POPULATION STRUCTURE OF *UROMYCES APPENDICULATUS* IN NORTH DAKOTA

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

North Dakota is the lead producing state of common bean in the USA, accounting for 43% of national production. Bean rust is caused by the autoecious, macrocyclic fungus *Uromyces appendiculatus* (Pers.:Pers.). All pathogen spores stages have been observed in North Dakota, but it is unclear how frequently sexual reproduction occurs. Traditionally, genetic resistance is the preferred management method. Sixty-seven percent of the 119 *U. appendiculatus* single pustule isolates collected in 2015 and 2016 were classified phenotypically as race 20-3. Virulence phenotypes of race 20-3 isolates ranged from hypersensitive to small pustules (0.2-0.3mm) on Early Gallatin, PC-50, Mexico 235, and Mexico 325 rust differential lines. This variation suggests more pathogen diversity is present than is discernable via traditional race classifications. The remaining 33% of *U. appendiculatus* isolates belonged to 18 additional races. Genome Wide Association Studies were conducted with the advanced breeding lines from the NDSU bean breeding program, the Middle American diversity panel, and a subset of Andean diversity panel reaction to races 20-3, 29-3 and 27-7. Significant SNP markers on chromosomes Pv01, Pv04, Pv06, Pv08, Pv10, and Pv11 were identified using genome wide association mapping. RAD-GBS was performed on 84 single pustule *U. appendiculatus* isolates using the Ion-Torrent S5 sequencing platform. A *de novo* assembly was performed on a single isolate of race 20-3 to generate reference sequence tags for variant calling. The relatedness measure using an identity by state (IBS) matrix suggested the presence of diversity within and among the isolates belonging to the same race, providing further evidence that the *U. appendiculatus* population in North Dakota is undergoing sexual reproduction and is more diverse than virulence phenotypes suggest. Results from this research increase our understanding of population dynamics and diversity in phenotype and genotype of *U. appendiculatus* and will assist common bean breeding for rust resistance.

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DEDICATION

I want to dedicate this dissertation to my parents that believed in me and supported me all the way. I want to dedicate this piece of work to my entire Monclova-Santana family, for being the first one to have a PhD and for the generations behind me, for all the dreams that they will fulfill. To God that put special people in my path to accomplish all of this. To my amazing and incredible husband that is my biggest cheerleader and supporter William Rodriguez, I could not have done it without you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. LITERATURE REVIEW

From a historic overview to a detailed description, this literature review aims to explore in depth the particularities of the *Uromyces appendiculatus:Phaseolus vulgaris* pathosystem. *Uromyces appendiculatus* (Pers.:Pers.) Unger is the causal agent of bean rust and has been reported worldwide. The pathogen reproduces sexually and has a repeating urediniospore stage. The disease had been reported to cause up to 100% losses in extreme cases. This review will cover *Uromyces appendiculatus, Phaseolus vulgaris* and the relationship between them, including advances in research, challenges and limitations.

1.1. Common bean

1.1.1. Origin and domestication

Common bean (*Phaseolus vulgaris* L.) is a New World legume, part of the Leguminosae (Fabaceae) family. Members of the family include lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), and faba bean (*Vicia faba* L.), among others. However, all of the above mentioned have the center of origin in the Old World; in contrast with common beans (Zohary and Hopf, 2000). The Leguminoceae (Fabaceae) family belongs to the order Fabales (Doyle, 2004; McClean et al., 2004). The Phaseoleae tribe part of the Phaseoloid subfamily, includes *Glycine, Phaseolus* and *Vigna*; this last two belonging to the Phaseolinae subtribe.

Phaseolus vulgaris includes common beans (dry edible beans) and garden beans. Common beans include kidney, navy and pinto market classes, among others; and garden beans include string, runner, snap, French and many more (Kelly, 2010). The *Phaseolus* genera includes five cultivated species, *P. vulgaris* (common bean), *P. coccineus* (scarlet runner bean), *P. polyanthus* (year-long bean), *P. acuatifolius* (tepary bean), and *P. lunatus* (lima bean), all of which are domesticated (Osorno and McClean, 2014).

Common bean is exclusive to the new world with two major domestication centers, Middle America and the Andes Mountains (Doyle, 2004; Gepts, 2004; Kelly, 2010). Genome sequencing efforts support the dual domestication theory (Schmutz et al., 2014). Studies based on the content of the phaseolin protein have been used to determine genetic relationship among market classes. The wild type bean is considered the most ancestral, originating in Southern Ecuador and Northern Peru (Kelly, 2010). It is also believed that this ancestral wild type was moved to Middle America and to the Andean region 100,000 years ago; resulting in the two domestication centers (Kelly, 2010; Schmutz et al., 2014). The Middle American gene pool is more genetically diverse than the Andean gene pool, but both are divided into races (Kelly, 2010). Races of common bean from the Middle American gene pool are small seeded when compared to larger Andean seed types. The Middle American gene pool navy and black beans which belong to the Mesoamerican race; great northern and pinto beans which belong to the Durango race; and pink and small red beans which belong to the Jalisco race (Gepts, 2004). In addition, climbing beans belong to Guatemala race (Kelly, 2010). The Andean gene pool is divided into three races Nueva Granada, Peru and Chile. Nueva Granada race includes large seeded kidney beans, snap beans and bush cranberry beans. The Peru race includes yellow beans such as Mayacoba and Canario. The Chile race includes vine cranberry beans (Becerra et al., 2011; Kelly, 2010).

Evolutionarily, wild type *P. vulgaris* across Central and South America have different morphological characteristic when compared to domesticated lines. Wild populations of *P. vulgaris* differ in seed size, shape and color; display seed dormancy, many are photoperiod sensitive, they mainly display a climbing growth habit, have smaller leaf size, and seeds are disseminated from pods via explosive dehiscence (Singh et al., 1991; Mamidi, 2010). The seed storage protein phaseolin has been used to distinguish wild from domesticated populations (Gepts,

2004). The Middle America pool has a single phaseolin type, in divergence with their wild progenitor which displays 15-20 phaseolin types (Gepts, 2004).

Agronomic characteristics such as seed size and growth habit have been the most important traits for domestication efforts (Gepts, 2004). Navy, black, and pinto beans are the most important market classes in North America and have been the most intensively bred (NASS, 2019; Liebenberg and Preterious, 2010). However, the genetic diversity within these market classes is increasing through the introgression of new sources of germplasm. For example, many varieties have been released as rust resistant where genes from both Andean and Middle American origin have been combined (Pastor-Corrales, 2003).

1.1.2. Production and economic importance

Brazil, the USA and Mexico are the leading common bean producing countries (FAO, 2019). In 2018 1,386,112, tonnes of common bean (*Phaseolus vulgaris* L.) were produced in the USA. North Dakota is the primary common bean producer in the USA. In 2018 North Dakota production was valued in $301,126,000 USD, representing 43% of the total USA production (NASS, 2019). North Dakota is followed by Michigan (16%), Minnesota (13%), Nebraska (10%), and Idaho (4%). The most popular market classes produced in USA in 2018 were: pinto (40%), black (21%), navy (15%), and great northern (5%). Other market classes including light/dark red kidney, pink, small red, cranberry, and small white are also produced in smaller quantities (NASS, 2019). Approximately 22% of USA common bean production was exported in 2017-2018 fiscal year, most commonly navy (30%), pinto (25%), and black (23%) beans (USDA- ERS, 2019). Twenty percent of the total dry bean production in North Dakota goes for exportation for international markets. USA habitants consume approximately 7.5 pounds of dry bean annually per

capita. Pinto, navy, great northern, red kidney and black beans are the preferred market classes of consumption (usdrybeans.com).

