_6963716, 25 candidate genes were identified in the reference genome assembly (v2.1) of G19833 (Table 7). The orthologs of these protein sequences in the UI 111 reference genome were compared, the sequences differences are noted in Table 7. The genomic scaffolds developed from sequencing a 10X library of UI 114 were searched, and the UI 114 orthologs to the G19833 were discovered. The sequences of the UI 114 and UI 111 orthologs were identical. This was expected since UI 111 is one of the two parents of UI 114.

Table 7. Observed differences in the translated amino acid sequences for the candidate genes between the G19833 and UI 111 reference genomes.

G19833 Gene Models	UI 111 Gene Models	Differences Observed
Phvul.007G071900.1	PvUI111.07G072800.1	No Differences
Phvul.007G072000.1	PvUI111.07G072900.1	4 Residues Changed
Phvul.007G072100.1	PvUI111.07G073000.1	No Differences
Phvul.007G072200.1	PvUI111.07G073100.1	5 Residues Changed
Phvul.007G072300.1	PvUI111.07G073200.1	No Differences
Phvul.007G072400.1	PvUI111.07G073300.1	No Differences
Phvul.007G072500.1	PvUI111.07G073400.1	No Differences
Phvul.007G072600.1	PvUI111.07G073500.1	2 Residues Changed
Phvul.007G072700.5	PvUI111.07G073600.1	2 Residues Changed
Phvul.007G072801.1	PvUI111.07G073700.1	1 Residue Changed
Phvul.007G072900.1	PvUI111.07G073800.1	No Differences
Phvul.007G073000.3	PvUI111.07G073900.1	No Differences
Phvul.007G073100.1	PvUI111.07G074000.1	No Differences
Phvul.007G073200.1	PvUI111.07G074100.1	No Differences
Phvul.007G073300.1	PvUI111.07G074200.1	1 Residue Changed
Phvul.007G073400.1	PvUI111.07G074300.1	No Differences
Phvul.007G073500.1	PvUI111.07G074400.1	6 Residues Changed
Phvul.007G073600.1	PvUI111.07G074500.1	3 Residues Changed
Phvul.007G073700.1	-	Unable to compare - gene likely only present in G19833
Phvul.007G073800.1	PvUI111.07G074600.1	3 Residues Changed
Phvul.007G073900.1	PvUI111.07G074700.1	19 AA Insertion; 1 Residue Changed
Phvul.007G074000.2	PvUI111.07G074800.1	2 Residues Changed
Phvul.007G074100.1	PvUI111.07G074900.1	3 Residues Changed
Phvul.007G074200.2	PvUI111.07G075000.1	No Differences
Phvul.007G074300.1	PvUI111.07G075100.1	183 AA Deletion; 2 Residues Changed

Prediction of gene structure and function was made based on potential PANTHER and Pfam domains found in Phytozome v13 (Table 8) and used to assess the likelihood of genes being involved in plant pathogen defense responses. These analyses suggested that Phvul.007G072700, Phvul.007G072801, Phvul.007G074000, Phvul.007G074100, and Phvul.007G074300 were the most likely candidate genes for *Ur-6*.

Table 8. Predicted gene functions for candidate genes.