1.2. Common bean rust

1.2.1. Taxonomy of *Uromyces appendiculatus*

In 1795, *Uromyces appendiculatus* (Pers.:Pers.) Unger was first discovered in Germany by Persoon and named *Uredo appendiculata phaseoli* (Boerema and Verhoeven, 1979; Liebenberg and Pretorius, 2010; Schwartz et al., 1980). In 1801, the name was changed to *Uredo appendiculata* Pearson. ex Persoon subsp. *appendiculata* (Boerema and Verhoeven, 1979; Liebenberg and Pretorius, 2010). In 1804 and 1815, the organism was described as *Puccinia phaseoli (*Pers. ex Pers) Rebentisch, and *Hypodermium appendiculatus* (Pers. ex Pers.) Link, respectively (Boerema and Verhoeven, 1979; Liebenberg and Pretorius, 2010). In 1849, *Uromyces appendiculatus* (Pers. ex Pers.) Fries was first introduced (Boerema and Verhoeven, 1979). In 1854, *Uromyces phaseolorum* Tulasne nomenclature was published while describing *Uredo appendiculata* var *phaseoli* Pers. (Boerema and Verhoeven, 1979). *Uromyces phaseoli* (Pers. ex Pers.) Winter var. *phaseoli*; or in Latin America, var. *typical* was introduced in 1880 and became one of the most used names in publications for this organism (Boerema and Verhoeven, 1979; Schwartz et al., 1980). *Uromyces appendiculatus* (Pers.:Pers.) Unger was the accepted nomenclature for common bean rust at the International Code of Nomenclature, (Boerema and Verhoeven, 1979; Liebenberg and Pretorius, 2010; Schwartz et al., 1980).

1.2.2. Ecology of the pathogen

Environmental conditions including relative humidity, temperature, light, nutrition of host, pH, ion concentration and leaf age play a role on the development of the disease (Liebenberg and Pretorius, 2010). Temperature, humidity and dew point are the major environmental factors

affecting pathogen infection and disease development. Temperatures ranging from 17ºC to 25ºC (65º to 85ºF) are considered optimal for spore germination as long as relative humidity is constant at 95% or higher for a period of seven to eight hours; higher or lower temperatures will inhibit germination (Liebenberg and Pretorius, 2010; Markell, 2009; Venette and Lamey, 1995). When incubation temperatures rose to 21-24ºC, the number of pustules decreased; no pustules developed above 27ºC (Liebenberg and Pretorius, 2010; Venette and Lamey, 1995). Temperature changes can be detrimental to urediospore germination, even a 5º to 6 ºC change for periods of 4 to 6 hours had been reported to reduce urediniospore germination and germination is inhibited at very low temperatures (1.8 to 4ºC) (Pastor-Corrales and Liebenberg, 2010; Schwartz et al., 1999; Venette and Lamey, 1995;). Appressorium formation is optimal at 16º to 21ºC and appresorium formation is decreased at 24ºC (Liebenberg and Pretorius, 2010; Venette and Lamey, 1995). *U. appendiculatus* has coevolved with its host; therefore specific pathogen races differ in optimal temperature and resulted in different disease development rates when inoculated at the same temperature when inoculated under greenhouse conditions. Therefore, consistent disease screening under greenhouse conditions can be challenging (Acevedo et al., 2005; Liebenberg and Pretorius, 2010; Wright et al., 2008).

Uromyces appemdiculatus requires free moisture to cause infection (e.g. morning dew). Humidity is a key factor for disease development, relative humidity higher than 96% is optimal (Pastor-Corrales and Liebenberg, 2010; Schwartz et al., 1999; Venette and Lamey, 1995). Relative humidity less than 95% can reduce urediniospore germination (Venette and Lamey, 1995). Leaf surface wetness is crucial as urediniospores require hydration to germinate (Liebenberg and Pretorius, 2010). Germination of teliospores depends on the rehydration for 3 to 5 days under

conditions of high relative humidity (Pastor-Corrales and Liebenberg, 2010; Schwartz et al., 1999; Venette and Lamey, 1995).

Rust symptoms include rusty colored pustules on the susceptible host *P. vulgaris*. In some instances, a yellow or green halo is visible around a pustule. In addition, on a highly susceptible host, the pathogen produces a spore ring around the initial pustule, which is also a source of secondary inoculum (Hater and Zaumeyer, 1941; Pastor-Corrales and Liedenberg, 2010). Pustules can be produced on leaves, petioles, stem and pods. Earlier infections spread to more plant tissues when environmental conditions remain favorable (Pastor-Corrales and Liedenberg, 2010).

1.2.3. *Uromyces appendiculatus* life cycle

Uromyces appendiculatus is an autoecious, macrocyclic, obligate parasite (biotroph). The life cycle includes the production of asexual and sexual spores on a single host, common bean. Uredinia pustules are amphigenous and produce dikariotic (n + n) and golden brown or cinnamon colored urediniospores (Venette and Lamey, 1995; Pastor-Corrales and Liedenberg, 2010). Urediospore dimensions can range from 20-33 ×18-29 μm, but are typically 24-33 × 20-27 μm and obovoid to ellipsoid. They contain a thick wall of 1.5-2.0 μm, echinulate, equatorial pores or slightly above with smooth caps (Cummins, 1978). They germinate in the dark when temperatures and humidity are favorable to form a germ tube. Urediniospores are considered the primary inoculum for the disease, these can be windblown or mechanically spread from infected fields (Pastor-Corrales and Liedenberg, 2010; Venette and Lamey, 1995). Infected plant debris is likely the primary inoculum source.

Urediniospores of *U. appendiculatus* produce an appresorium; a specialized melanized cell structure that plays a crucial role in pathogen infection. An appresorium is only formed from an *U. appendiculatus* urediniospore as a result of topographic stimuli of the bean leaf with an

epidermal cell ridge height from 0.4 to 0.8 μm. The guard cells of the stoma serve as the final cue for formation (Allen et al., 1991; Read et al., 1997). After the appresorium is formed, an infection hyphae grows intracellularly and produces a haustorium; a specialized structure that absorbs nutrients from host cell in biotrophic fungi (Venette and Lamey, 1995; Pastor-Corrales and Liedenberg, 2010). Colonization continues to adjacent cells to create a sorus (Venette and Lamey, 1995). The sorus increases in size, giving rise to single-celled, thin-walled urediniospores. Seven to 10 days after urediniospore germination, the sorus will burst open to create uredinia, also known as pustules. Pustules are a small, raised circular to oval structures containing urediniospores.

Uromyces appendiculatus produce pustules on the upper and lower side of the leaf and usually range in size from 0.2 to 1.2 mm in diameter, depending on host:pathogen interactions (Yarwood 1961). Susceptible bean lines that do not carry resistant genes tend to develop bigger pustules than partially resistant lines. Common bean resistant lines can produce a hypersensitive response to pathogen infection. Urediniospores serve as repeating inoculum for the disease, where new pustules develop 10 to 15 days after the initial pustules (Pastor-Corrales and Liedenberg, 2010; Venette and Lamey, 1995). Urediniospores have been reported to survive harsh winters on plant debris and produce a viable inoculum for next season (Gross and Venette, 2001; Schwartz et al., 1999).

Uromyces appendiculatus is an autoecious rust, forming all five spore stages on the same host. After the pustule matures, it produces a thick-walled, dark colored, smooth, and single celled telium that serves as the survival structure of the pathogen (Venette and Lamey, 1995; Pastor-Corrales and Liedenberg, 2010). The telium is circinate around uredinia or scattered, pulverulent, and exposed. The telium produces black to brown, diploid (2n), ovoid, broadly ellipsoid or globoid teliospores that are 24-35 to 20-29 μm in size. Teliospores are thick-walled 2-4 μm thick on the

sides and 5-11 μm thick at the pore, or umbo. The pore is defined, pale, chestnut brown, with few to numerous verrucae randomly distributed or in lineal to even fused ridges, rarely smooth. The teliospore pedicel is hyaline or colorless and approximately 45 μm long (Cummins, 1978). Teliospore germination requires a resting, or dormant period (Liebenberg and Pretorius, 2010). Teliospores do not infect the host, rather the germ tube serves to produce a basidium. Karyogamy occurs in the teliospore and meiosis in the basidium to produce four haploid (n) basidiospores (Gold and Mendgen, 1984; Schwartz et al., 1999). Basidiospores land on young leaf surface and germinate to produce a germ tube and appresorium (Gold and Mendgen, 1984). This appresorium directly penetrates the plant tissue with a penetration peg, in contrast with the appressoria produced by urediniospores (Liebenberg and Pretorius, 2010). Colonization of the intercellular and intracellular space takes place within 72 hours. The spermagonia (pycnia) (n) fuses with spermatia (pycnidiospores) (n) from the opposite mating type (Gold and Mendgen, 1984). This produces a chlorotic lesion with some leaf malformation. Pycniospores ("+" or "-" mating type) are elliptical, hyaline and smooth walled, produced in an opaque white nectar by the spermagonia, accompanied by receptive hyphae (Gold and Mendgen, 1984). The fusion of primary mycelium of opposite mating types results in the production of a white cup (aecia) on the lower side of leaf. Aecia have a short, whitish peridium with erose margins (Cummins, 1978). Dikariotic aeciospores are produced inside the aecia. Aeciospores range from 18-33 to 16-24 μm, ellipsoid to oblong ellipsoid or angularly globoid. The aeciospore (n + n) wall is 1.0-1.5 μm thick, colorless, and finely verrucose (Cummins, 1978). Aeciospores land on healthy common bean tissue and infect, resulting in the production of uredinia, completing the *U. appendiculatus* life cycle (Pastor-Corrales and Liedenberg, 2010; Venette et al, 1978; Venette and Lamey, 1995).

Uromyces appendiculatus pathogen genetics suggest that it has coevolved with the host and there are three major groups Middle American, Andean and a combination of both (Acevedo et al., 2005; Wright et al., 2008). *Uromyces appendiculatus* has a very important interaction with its host which has one center of origin in Middle America but two centers of domestication, Mesoamerica (Central America area) and the Andean mountains (Peru area) (Kelly, 2010; Pastor-Corrales and Liebenberg, 2010). The determination of the center of diversity of the pathogen and the aggrupation of races accordingly to the center of domestications of the host supports the theory of coevolution of the two (Acevedo et al., 2005; Wright et al., 2008). Some rust races from Andean background are unable to produce teliospores under greenhouse conditions. They clearly are evolutionarily deferred from teliospore producing races of Middle American background (Lu and Groth, 1987). The genome of *U. appendiculatus* is currently not available: however, genome size is estimated at 560Mb, roughly 6.9 times larger than *Puccinia graminis* (94Mb) (Eilam et al., 1992; Puthoff et al., 2008). *De novo* assembly of genomes of two isolates race 5-0 and 31-1 resulted in genome sizes of 587.6 and 546.7 Mb, respectively, confirming previous estimates (Hurtado-Gonzales et al., 2018). In comparison, the host *P. vulgaris,* is diploid with 11 chromosomes with a genome size of 450 to 650 Mbp/haploid genome (Gepts, 1999). This limits the power of pathogen population studies performed using genomic methods.

1.2.4. *Uromyces appendiculatus* race characterization and distribution

Uromyces appendiculatus undergoes sexual reproduction in addition to the production of asexual dikariotic spores produced repeatedly over the growing season; therefore, it is reasonable to conclude that a great genetic variation exists (Schwartz, et al., 1980). *U. appendiculatus* is classified into races based on the host:pathogen interaction; which serve as sub-specific classification of isolates based on virulence. Host resistance had been observed widely and it is

believed that resistance and virulence, fit the gene-for-gene theory; thus a differential set is appropriate for race characterization (Flor, 1971; Pastor-Corrales, 2002; Schwartz et al., 1980). A reaction is classified as resistant when the uredinia, or pustule, diameter is less than 0.3 mm (scale number 3), also when the reaction on a resistant host is immune (no symptoms) or hypersensitive (necrotic spots). When pustules are greater than 0.3 mm (up to full size of 1.2 mm) the bean line is considered susceptible (Stavely et al., 2002). The disease scale combines qualitative and quantitative assessments data to record virulence phenotype using a 1 to 6 scale (Mmbaga et al., 1999).

In 1983 at the International Rust Workshop, a common bean differential set was developed including 20 lines (Stavely et al., 1983). A year later, line Mountaineer White Half Runner was removed because it did not differentiate from Kentucky Wonder 780 (KW 780), reducing the set to 19 accessions (Stavely, 1984). Later, at the International Rust Workshop in 2002 a new differential set was developed (Steadman et al., 2002). Many of the original differential lines were removed due to repetition of genes. From the original differential set, lines Golden Gate Wax, Early Gallatin, Redland Pioneer, Mexico 235, Mexico 309, Aurora and Compuesto Negro Chimaltengo (CNC) were included in the new differential set developed in 2002. This current differential set includes 12 lines, reducing the number and the duplication in genes represented (Steadman et al., 2002; Table 2). Each common bean line carries one or more specific resistant genes, some of which have not been characterized. The current differential set used to determine *U. appendiculatus* race is divided into two sub-sets containing lines from Andean and Middle American gene pools. Every line has been assigned a binary value to represent the line on which the isolate is virulent (Steadman, et al., 2002; Table 2). A virulence phenotype is recorded using the binary number assigned to that differential line and the race is determined by adding the

numbers within each gene pool. The first number corresponds to the reaction against lines originating from the Andean gene pool and the second number corresponds to the Middle American gene pool. Any given number combination designates one set of gene combinations, making each combination unique and able to be directly translated to determine the set of resistance gene(s) overcome (Steadman, et al., 2002). For example, rust race 7-3 is virulent on Andean differential lines Early Gallatin (binary value = 1), Redlands Pioneer (2) and Montcalm (4) equal to 7; and GN11410 (1) and Middle American differential lines Aurora (2) resulting in 3.

Bean rust had been reported in many countries in North America, Latin America, Asia, Europe, Africa, Australia and New Zealand with some specific diversity based on location (Jochua, et al., 2008; Liebenberg and Pretorius, 2010; Markell, 2008). Races 31-7 and 22-2 were reported in Dominican Republic (Pastor-Corrales personal communication). Puerto Rico first reported rust in 2009, where only one race, 19-63 was found (Vega et al., 2009). *U. appendiculatus* races identified in Brazil, 21-3, 29-3, 53-3, 53-19, 61-3, 63-3, and 63-16, are highly virulent on the Andean genes (DeSouza et al., 2011; DeSouza et al., 2007). Honduras was determinate to be the center of highest virulence diversity of *U. appendiculatus*, the center of origin (Acevedo et al., 2013). Ninety-one races were identified in Honduras including highly virulent race 22-52 (108), the only race currently identified as virulent on *Ur-11* (Acevedo et al., 2013). In Africa, races 15-2, 21-0, and 29-0 were reported in Egypt (Pastor-Corrales, personal communication). Kenya reported seven *U. appendiculatus* races, 29-0, 29-1, 29-3, 31-1, 31-3, 31-11, 61-1, and 61-3, in 2016 (Nyang'au et al., 2016). Races in Uganda are have been shown to be from Andean background since Andean beans are preferred (Odogwu et al., 2017). In 1935, bean rust was prevalent across the USA in many common bean growing states (Harter et al., 1935). In recent

years, rust is common in Nebraska, Michigan, and North Dakota (Jochua et al., 2008; Markell et al., 2009; Venette et al., 1998; Wright et al., 2009).

1.2.5. *Uromyces appendiculatus* in North Dakota

Common bean production started in North Dakota around 1970 and rust epidemics have been reported since 1975 (Venette and Lamey, 1994). In 1980 and 1981, disease was severe enough that some fields were not harvested. In 1994, a 16% yield loss, about $13.7 million USD, was reported in the state due to bean rust. In 1996, 6% yield loss resulted in a reported economic impact of $12 million USD (Gross and Venette, 2001; Venette and Lamey, 1994). More recently, rust prevalence has increased since 2013 ranging from 13% to 40% of acres reported by growers; the highest being reported in 2016 (Knodel et al., 2014; 2015; 2016; 2017; 2018; 2019).