G19833 V2.1 Gene Name	UI 111 V1.1 Gene Name	arabi-define	Likely Function
Phvul.007G071900	PvUI111.07G072800	NIN like protein 7	NLP7 / NIN-like protein 7
Phvul.007G072000	PvUI111.07G072900		oxidoreductase/transition metal ion-binding protein
Phvul.007G072100	PvUI111.07G073000	Ribosomal protein L36e family protein	Ribosomal protein L36e family protein
Phvul.007G072200	PvUI111.07G073100		ATP-binding, maybe myosin
Phvul.007G072300	PvUI111.07G073200	U-box domain-containing protein kinase family protein	U-box domain-containing protein kinase family protein / Both ubiquinase and kinase / Possbile lectin
Phvul.007G072400	PvUI111.07G073300	C2H2-like zinc finger protein	C2H2 ZFP - abiotic stress response
Phvul.007G072500	PvUI111.07G073400		Unknown, could be chromatid maintenance, transcription factor, or involved in respiration as electron acceptor
Phvul.007G072600	PvUI111.07G073500	NAD(P)-binding Rossmann-fold superfamily protein	Electron acceptor in Mitochondria
Phvul.007G072700	PvUI111.07G073600	UB-like protease 1B	Protease
Phvul.007G072801	PvUI111.07G073700	Protein kinase superfamily protein	MAPKKK
Phvul.007G072900	PvUI111.07G073800	Alpha-L RNA-binding motif/Ribosomal protein S4 family protein	Ribosomal protein
Phvul.007G073000	PvUI111.07G073900	Divalent ion symporter	Arscenic or Sodium pump - likely for abiotic stress
Phvul.007G073100	PvUI111.07G074000	hydroxyproline-rich glycoprotein family protein	Hydroxyproline-Rich Glycoprotein - defence against fungi
Phvul.007G073200	PvUI111.07G074100	Bacterial sec-independent translocation protein mttA/Hcf106	Thylaokoid membrane formation
Phvul.007G073300	PvUI111.07G074200	Methyltransferase MT-A70 family protein	Methyltransferase - possibly a transcription factor for karyogamy
Phvul.007G073400	PvUI111.07G074300	detoxifying efflux carrier 35	MATE transporter - more likely for abiotic stress than biotic
Phvul.007G073500	PvUI111.07G074400	SCARECROW-like 8	promotes growth, gibberilic acid sensitive
Phvul.007G073600	PvUI111.07G074500		Unknown, possibly Arm protein or some sort of enzyme?
Phvul.007G073700	-	vacuolar ATP synthase catalytic subunit-related / V-ATPase-related / vacuolar proton pump-related	Proton pump, but possibly kinase or protease?
Phvul.007G073800	PvUI111.07G074600	ferric reduction oxidase 2	abiotic stress response
Phvul.007G073900	PvUI111.07G074700	ferric reduction oxidase 4	abiotic stress response
Phvul.007G074000	PvUI111.07G074800	Protein kinase superfamily protein	kinase
Phvul.007G074100	PvUI111.07G074900	Poly (ADP-ribose) glycohydrolase (PARG)	PARG - possible response to pathogens
Phvul.007G074200	PvUI111.07G075000	beta-hydroxylase 1	chloroplast enzyme
Phvul.007G074300	PvUI111.07G075100	Pectin lyase-like superfamily protein	Pectin lyase - possible disease resistance

DISCUSSION

Mapping the *Ur-6* locus

(Stavely 1984) and (Stavely et al. 1989) discovered that GGW conferred resistance 14 of 27 races of the bean rust pathogen. All of the resistant responses exhibited a hypersensitive response with a 2 or 2^+ score. This resistance response in GGW has historically been assigned to the *Ur-6* gene. The same screening response discovered that Olathe also provide hypersensitive resistance to the same 14 races, as well as hypersensitive resistance to race 39. In addition, the pinto cultivar Olathe exhibited intermediate, small pustule resistance (score = 3) to five races in which GGW exhibited larger pustules (score = 4-5). When the new bean rust differential set was developed in 2002, Olathe was dropped since it was considered to have essentially the same resistance response. The Olathe resistance response was then assigned the symbol *Ur*-6+, and it was generally thought at the time that it was an allele of the GGW *Ur-6* gene and designated *Ur-6*+. Subsequent mapping efforts used Olathe as a source of *Ur-6* resistance, and the gene was assigned to chromosome Pv11 (Park et al. 2004). Using the limited mapping data available at the time (McClean et al. 1994), it was determined that the Olathe source of *Ur-6* maps to what is now named Pv11 (Miklas et al. 2002). *Ur-3*, a bean rust resistance gene with broad race specificity, was mapped to a narrow interval on Pv11 (Hurtado-Gonzales et al. 2017) in the NLR cluster at the end of the chromosome. Since *U-3* mapped at a distance >50 cM than the Olathe *Ur-6*+ gene, it is thought that *Ur-6*+ must be located elsewhere on Pv11. Therefore, while the initial goal of the research was to map the GGW-derived *Ur-6* gene, determining its relationship to *Ur-6*+ would be of interest if suitable data could be derived.