Urediniospores of *U. appendiculatus* have been reported to survive North Dakota winters; this includes a period of several months with temperatures below freezing (Gross and Venette, 2001). While uncommon, evidence of sexual reproduction, including pycnia and aecia had been reported in North Dakota (Venette et al., 1978). The *Ur-3* gene was incorporated into germplasm developed and adapted to North Dakota, and varieties of pinto, navy, black, kidney and small red beans were released as rust resistant since the 1980's. In 2002, five rust races were reported in North Dakota based on the original rust differential set containing 19 common bean lines. Races 52, 54, 69, 70, and 71 are virulent on known resistance genes *Ur-6*, *Ur-6+*, *Ur-4* and *Ur-13* (Gross and Venette, 2002; Stavely et al., 1983). These races were also virulent on other unknown genes from differential bean lines US3, CSW643, Pinto650, KW765, KW780 and KW814. Evaluations performed in North Dakota from 1996 to 2000 confirmed that no races were virulent on resistance gene *Ur-3* at the moment of screening (Gross and Venette, 2002). Furthemore, pinto beans such as Lariat (2007), Stampede (2007), and ND-307 (2008) were marketed as rust resistant, due to the

Ur-3 gene (Osorno et al., 2009b; 2010). Navy bean varieties such as Norstar (1991) and Avalanche (2008) (Grafton et al., 1993; Osorno et al., 2011); along with black bean Eclipse (2004) were resistant to rust races present at that time in the state (Osorno et al., 2009a).

In 2008, a new *U. appendiculatus* race, 20-3, was identified in North Dakota, resulting in susceptibility of at least 27 of the most commonly grown varieties in North Dakota (Markell et al., 2009). Race 20-3 is virulent on *Ur-6, Ur-7, Ur-3,* and unknown gene from Montcalm. Recently, line ND-Falcon was released in 2019 with resistant to the current pathogen population, since it carries resistant gene *Ur-11* (Osorno, 2019). In 2018, known susceptible cultivars Lariat, Stampede, ND-307 and Eclipse are still grown in the state on 481, 406, 161 and 3433 ha, respectively, regardless of proven susceptibility (Knodel et al., 2019). This wide spread susceptibility represented a threat of dry bean rust epidemics return to North Dakota (Markell et al., 2009). Hence, understanding *U. appendiculatus* population dynamics and interaction with host *P. vulgaris* is extremely important.

Pathogen diversity based on location had been proposed and studied, stipulating that some geographical areas include specific races not present in other areas. Jochua and contributors found high levels of diversity when studying isolates form multiple countries, where some races were unique to a specific country and location. Isolates from tropical and subtropical regions had higher virulence and greater number of races than from temperate regions (Jochua et al., 2008). This evidence suggest that *U. appendiculatus* races can be geographically delimited, where host cultivars grown in the region play a role in virulence of races on specific genes (McClean et al., 1995). In 2008, Alleyne and collaborators found 10 polymorphic fragments that separated isolates by state, Nebraska and Colorado, in terms of the reaction to bean cultivar Olathe that carries *Ur-6* gene. The 10 polymorphic fragments were present on Nebraska isolates but absent on Colorado

isolates; however, polymorphic fragment marker were unable to distinguish between virulence or avirulence of isolates on *Ur-6* (Alleyne et al., 2008). This provides further evidence that genetic differences exist in race composition even at local level. Local events such as varieties grown and environmental and cultural practices can influence the virulence composition within and among pathogen populations (Alleyne et al., 2008). More recent research based on 16 simple sequence repeats (SSRs) targeting 77 alleles on the *U. appendiculatus* genome classified 46 races into two major clusters, Andean and Middle American (Hurtado-Gonzales et al., 2018).

1.3. Disease management

1.3.1. Host resistance

Host resistance is the most effective disease management tool available and because *U. appendiculatus:P. vulgaris* follows the gene for gene theory, researchers and breeders have focused attention on finding major genes. *U. appendiculatus* resistant genes have been mapped to six of 11 *Phaseolus vulgaris* chromosomes (Miklas et al., 2006; Pastor-Corrales and Steadman, 2015). Genome Wide Association Studies (GWAS) have localized multiple disease resistant genes in common bean, where in many cases rust and anthracnose resistance are shared by the same locus (Pastor-Corrales and Steadman, 2015; Miklas et al., 2006). Resistance gene *Ur-3* present in rust differential line Aurora; *Ur-6* present in Golden Gate Wax, *Ur-7* present in GN1140, and *Ur-11* present in PI181196 are clustered at chromosome Pv11 (Miklas et al., 2006; Pastor-Corrales and Steadman, 2015; Souza et al., 2008). Genes *Ur-3* and *Ur-11*, and *Ur-6* and *Ur-11* are epistatic (Pastor-Corrales and Steadman, 2015). Fine mapping localized *Ur-3* gene on chromosome Pv11 between 46.96 and 47.01 Mb and about a 46.5kb in size (Hurtado-Gonzales et al., 2017). Fine mapping localized *Ur-11* at 3.9 cM on Pv11 (Pastor-Corrales and Steadman, 2015). *Ur-5*, present in Mexico 309, and *Ur-14*, present in Ouro Negro, are located on chromosome Pv04 (Miklas et

al., 2006; Pastor-Corrales and Steadman, 2015; Souza et al., 2008). Anthracnose and rust resistance genes such as *Ur-5, Ur*-Dorado-108, *Ur*-14, *Co-3, Co-9, Co-y* and *Co-z* are clustered on Pv4 (Liebenberg and Pretorius, 2010). Fine mapping later localized gene *Ur-5* at 1.9 cM on linkage group Pv04 in Mexico 309 and *Ur-14* at 0.8 cM in Ouro Negro (Pastor-Corrales and Steadman, 2015). *Ur-4*, present in Early Gallatin, is located on Pv06; *Ur-13*, present in Redland Pioneer, is located on Pv08 (Pastor-Corrales and Steadman, 2015). *Ur-9* and *Ur-12* present in Line Pompadour Checa 50 (PC50) are located in on Pv01 and Pv07, respectively (Pastor-Corrales and Steadman, 2015). Andean gene *Ur-9* on Pv01 is located at 34.6 cM, and is associated with three anthracnosis genes *Co-I, Co-x,* and *Co-w* (Liebenberg and Pretorius, 2010). A recently identified gene, *Ur-13* present in PI310762, is located on Pv04 (Pastor-Corrales and Steadman, 2015). Resistance genes *Ur-3, Ur-5, Ur-7, Ur-11, Ur-13* and *Ur-14* are from Middle American origin has and have been incorporated into Andean cultivars (Pastor-Corrales and Steadman, 2015). Resistance gene *Ur-4, Ur-6, Ur-9* and *Ur-12* are from Andean origin (Pastor-Corrales and Steadman, 2015). We can expect more genes to be described as more line are being screened for rust resistance. The *Ur-11* gene is effective against most *U. appendiculatus* races, where only Honduran race 108 (22-52) is virulent on the gene. Thus *Ur-11* has been targeted as part of breeding programs. Successful implementation of *Ur-11* by the NDSU bean breeding program was accomplished and the cultivar ND-Falcon, released on 2019 (Osorno, 2019). USDA breeding program at Beltsville, MD had released multiple great northern lines, such as BelmiNeb-RMR-4 containing rust resistant genes *Ur-4*, *Ur-6* and *Ur-11* (Hurtado-Gonzales et al., 2017; Pastor-Corrales, 2003).

GWAS is an excellent, relatively new tool to identify and localize new sources of resistance. GWAS studies conducted on the Guatemalan climbing bean germplasm found multiple

regions that confer resistance to three *U. appendiculatus* races (Montejo-Dominguez, 2017). Resistance to race 20-3 was found associated to regions on chromosome Pv02 and Pv04. While resistance to race 63-1 was associated to regions at Pv10 and Pv04. Finally, resistance to race 31-1 (53), was associated to regions atn Pv04 and Pv02 (Montejo-Dominguez, 2017). Regions on Pv04 were different per *U. appendiculatus* race. While region on Pv02 was shared in reaction to 20-3 and 31-1. This study provided possible regions in the Guatemalan germplasm for breeding purposes.

Disease resistance genes have been moved from tepary bean (*P. acutifolius*) to common bean (*P. vugaris*) (Kelly, 2010). Specifically, genes conveying resistance to *U. appendiculatus* have been transferred from tepary to common bean via interspecific hybridization (Miklas and Stavely, 1998). Tepary beans expressed a single locus exhibiting incomplete dominance to *U. appendiculatus,* resulting in an immune or hypersensitive response (Miklas and Stavely, 1998). Tepary bean accession G40022 was reported to contain a *U. appendiculatus* resistance gene effective against all currently identified pathogen races (Pastor-Corrales and Steadman, 2015). This could represent a new source of resistance available to be integrated into common bean germplasm.

Many scientists have warned about the use of disease resistance based on one resistance gene. This often shortens the life span of the gene, such as in the case of *Ur-3* gene in the USA (Markell, et al., 2009; McMillan, et al., 2003). Thus, gene pyramiding is the most recommended approach for breeding programs (Alleyne et al., 2004; McMillan et al., 2003; Pastor-Corrales and Steadman, 2015; Souza et al., 2008; Stavely, 1984). Successful gene pyramiding in beans has been accomplished for resistance to *bean common mosaic virus* (BCMV), anthracnosis and rust (Souza et al., 2008; Kelly et al., 1995). For example, the great northern line BelmiNeb-RMR-4 is resistant

to rust and BCMV containing rust resistant genes *Ur-4, Ur-6* and *Ur-11*; and BCMV resistant gene *bc1*^{2} *(*Hurtado-Gonzales et al., 2017; Pastor-Corrales, 2003). Many other bean cultivars from pinto, great northern, and navy market classes have been released with multiple rust resistant genes *(*Hurtado-Gonzales et al., 2017; Pastor-Corrales, 2003). However, the life span of these pyramided genes remains uncertain.

Partial resistance had been described on bean cultivars Nodak and Upland, where uredinia were smaller than the susceptible check 14 days post inoculation (Statler and McVey, 1987). This partial resistance is the analog of what is considered slow rusting in *Puccinia graminis*; where smaller pustules produce fewer urediniospores. This small pustule reaction is believed to be more stable, polygenic, and non-race specific resistance (Liebenberg & Pretorius, 2010). Adult plant resistance (APR) is a specific type of partial resistance expressed as small pustules on leaves grown beyond the third trifoliolate stage (Jung et al., 1998). These APR genes are different from the vertical resistance genes, and also have been mapped independently from pubescent genes; that were once believed to be linked (Jung et al. 1998). Cultivar PC-50 that contains *Ur-9* and *Ur-12* (Sandlin and Steadman, 1994). *Ur-9* confers the specific resistance, while *Ur-12* confers APR (Jung et al., 1998).

Recent studies using small interfering RNA (siRNA) provide resistance to bean rust (Cooper and Campbell, 2017). Cooper and Campbell, found four genes in *U. appendiculatus* that promote pathogenicity, and the inhibition of those genes can confer resistance. Using Bean Pod Mottle Virus (BPMV) they delivered the effectors involved in haustorium formation and functionality. Basically, BPMV activate a gene-specific silencing signal in a common bean plant that could be transmitted back to the fungus and inhibit haustorium production (Cooper and

Campbell, 2017). These findings could mean the next step in achieving more efficient and effective disease resistance.

Most of the host:pathogen interactions between common bean and *U. appendiculatus* remain largely unknown; however, some advances have been made. Ayyappan and collaborators (2015) reported two histone modifications, histone methylation H3K9 and acetylation H4K12 on common beans infected with *U. appendiculatus*. $H3K9_{me2}$ methylation modification was found bound to disease resistance family of proteins which includes the leucine rich repeats (LRR) family, nucleotide binding adaptor (NB-ARC) shared by APAF-1, R proteins and *Caenorhabditis elegans* death-4 protein (CED-4) domain containing Toll Interleukin Receptor- Nucleotide Binding Site- Leucine Rich Repeats (TIR-NBS-LRR) class of proteins localized on bean chromosomes Pv01, Pv07, Pv08, Pv10 and Pv11. Conversely, $H4K12_{ac}$ target only NB-ARC domain with a disease resistance protein at chromosome Pv11. For example, the *Ur-3* gene located on bean chromosome Pv11 included three regions that encode to NB-ARC with LRR regions (Hurtado-Gonzalez et al., 2017). NB-ARC regulates the activity of R-proteins. Both methylation and acetylation had been related to hypersensitive response-like inducing proteins that are highly produced during early infection, particularly 12 hours after infection (Ayyappan et al., 2015).

1.3.2. Fungicides

Fungicide application is crucial to control the disease where rust-susceptible cultivars are grown and the environment is conducive for disease development. Fungicide applications are recommended when the average pustule quantity is about two per leaf and the plant has not reached the pod fill stage (Markell and Pasche, 2017; Venette and Lamey, 1995). If the disease has progressed to 40 spots per leaf and the plants are in the bloom to small pod stage, it is not economically viable to apply fungicides for rust management. Currently, protectant fungicides

such as chlorothalonil and maneb; and systemic fungicides as Endura, Folicur, Headline and Quadris applied at least 5 gallons of water per acre are recommended in Colorado (Schwartz et al., 2011). In North Dakota, Triazoles (FRAC 3) such as Proline (prothioconazole), and Folicur (tebuconazole); strobilurins (FRAC11) such as Headline (pyraclostrobin) and Quadris (azoxystrobin) have been very effective (Markell et al., 2012; Markell, 2009). Fungicides Topsin, Endura and Omega reduce rust but they are less effective than triazoles and strobilurins (Markell et al, 2012). Alternating fungicide modes of action are highly recommended to prevent resistance development (Liebenberg and Pretorius, 2010).

1.3.3. Cultural practices

Practices such as crop rotation, removal of volunteer plants, change of planting time to less conducive conditions, and removal of plant debris by deep plowing are recommended to reduce disease incidence and severity (Pastor-Corrales and Liebenberg, 2010). However, more growers are moving to minimum tillage. Intercropping with non-host crops are also recommended practices, although this might be a challenge in large farming operations. Crop rotation for three to four years has been proven to reduce the amount of surviving uredinospores (Venette and Lamey, 1995). Change of planting time allows based on environmental conditions, helps to avoid conditions that are conducive to disease. Thus, if the disease appears later in the season it is less detrimental.

1.4. Conclusions

Bean rust is present in every common bean growing country, each one with race specificity influenced by the preferred market classes grown. Pathogen population diversity is highly influenced by sexual recombination and heterokaryotic, polycyclic urediniospores. Hence the study of the *U. appendiculatus* population structure and race composition is crucial in

understanding population dynamics. Pathogen diversity studies increase our understanding of the host:pathogen interaction and have led to the identification of new sources of resistance, which is crucial to common bean production. Resistance based on a single dominant gene has been overcome by new pathogen races, such is the case of *Ur-3* gene in North Dakota. Thus, a different approach to breeding is needed, like gene pyramiding and quantitative trail loci (QTL). GWAS studies can provide details of uncharacterized resistance regions associated with disease resistance to further incorporate on breeding programs. The objective of this research is to characterize *U. appendiculatus* races in North Dakota; identify resistance and genomic regions associated with resistance to the *U. appendiculatus* races most frequently identified in North Dakota; and determine the *U. appendiculatus* population structure in North Dakota.