To map the GGW *Ur-6* gene, we utilized previously generated SNP data, and phenotypic data for the bean rust race 47 resistance response of the MDP to initially located *Ur-6* using association genetics. A single SNP peak was located on chromosome Pv07 at position 7,287,411.

The GWAS results confirm that the rust race 47 resistance is a qualitative trait controlled by a single gene. The presence of a single strong GWAS peak with a precipitous drop in signal strength as SNPs are further from the peak SNP is the most obvious sign of a qualitative trait. If rust race 47 resistance were not controlled by a single qualitative trait, multiple peaks with higher *p*-value would be observed (Oladzad et al. 2019a).

Using the interval around the peak SNP, InDel markers polymorphic between GGW and UI 114 between positions 5,005,158 and 10,826,415 were identified, and PCR primers were designed to amplify polymorphic fragments between the parents and members of a GGW x UI 114 F_2 population. The F_2 polymorphism data was used to generate a genetic linkage map that placed for *Ur-6* in a genomic region of 247,289 bp between InDel markers Pv07_6714298 and Pv07_963716. A total of 25 candidate genes were identified within this 247 kb region. An ortholog of one of the G19833 genes, *Phvul.007G076700*, was absent from UI 111.

The genomic region was defined based on recombination breakpoints for the two InDel markers in two members of the F_2 population. Because there is only a single rust resistance gene, *Ur-6*, present in the F_2 population, this mapping process is protected from the confounding factors of other rust resistance genes that may exert epistatic effects. This population possesses the potential to be used for further screening, as more markers may be developed in this genomic locus to screen the F_3 families derived from the two informative F_2 plants (P2-B5 and P3-D2).

The MDP was subsequently screened with the two markers (Pv07_6714298 and Pv07_963716) that mapped closest to *Ur-6* to determine the haplotype within this narrow region.

A total of 166 genotypes susceptible to race 47 also had the susceptible haplotype, and 36 genotypes resistant to the race expressed the resistant haplotype. In addition, 20 resistant genotypes had the susceptible haplotype. This suggests that another gene(s) is present in these resistant genotypes other than the GGW derived *Ur-6* gene that is providing resistance. There was no clear evidence from a pedigree analysis and registration data regarding the rust resistance of these genotypes as to the source of that resistance. In addition, there were 20 genotypes that were susceptible to the race 47 that contained the resistant haplotype. Again, the reason for this is not clear, and awaits more in-depth haplotype analysis determined if a recombination event occurred that broke the linkage between the resistant markers and the dominant *Ur-6* resistance allele. A number of the MDP genotypes were known to possess race 47 resistance from Olathe.

Olathe, the source of *Ur-6*+ gene, was determined to have the susceptible haplotype found in UI 114 across the entire mapped 5.8 Mb *Ur-6* region. This strongly suggests, based on the haplotype analysis, that *Ur-6* and *Ur-6*+ are not allelic but rather two different genes that provide resistance to race 47. The determination of whether *Ur-6* and *Ur-6*+ are alleles or separate genes may be accomplished by generating an F_2 population from a cross of Golden Gate Wax (*Ur-6*) and Olathe (*Ur-6*+) and screening with bean rust race 47. If *Ur-6* and *Ur-6*+ are alleles, one would expect to observe resistance among the entirety of the F_2 population, though it is theoretically possible for there to be a 1:1 ratio of resistant to susceptible in the population if the effect of underdominance were to occur. Alternatively, if *Ur-6* and *Ur-6*+ are independently assorting genes, one would expect to observe a 15:1 ratio of resistant to susceptible phenotypes in the F_2 population. However, alternative ratios derived from the classical 9:3:3:1 ratio observed in dihybrid crosses may be observed if there are epistatic interactions between *Ur-6* and *Ur-6*+.

Bearing in mind the issues posed by the *Ur-6+* resistance gene conferred by Olathe, it is important to ensure that the *Ur-6* mapping efforts are not compromised by the Olathe bean rust resistance gene. The similarity of the marker scores between Olathe and UI 114, along with the dissimilarity of the marker scores between Olathe and GGW, confirms the markers in linkage disequilibrium with the phenotype being mapped in the bi-parental population are not mapping *Ur-6+*.

Putative Candidate Genes

Among the 25 candidate genes within the interval that defines the *Ur-6* locus, five were considered to be likely candidates based on their putative involvement in the plant disease response and possessing changes to the protein sequences that were polymorphic between resistant and susceptible genotypes. These genes are: *Phvul.007G072700*, *Phvul.007G072801*, *Phvul.007G074000*, *Phvul.007G074100*, and *Phvul.007G074300*.

Phvul.007G072700 is a G19883 (resistant parent) protease that differs from its UI 114 (susceptible parent) ortholog PvUI111.07G073600 by two amino acid residues. At residue 158, the serine present in G19833 is cysteine in UI 114. This change in amino acid is present in the middle of the C-terminal catalytic domain of the Ulp1 protease family. At residue 302, the glycine present in G19833 is arginine in UI 114, a change from a hydrophobic amino acid to a charged one. This change is present in a region annotated as a sentrin/SUMO-specific protease domain.

SUMO (small ubiquitin-like modifier) proteases like Phvul.007G072700 have been associated with plant pathogen identification and defense responses (Sadanandom and Morrell 2019). Plant pathogens have been found to employ effector proteins capable of deSUMOylating target proteins in plant cells, preventing plant SUMO proteases from targeting the effected

proteins and thereby preventing the elicitation of a hypersensitive response from the plant (Kim et al. 2013; Orth et al. 2000). Further, SUMO proteases have been found to have a role in PAMP detection, where the detection of a PAMP in *Arabidopsis* triggered the degradation of a key SUMO protease, allowing for the increased SUMOylation of a receptor protein, triggering a signaling cascade that induces a pathogen defense response (Orosa et al. 2018). This role would suggest that Phvul.007G072700 would have a role in a direct extracellular pathogen perception mechanism.

The G19833 protein Phvul.007G072801 is a kinase that differs from its UI 114 ortholog PvUI111.07G073700 by one amino acid residue. At residue 200, the serine present in G 19833 is threonine in UI 114. This alteration occurs outside the protein kinase domain identified in the protein.

The G19833 protein Phvul.007G074000 is a kinase that differs from its UI 114 ortholog PvUI111.07G074800 by two amino acid residues. At residue 24, the threonine present in G19833 is serine in UI 114. This alteration occurs outside of the protein kinase domain identified in the protein. At residue 30, the phenylalanine present in G 19833 is leucine in UI 114. This alteration occurs outside of the protein kinase domain identified in the protein.

Kinases are involved in many types of intracellular signaling cascades and can therefore be involved in eliciting a plant pathogen defense response in many ways. Kinase-activated signaling pathways have been found to act upstream of salicylic acid synthesis and pathogen defense (Rasmussen et al. 2012; Tang et al. 2005; Asai et al. 2002). Further, kinase signaling cascades have been found to connect chitin-activated pattern-recognition receptors to the production of reactive oxygen species (ROS) as an immune response in both rice and *Arabidopsis* (Kawasaki et al. 2017). Due to the role of kinases in signaling cascades, it is

possible for these proteins to be involved in many different mechanisms of active plant pathogen resistance

The G19833 protein Phvul.007G074100 is a Poly (ADP-ribose) glycohydrolase (PARG) protein that differs from its UI 114 ortholog Phvul.007G074100 by three amino acid residues. At residue 41, the leucine present in G19833 is valine in UI 111. This alteration occurs within the PARG domain identified in the protein. At residue 232, leucine present in G19833 is tryptophan in UI 114. This change from a hydrophobic residue to an amphipathic residue also occurs within the PARG domain. And in addition, at residue 518 in the PARG domain the serine present in G19833 is converted to glycine in UI 111. This changes a polar residue to a hydrophobic residue.