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2. *UROMYCES APPENDICULATUS* RACE CHARACTERIZATION IN NORTH DAKOTA

2.1. Introduction

In 2018, 1,470,784 tons of common beans (*Phaseolus vulgaris* L.) were produced in the United States. North Dakota is the primary common bean producer in the USA, producing 493,416 tons in 2018, valued at $301 million USD and representing 33% of the total nation's production (NASS, 2019). Walsh (16%), Grand Forks (14.3%), and Pembina (12%) counties located in the northeastern side of North Dakota lead common bean production (NASS, 2019).

Common bean production started in North Dakota around 1970 and rust epidemics have been reported since 1975 (Venette and Lamey, 1994). In 1980 and 1981 disease was severe enough that some fields were not harvested. In 1994, a 16% yield loss, about $13.7 million USD, was reported in the state due to bean rust and 6% yield loss, with an economic impact of $12 million USD, was reported in 1996 (Gross and Venette, 2001; Venette and Lamey, 1994). More recently, rust prevalence started to increase in 2013 and up to 40% of acres were affected as reported by growers in 2016, the highest percent reported since 2013 (Knodel et al., 2014; 2015; 2016; 2017; 2018; 2019).

Uromyces appendiculatus (Pers.:Pers.) Unger, an autoecious, macrocyclic obligate biotroph, is the causal agent of bean rust. Urediniospores of *U. appendiculatus* have been reported to survive North Dakota winters; this includes a period of several months with temperatures below freezing (Gross and Venette, 2001). While uncommon, evidence of sexual reproduction, including pycnia and aecia has been documented in North Dakota (Venette et al., 1978). *U. appendiculatus* has been reported to undergo sexual reproduction in Colorado (McMillan et al., 2003; Schwartz et al., 1990; 1999); Oregon (Zaumeyer et al., 1957); New York (Jones, 1960); and Nebraska (Schartz

et al., 1994) in addition to North Dakota (Venette et al., 1978) based on reports of aecial and pycnial sexual stages of *U. appendiculatus*, usually on volunteer bean plants. Five rust races were reported in 2002 in North Dakota based on the original rust differential set containing 19 common bean lines (Stavely et al., 1983). Races 52, 54, 69, 70, and 71 are virulent to one or more known resistance genes *Ur-6, Ur-6+, Ur-4* and *Ur-13* (Gross and Venette, 2002; Stavely et al., 1983). These races were also virulent on other unknown genes from differential bean lines US3, CSW643, Pinto650, KW765, KW780 and KW814. Due to redundancies in phenotypic reactions, a new differential set containing 12 bean lines was developed in 2002 (Steadman, et al. 2002). This set contains equal representation from both bean gene pools, Andean and Middle American, and each line has an assigned binary value resulting in a unique number combination to identify the *U. appendiculatus* race.

Rust represents a limitation to common bean production across the world. Resistance gene *Ur-3* was widely used in breeding programs in Michigan and North Dakota due to the resistance response to the pathogen population at that time (McMillan et al., 2003; Venette et al., 1998; Wright et al., 2009). Virulence surveys in North Dakota from 1996 to 2000 confirmed that no races were virulent on resistance gene *Ur-3* (Gross and Venette, 2002). Therefore, the *Ur-3* gene was incorporated into germplasm developed and adapted to North Dakota, and varieties of pinto, navy, black, kidney and small red beans were released as rust resistant. Pinto beans such as Lariat (2007), Stampede (2007), and ND-307 (2008) were marketed as rust resistant, due to the incorporation of *Ur-3* gene (Osorno et al., 2009; 2010). Navy bean varieties such as Norstar (1991) and Avalanche (2008) (Grafton et al., 1993; Osorno et al., 2011); along with black bean Eclipse (2004) were resistant to rust races present at that time in the state (Osorno et al., 2009).

In 2008 a new rust race, 20-3, was first identified in North Dakota, and was virulent on 27 of the most commonly grown varieties in North Dakota (Markell et al., 2009). Race 20-3 is virulent on *Ur-6, Ur-7, Ur-3,* and unknown gene from Montcalm. This wide spread virulence on *Ur-3* gene represented a threat of the return of dry bean rust epidemics to North Dakota (Markell et al., 2009). In addition, 99.1% of North Dakota hectares are planted with Middle American origin beans (Knodel et al., 2019). Hence, understanding *U. appendiculatus* population dynamics and interaction with host *P. vulgaris* is extremely important.

It is crucial to characterize the *U. appendiculatus* population structure to aid in the identification of new sources of host resistance and select the most appropriate resistant genes to incorporate into germplasm. In addition, the high level of diversity present during previous *U. appendiculatus* race evaluations; evidence of sexual reproduction in the pathogen population; the lack of resistant varieties in most market classes; and the increasing prevalence of rust in North Dakota, make the research conducted here relevant. The objective of this research is to determine the frequency of virulence to rust resistance genes in the *U. appendiculatus* population from North Dakota.

2.2. Materials and methods

2.2.1. Pathogen collection

Common bean fields were surveyed during the 2015 and 2016 growing seasons in the main growing counties of North Dakota (Grand Forks, Pembina, Wells, Walsh, Benson, Ramsey, Traill, and Nelson) (Figure 2.2A). Twenty one fields were surveyed in 2015 and 38 fields in 2016. Prevalence and severity of bean rust was recorded visually in each field using a 0 to 100% scale. Prevalence was recorded based on observations made across each field. Each field location was recorded using a Geographical Positioning System (GPS). Diseased leaf samples collected were

dried and pressed in a paper towel and stored in an envelope in the fridge. At least 2 miles separated each field; 1 to 4 sample sites were surveyed in each field with no less than 100 meters in distance between them; a sample consisted of 3 to 5 infected leaves. Each sample and isolate was identified with a chronologically assigned number referring to field, followed by sample number within the field (Table 2.3). To prepare the inoculum, urediniospores collected from pustules on field samples were suspended in 5% tween 20 diluted in distilled water as a surfactant and inoculated onto susceptible pinto PI14 (UI 114) (Singh, University of Idaho) using a cotton swab when primary leaves were fully expanded. Inoculated plants were incubated in humidity chambers overnight in darkness with misting for 15 sec every 4 min at 21±1 ºC to increase humidity and aid infection. Plants were moved to a greenhouse room (14 h days at 17.5± 1.5ºC; 10 h night at 17.2± 2ºC) for disease development in fiberglass boxes topped with net fabric to contain *U. appendiculatus* urediniospores produced from new lesions. To collect single pustule isolates, pustules were cut with a sterile scalpel when developed but still closed, typically 7 to 10 days post-inoculation, five single pustules from each plant were collected and placed each in a separate wax paper envelop and identified with a single pustule number (SP#) (Table 2.5; 2.6). This process was repeated 2 to 3 times as necessary to increase urediniospores for further phenotypic and genotypic evaluations. To store isolates, *U. appendiculatus* urediniospores were collected with a vacuum (Welch Vacuum by Gardner Denver, Model No. 2522B-01) and saved in a size 00 gel caplet (Gallipot Inc. Item #802781) inside a 2.0mL plastic cryogenic vial (Corning Inc. #430659). Vials with urediniospores were placed on a desiccator for 48 to 72 h to reduce moisture and finally stored at -20ºC. A density heat map was developed to plot density of samples collected per geographical area using Tableau® (Figure 1.1; Tableau Software, 2019).

2.2.2. Race characterization

The virulence phenotype of single pustule isolates of *U. appendiculatus* were characterized using the 12 line common bean differential set (Steadman et al., 2002) (Table 2.1). To break dormancy of spores, urediniospores were heat shocked in a water bath for 10 min at 15.5ºC. The *U. appendiculatus* inoculum was prepared by adding about 0.9 mL of Soltrol oil to each gel caplet containing approximately 2 mg of urediniospores. The urediniospore solution was shaken and sprayed onto primary leaves using a pump sprayer (Welch Vacuum by Gardner Denver, Model No. 2522B-01). Soltrol was allowed to dry for approximately 20 min before plants were incubated in misting chambers following the previously described parameters. Rust reaction was scored 14 days post-inoculation by measuring ten arbitrarily chosen pustules on primary leaflets of each plant using a 6× Pocket Comparator (Edmund Optics Inc., Cat. #30-585). Ten uredinial diameters were averaged for each plant and converted to a reaction type on a 1 to 6 scale (Mmbaga et al., 1996; Stavely et al., 2002) (Table 2.2). A disease reaction equal to or greater than 4,3 was considered susceptible (Figure 2.1). Spores were collected from all plants using a vacuum, stored in a size 00 gel caplet inside a 2.0mL plastic cryogenic vial and stored at -20ºC.