PARGs are involved in a wide variety of plant cellular processes, but most pertinent to the current discussion is their role in inducing programmed cell death to effect a hypersensitive response (D'Silva et al. 1998). Further, PARGs are capable of regulating gene expression in plants, and multiple *Arabidopsis* PARGs have been found to regulate the expression of pathogen defense genes. Knockout-mutants of these PARGs having increased pathogen susceptibility (Feng et al. 2015). If Phvul.007G074100 were the *Ur-6* gene, this would indicate that *Ur-6* most likely functions through a mechanism based on the passive loss of susceptibility by host reprogramming.

The G19833 protein Phvul.007G074300 is a pectin lyase with multiple polymorphisms relative to its UI 114 ortholog PvUI111.07G075100. At residue 262, the glycine present in G19833 is arginine in UI 114. This change from a hydrophobic residue to a charged residue occurs within the pectin lyase domain and immediately next to a parallel beta-helix repeat. At residue 17, the serine present in G19833 is arginine UI 114. The most significant polymorphism is a five amino acid insertion in susceptible UI 114 PvUI111.07G075100 protein between

residues 29 and 30 of Phvul.007G074300. This insertion is 15 amino acids upstream of the pectate_lyase_3 PF12708 domain. At residue 70, the arginine present in G19833 is serine in UI 114. At residue 74, the glutamine present in G19833 is serine in UI 114, a change which is not consistent with the Hidden Markov Model for this domain. These polymorphisms are in the pectate_lyase_3 domain. Elsewhere in the protein, at residue 283, the threonine present in G19833 is alanine in UI 114. This change from a polar residue to a hydrophobic residue occurs within the protein's identified glyco_hydro_28 PF00295 domain.

Typically when discussing plant pathogen defense, pectin lyases are thought of as virulence agents employed by pathogens to degrade plant cell walls (Barras et al. 1994). However, pectin lyases have been found to be expressed with protein kinases linked to signaling cascades for plant pathogen defense responses in *Arabidopsis* (Chinchilla et al. 2007). In Arabidopsis, a pectin lyase-like gene, *PMR6*, acts as a susceptibility factor, and the mutant version of this gene provides recessive resistance (Vogel et al. 2002). The mutant allele is suggested to minimize cell wall breakdown which in turn results in a thicker cell wall that prevents the pathogen from establishing a physical presence in the host. It may be the case that the reduced activity of the Phvul.007G074300 resistant allele, may result in a thicker cell. This is consistent with the very low gene expression level of this gene based on the FPKM level in young unifoliate and trifoliate leaves (https://phytozome-next.jgi.doe.gov/info/Pvulgaris_v2_1).

Identification of *Ur*-6 InDel Marker

In screening the F_2 population with InDel markers, the marker Pv07_6963716 was completely linked with the *Ur-6* phenotype. Presently, the only issue faced with implementing this marker into a breeding program is the potential for conflict with *Ur-6+* in breeding populations. This issue can be ameliorated by ensuring that the bean rust race 47 resistance is

indeed from a *Ur-6* source and not another gene, such as *Ur-6+* which also provides race 47 resistance.

CONCLUSIONS

This study used a combined a GWAS in the Middle American Diversity Panel and screening an F_2 population to identify the indel marker Pv07_6963716 marker that cosegregates with the *Ur-6* bean rust resistance gene in common bean. The *Ur-6* gene was mapped to a 251.5 kb interval on chromosome Pv07. The validation of this marker on 277 genotypes in the Middle American Diversity panel revealed that the marker is accurate, but the potential for usage as a marker is affected by the presence of *Ur-6*+ in the Middle American gene pool. In this screening, it was revealed that landrace G19833, the genotype used for the primary common bean reference genome, possesses the dominant resistance *Ur-6* allele, and that UI 111, the genotype used for the newly published pinto reference genome, possesses the recessive *Ur*-6 allele. This marker could be of use for combining *Ur-6* with other bean rust resistance genes. The *Ur-6* locus contains 25 potential candidate genes, five of which have been identified as being likely candidates due to their previously describe involvement in plant pathogen defense and the presence of polymorphisms between resistant and susceptible genotypes.

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APPENDIX

Table A1. Average bean rust race 47 reaction scores for MDP genotypes.

MDP Entry #	Variety	Avg. Score
1	BelMiNeb-RR-2	2+ , 2
2	BelMiNeb-RMR-3	N/A
3	BelDakMi-RR-5	N/A
5	BelMiNeb-RMR-7	N/A
6	BelMiNeb-RMR-4	N/A
7	BelNeb-RR-1	N/A
8	BelMiNeb-RMR-8	N/A
9	AC Redbond	N/A
10	AC Black Diamond	N/A
11	AC Island	N/A
12	AC Early Rose	N/A
13	AC Polaris	N/A
14	AC Resolute	N/A
15	AC Earlired	N/A
16	Bill Z	N/A
17	Ouray	N/A
18	Grand Mesa	N/A
19	Fisher	N/A
20	Montrose	N/A
21	Olathe	N/A
22	Shiny Crow	N/A
23	San Juan	N/A
24	Croissant	N/A
25	Arapaho	N/A
26	DOR 364	N/A
27	Xan 176	N/A
28	PR 0340-3-3-1	N/A
29	Amadeus 77	N/A
30	Morales	N/A
31	Verano	N/A
32	DPC-4	N/A
33	PR 0443-151	N/A
35	BTS	N/A
37	IBC 301-204	N/A
38	CENTA Pupil	N/A
39	INTA Precoz	N/A
40	Dehoro	5, 4, 3
41	Aifi Wuriti	2+, 4, 3
42	TARS09-RR004	2+, 2
43	TARS09-RR007	4, 3
44	TARS09-RR023	2+, 2++

Table A1. Average bean rust race 47 reaction scores for MDP genotypes (continued).

MDP Entry #	Variety	Avg. Score
45	TARS09-RR029	4, 3
47	F07-004-9-1	4
48	F07-449-9-3	4
49	F07-014-22-2	4
50	F04-2801-4-6-6	4, 5
51	F04-2801-4-5-1	4, 5
52	I06-2575-17	4, 5
53	F04-2801-4-1-2	4
54	Michelite	4
55	Sanilac	4,5
56	Seafarer	4
60	Swan Valley	2+
61	Neptune	2+
62	Domino	2+, 2
63	Black Magic	1
65	Bunsi	2+, 2++
66	C-20	4
67	Laker	2+, 4
68	Mayflower	4, 5, 2
69	Blackhawk	5, 4
70	Sierra	4, 5
73	Aztec	4, 2+
74	Huron	4
75	Raven	4
77	Newport	4
78	Mackinac	5, 4
79	Kodiak	2+
80	Matterhorn	5, 4
84	Phantom	4, 5
85	Jaguar	5, 4
86	Seahawk	4
87	Condor	5, 4
88	Zorro	4, 5
89	Santa Fe	4
90	B05055	5, 4
91	P07863	5, 6
92	T-39	4
93	Merlot	4, 5
94	Sedona	4
96	Cornell 49-242	4
97	N05324	No Plant
99	S08418	2+, 2++
104	115M (Black Rhino)	2, 2+

Table A1. Average bean rust race 47 reaction scores for MDP genotypes (continued).