Table 2.1. Common bean differential set used to determine virulence phenotype of *Uromyces appendiculatus* isolates collected in North Dakota during 2015 and 2016.

Differential number	Accession[a]	Resistance gene	Gene pool	Binary value
1	Early Gallatin	*Ur-4*	Andean	1
2	Redland Pioneer	*Ur-13*	Andean	2
3	Montcalm	Unknown	Andean	4
4	PC-50	*Ur-9, Ur-12*	Andean	8
5	Golden Gate Wax	*Ur-6*	Andean	16
6	PI 260418	Unknown	Andean	32
7	GN 1140	*Ur-7*	Middle America	1
8	Aurora	*Ur-3*	Middle America	2
9	Mexico 309	*Ur-5*	Middle America	4
10	Mexico 235	*Ur-3+*	Middle America	8
11	CNC[b]	*Ur-CNC*	Middle America	16
12	PI 181996	*Ur-11*	Middle America	32

[a]Differential accession based on Steadman et al., 2002.

[b]CNC = Compuesto Negro Chimaltenengo.

Table 2.2. Disease scale used to determine virulence phenotype of *Uromyces appendiculatus* isolates collected in North Dakota during 2015 and 2016 on each of the 12 common bean differential lines.

Reaction type [a]	Symptom description[b]	Score[c]	Rust reaction
1	No visible symptom	1.1	Resistant
2	Necrotic spots without sporulation	2.1	Resistant
2,3	Reaction 2 with few type 3	2.4	Resistant
3,2	Reaction 3 with few type 2	2.7	Resistant
3	Uredinia <0.3 mm in diameter	3.1	Resistant
3,4	Reaction 3 with few type 4	3.4	Resistant
4,3	Reaction 4 with few type 3	3.7	Susceptible
4	Uredinia 0.3-0.49 mm in diameter	4.1	Susceptible
4,5	Reaction 4 with few type 5	4.4	Susceptible
5,4	Reaction 5 with few type 4	4.7	Susceptible
5	Uredinia 0.5-0.8 mm in diameter	5.1	Susceptible
5,6	Reaction 5 with few type 6	5.4	Susceptible
6,5	Reaction 6 with few type 5	5.7	Susceptible
6	Uredinia 0.8- 1.2 mm in diameter	6.1	Susceptible

[a]Reaction type based on pustule diameter.

[b]Modified from Stavely et al., 2002.

[c]Quantitative disease score based on Mmbaga et al. 1996

Figure 2.1. Examples of *Uromyces appendiculatus* virulence phenotype reactions; hypersensitive response (A), pustule size 0.5 mm (reaction type = 5) (B) and 1.0 mm (reaction type = 6) (C).

2.2.3. Data analysis

To visually represent the data points, a geographical heat map of sample locations was developed using web map generator eSpatial to plot concentration of samples collected per location. Virulence score was determined by converting disease reaction type to quantitative virulence score (Table 2.2) (Mmbaga et al., 1996). The virulence score was averaged across all 12 differentials to determine Mean Disease Severity (MDS). The MDS for each isolate was used to compare *U. appendiculatus* isolates quantitatively. Bean differential PI290418 was not used to calculate the MDS on some isolates due to limited accessibility to viable seed. In that case, MDS was calculated based on 11 differentials. Mean Disease Severity (MDS) was used to plot isolate virulence in a geographical setting using Tableau® (Tableau Software, 2019). Latitude was used as the Y axis, and longitude as the X axis. MDS was used as the parameter for size and for color of each point. Higher MDS were marked by larger size and a warmer color of the spectrum.

2.3. Results

In 2015, 21 fields were surveyed across seven North Dakota common bean growing counties where production is concentrated (Table 2.3; Figure 2.2A and B). In 2016, 38 fields were surveyed across 13 common bean growing counties. Across the fields included in this survey, in

2015 rust prevalence was higher than in 2016; on the contrary rust severity was higher in 2016 than in 2015. Benson County had one of the highest rust prevalence and severity during both years. In 2015, rust prevalence reached 100% in Benson, Eddy and Wells counties. Likewise, disease severity in these counties were the highest observed reaching 50%. Walsh and Pembina counties also had high prevalence of 90% and 80%, respectively. Rust prevalence was lowest in Walsh County.

In 2016, the highest prevalence and severity were observed on fields in Ramsey County reaching 80% prevalence, with severity of 70%. Highest prevalence was 80% on Benson and Ramsey Counties, followed by Pembina County with 70%. Fields in Traill County had the lowest prevalence among counties where the disease was observed. The highest severity was present in Dickey (80%), Ramsey (70%), and Benson (50%), while fields in Pembina County had the lowest. No rust was observed in fields in Foster, Grand Forks, Walsh, Sheridan and Pembina counties (Table 2.3).

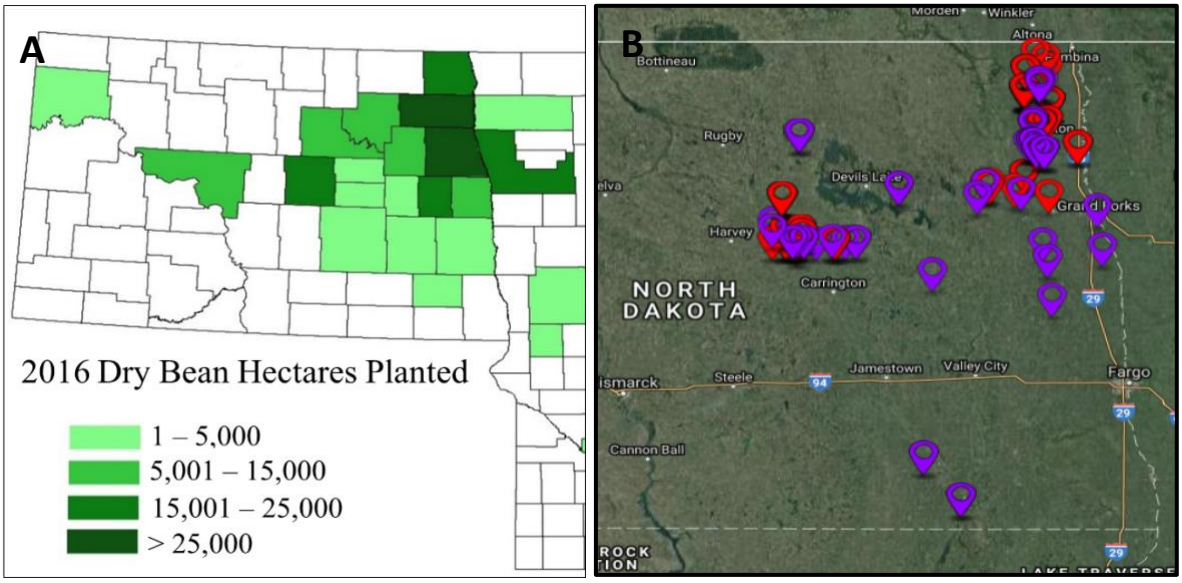

Figure 2.2. Common bean acres planted in North Dakota in 2016 (A) and sampling location of dry bean fields surveyed in 2015 (red) and 2016 (purple) (B).

Table 2.3. Percentage common bean rust prevalence and severity at each sampling site within fields surveyed in 2015 and 2016. Each sample is identified by collection year, field and sample number. Global Positioning System (GPS) coordinates are included with town (nearest to sampled field) and county in North Dakota.