MDP Entry #	Variety	Avg. Score
106	Puebla 152	3, 4
109	Poncho	5, 6, 4
110	Topaz	2+
111	Buckskin	5, 4, 6
112	Flint	5, 4
113	Fargo	2+, 4, 2++
114	Agassiz	5, 4
115	Remington	2+
120	La Paz	5, 4
121	Baja	N/A
122	Durango	4, 5, 2+
123	Sonora	4, 5, 2+
124	Shania	N/A
125	Bandit	5
126	Loreto	5
127	Schooner	5
128	Ensign	5 , 2+ , 4
129	Voyager	4 , 2+
130	Seabiskit	1
131	Pink Floyd	2+
132	Red Ryder	5 , 4
133	Medalist	5 , 4
134	Navigator	4 , 5
135	Ivory	5 , 4
136	Beryl	4 , 5
137	Beryl R	2+
138	Marquis	4
139	Sapphire	5 , 4
140	Ember	5 , 6
141	Garnet	5
142	ROG 312	4
143	Desert Rose	2+
145	Midnight	1
146	Black Knight	1
156	SDPI-1	1
157	Shoshone	1
158	UI-3	5 , 4
159	UI-37	5 , 4
160	UI-537	5
161	Common Pinto	5 , 4
162	Common Red Mexican	5
163	IP08-2	5 , 6

Table A1. Average bean rust race 47 reaction scores for MDP genotypes (continued).

MDP Entry #	Variety	Avg. Score
164	Kimberly	2+ , 4 , 3
165	Sawtooth	4 , 3
166	UI-123	5 , 4
167	UI-126	4 , 5
168	UI-196	4 , 5
169	UI-228	5 , 4
170	UI-239	5 , 4
172	UI-906	4 , 5
173	UI-911	5 , 4
174	US-1140	4 , 3
176	UI-59	N/A
177	UI-111	4 , 3 , 5
178	UI-114	N/A
179	UI-425	4 , 3
180	BelNeb-RR-2	2+ , 3
181	BelMiNeb-RR-1	4 , 3 , 2+
182	BelMiNeb-RR-2	2+ , 2
184	BelMiNeb-RMR-5	2+
185	GN#1Sel27	N/A
186	GN Harris	4 , 5 , 3
187	GN Star	4 , 5 , 3
189	Tara	N/A
190	Starlight	4 , 3
191	Emerson	4 , 5
192	Weihing	2+ , 4
193	ABC-Weihing	2+ , 4
194	Coyne	2+ , 4
195	ABCP-8	3 , 2+ , 4
196	Chase	3 , 4 , 2
197	ABCP-15	2+ , 4 , 3
198	ABCP-17	3 , 2 , 4
199	NE1-09-13	4 , 3
200	NE1-09-19	4
201	NE1-09-20	4 , 3
202	NE1-09-22	4 , 3 , 2+
203	NE2-09-1	2+
204	NE2-09-3	2+
205	NE2-09-4	2+ , 2
206	NE2-09-8	2+ , 2
207	NE2-09-10	4 , 3
208	NE1-09-9	4
209	AC Pintoba	2+ , 4

Table A1. Average bean rust race 47 reaction scores for MDP genotypes (continued).

MDP Entry #	Variety	Avg. Score
210	AC Ole	2+ , 4
211	Win Mor	2+ , 4
212	AC Scarlet	4 , 3
213	Morden 003	4 , 3
214	ICB-12	2+ , 4 , 3
215	A-55	4 , 3
216	I9365-31	3
217	92BG-7	3 , 4
218	ICB-10	2+
219	ICB-3	4
220	JM-24	4 , 3
221	USWA-12	4 , 3
222	Quincy	2+ , 4
223	Burke	2+
224	TARS-VCI-4B	2+
225	JM-126	N/A
227	Pindak	4 , 3
228	Nodak	4 , 3
229	Holberg	4 , 3
230	92US-1006	4 , 3
231	Othello	No Plant
232	NW-590	4 , 3
233	NW-410	4 , 3
234	PT7-2	2+ , 4
235	USPT-WM-1	2+ , 4
236	USPT-CBB-1	2
237	USPT-CBB-3	4 , 3 , 2+
238	USPT-ANT-1	4
239	USPT-CBB-5	4 , 3
240	Big Bend	5 , 4 , 6
241	Le Baron	5 , 4 , 6
242	NW-63	5 , 4 , 6
243	USRM-20	4 , 5
244	Coulee	5 , 6
257	USWA-13	5 , 4
258	NW-395	4 , 5
259	Hyden	4 , 5
260	USWA-50	2+ , 2
266	6R-42	4 , 5 , 6
267	Victor	5 , 4
268	USWA-61	4 , 5
269	I9365-25	2+ , 4

Table A1. Average bean rust race 47 reaction scores for MDP genotypes (continued).

MDP Entry #	Variety	Avg. Score
270	I9365-5	4
271	Rojo Chiquito	2+
272	Indeterminate Jamaica Red	4 , 3
273	Orca	4 , 5
278	Viva	4 , 5
279	Roza	4 , 5 , 6
280	Harold	4 , 5
281	Gloria	4 , 5
282	URS-117	4 , 5
286	A285	2
287	A801	2+ , 2
290	BAT 477	4 , 2+
291	SEA 10	2+ , 4
292	PK9-7	4 , 2+
293	PK915	4 , 5 , 6
294	SR7-3	5 , 4
295	SR9-4	4 , 2+
296	GN9-4	4 , 2+
297	GN9-1	2+ , 4
298	PT9-17	2+
299	Maverick	3 , 4 , 2+
300	Lariat	3 , 2+ , 4
301	Stampede	4
302	ND-307	4 , 2+
303	Frontier	N/A
304	Arthur	2+ , 4
305	Norstar	N/A
306	Avalanche	4
307	Eclipse	4 , 3
308	NDZ06249	2+
309	ND040494-4	4
310	ND021717	4
311	ND021574	4
312	ND041062-1	2+
314	ND060197	2+ , 4 , 3
316	Hatton	4
317	Crestwood	4
319	Reliant	4
320	Vista	4
321	Envoy	4
322	Blackjack	4
323	GTS-900	4

Table A1. Average bean rust race 47 reaction scores for MDP genotypes (continued).

MDP Entry #	Variety	Avg. Score
324	CDC Nighthawk	4
325	CDCWM-2	N/A
326	CDC Pinnacle	2+ , 4
327	CDC Whitecap	4
328	CDC Nordic	4
329	CDC Crocus	5 , 4
330	CDC Pintium	2+ , 4
331	CDC Expresso	4 , 5
332	CDC Jet	4 , 5
333	CDC Rosalee	4
334	CDC Camino	5 , 4
336	OAC Rex	4
339	Nautica	4 , 5
341	Fleetwood	4 , 2+ , 5
342	OAC Laser	4
343	OAC Gryphon	4
346	Lighthing	4 , 2+
349	Harrowhawk	4
350	AC Harblack	4
352	OAC Seaforth	4
353	AC Compass	4 , 2+
354	T9905	4 , 2+
355	T9903	4
356	HY 4181	4
357	Gemini	4
358	Orion	4 , 5
372	UCD 96114	4
373	UCD 9634	4 , 5
374	UCD 9623	4
375	Yolano	4
379	Max	2+ , 4
380	Jackpot	2+, 4
381	Gala	4 , 5
382	Sequoia	2+
383	Apache	2+
384	Fiesta	4 , 5
385	Bighorn	4
386	Buster	4 , 2+
387	Medicine Hat	2+ , 4
388	Windbreaker	4
389	Mariah	4
390	Focus	4 , 2+

Table A1. Average bean rust race 47 reaction scores for MDP genotypes (continued).

MDP Entry #	Variety	Avg. Score
391	Vision	4
392	Albion	4
393	Avanti	4
394	Midland	4
395	Black Velvet	4
403	McHale	4
J.K	Powderhorn	N/A
J.K	P12-606	N/A
P.M.	PK9-1	N/A
P.M.	3138	N/A
P.M.	Gypsy Rose(R11806)	N/A
P.M.	Longs Peak	N/A
P.M.	PT11-13	N/A
P.M.	PT9-22	N/A
P.M.	PT9-5-6	N/A
P.M.	R11801	N/A
R.S.	Aries	N/A
R.S.	Monterrey	N/A
R.S.	Santa Cruz	N/A
R.S.	Sinaloa	N/A
R.S.	Galeena	N/A
S.S.	Hungerford	N/A