Year	Sample ID	No. Isolates[b]	Prevalence (%)	Severity (%)	County
2015	UP15-1-1	0	10	15	Walsh
2015	UP15-1-2	1	10	15	Walsh
2015	UP15-1-3	0	5	5	Walsh
2015	UP15-1-4	3	0	10	Walsh
2015	UP15-1-5	4	12	18	Walsh
2015	UP15-1-6	3	15	7	Walsh
2015	UP15-2-1	4	35	25	Pembina
2015	UP15-2-2	3	80	10	Pembina
2015	UP15-2-3	3	60	7	Pembina
2015	UP15-2-4	0	-[c]	-	Pembina
2015	UP15-2-5	2	-	-	Pembina
2015	UP15-3-1	0	10	2	Walsh
2015	UP15-3-2	0	7	2	Walsh
2015	UP15-3-3	1	90	25	Walsh
2015	UP15-4-1	0	10	3	Walsh
2015	UP15-4-2	3	85	30	Walsh
2015	UP15-4-3	0	35	25	Walsh
2015	UP15-6-1	2	10	5	Grand Forks
2015	UP15-6-2	0	15	3	Grand Forks
2015	UP15-28	2	-	-	Grand Forks
2015	UP15-29	1	-	-	Nelson
2015	UP15-30	2	-	-	Walsh
2015	UP15-31	1	-	-	Pembina
2015	UP15-34	1	-	-	Pembina
2015	UP15-35	2	-	-	Pembina
2015	UP15-36	1	-	-	Pembina
2015	UP15-1w-2	0	20	3	Wells
2015	UP15-2w-1	1	90	10	Wells
2015	UP15-2w-2	2	100	50	Wells
2015	UP15-2w-3	0	100	30	Wells
2015	UP15-2w-4	3	100	35	Wells
2015	UP15-3w-1	0	60	10	Wells
2015	Up15-3w-2	0	40	10	Wells
2015	UP15-3w-3	0	55	50	Wells
2015	UP15-4w-1	2	90	50	Wells
2015	UP15-5w-1	0	20	5	Wells
2015	UP15-5w-2	0	20	2	Wells
2015	UP15-7w-2	1	100	40	Benson
2015	UP15-7w-3	1	100	50	Benson
2015	UP15-7w-1	1	60	20	Benson

(*continued on next page*)

Table 2.3. Percentage common bean rust prevalence and severity at each sampling site within fields surveyed in 2015 and 2016 (continued). Each sample is identified by collection year, field and sample number. Global Positioning System (GPS) coordinates are included with town (nearest to sampled field) and county in North Dakota.

Year	Sample ID	No. Isolates[b]	Prevalence (%)	Severity (%)	County
2015	UP15-7w-4	4	100	50	Benson
2015	UP15-8w-1	1	50	5	Wells
2015	UP15-8w-2	0	50	1	Wells
2015	UP15-8w-3	3	30	3	Wells
2015	UP15-9w-1	0	100	50	Eddy
2015	UP15-9w-2	2	100	50	Eddy
2015	UP15-9w-3	0	100	35	Eddy
2015	UP15-10w-1	0	100	30	Eddy
2015	UP15-10w-2	2	100	50	Eddy
2015	UP15-10w-3	1	60	10	Eddy
2016	UP16-1-1	0	10	20	Benson
2016	UP16-1-2	1	10	20	Benson
2016	UP16-1-3	0	10	20	Benson
2016	UP16-2-1	0	80	50	Benson
2016	UP16-2-2	0	80	50	Benson
2016	UP16-2-3	0	80	50	Benson
2016	UP16-3-1	1	80	70	Ramsey
2016	UP16-3-2	2	80	70	Ramsey
2016	UP16-4-1	0	0	30	Grand Forks
2016	UP16-5-1A	0	10	30	Traill
2016	UP16-5-1B	0	10	30	Traill
2016	UP16-5-3	1	10	30	Traill
2016	UP16-6-1	1	0	0	Pembina
2016	UP16-7-1	0	70	10	Pembina
2016	UP16-7-2	0	70	10	Pembina
2016	UP16-7-3	0	70	10	Pembina
2016	UP16-7-4	0	70	10	Pembina
2016	UP16-8-1	1	70	10	Pembina
2016	UP16-8-2	1	70	10	Pembina
2016	UP16-9-1	0	30	40	Walsh
2016	UP16-9-2	2	30	40	Walsh
2016	UP16-9-3	0	30	40	Walsh
2016	UP16-10-1	1	30	30	Walsh
2016	UP16-10-2	1	30	30	Walsh
2016	UP16-10-3	1	30	30	Walsh
2016	UP16-11-1	0	0	10	Walsh
2016	UP16-12-1	3	30	20	Walsh
2016	UP16-12-2	1	30	20	Walsh
2016	UP16-12-3	0	30	20	Walsh
2016	UP16-13-1	0	60	30	Walsh
2016	UP16-13-2	1	60	30	Walsh

(*continued on next page*)

Table 2.3. Percentage common bean rust prevalence and severity at each sampling site within fields surveyed in 2015 and 2016 (continued). Each sample is identified by collection year, field and sample number. Global Positioning System (GPS) coordinates are included with town (nearest to sampled field) and county in North Dakota.

Year	Sample ID[a]	No. Isolates[b]	Prevalence (%)	Severity (%)	County
2016	UP16-13-3	3	60	30	Walsh
2016	UP16-14-1	2	10	10	Walsh
2016	UP16-15-1	1	20	20	Walsh
2016	UP16-15-2	0	20	20	Walsh
2016	UP16-16-1	1	50	80	Dickey
2016	UP16-16-2	3	50	80	Dickey
2016	UP16-16-3	1	50	80	Dickey
2016	UP16-17-1	2	-	-	Dickey
2016	UP16-17-2	3	-	-	Dickey
2016	UP16-F5	1	-	-	Traill
2016	UP16-F12	2	-	-	Grand Forks
2016	UP16-F14	2	-	-	Nelson
2016	UP16-F15	2	-	-	Nelson
2016	UP16-F16	3	-	-	Traill
2016	UP16-F18	2	-	-	Traill
2016	UP16-F19	2	-	-	Eddy
2016	UP16-F20	1	-	-	Wells
2016	UP16-F22	2	-	-	Wells
2016	UP16-F23	1	-	-	Wells
2016	UP16-F27	2	-	-	Eddy
2016	UP16-F28	1	-	-	Giggs
2016	UP16-F31	1	-	-	Traill
2016	A	0	0	0	Foster
2016	B	0	0	0	Grand Forks
2016	C	0	0	0	Traill
2016	D	0	0	0	Walsh
2016	E	0	0	0	Walsh
2016	F	0	0	0	Walsh
2016	G	0	0	0	Sheridan
2016	H	0	0	0	Pembina

[a] UP= refers to *Uromyces appendiculatus* sample with collection year, second #= field, F= field, w=west, and third # = sample from field

[b] Number of single pustule isolates obtained per field sample collected.

[c] (-)= No data

Mean Disease Severity (MDS) was used to plot isolate virulence phenotype in a geographical platform (Figure 2.3B). Highest MDS were plotted in Walsh and Pembina counties. MDS of all isolates evaluated ranged from 1.9 to 4.6. An isolate originating in Grand Forks County had the highest MDS of 4.6 in 2016. In 2015, an isolate from Walsh County had the lowest MDS of 1.9. When combining both years *U. appendiculatus* isolates collected in Ramsey and Grand Forks had the highest average MDS ranging from 3.5 to 3.19 (Figure 2.3B). Field 24 in Pembina County had the highest average MDS of 4.4, while Walsh County field 4 had the lowest average MDS of 2.37. Walsh County had isolates with high MDS and isolates with low MDS, providing evidence that multiple races can be present at the same time with different virulence patterns. Likewise, majority of the bean production concentrates in Walsh, Pembina and Grand Forks counties, thus great race diversity is expected.

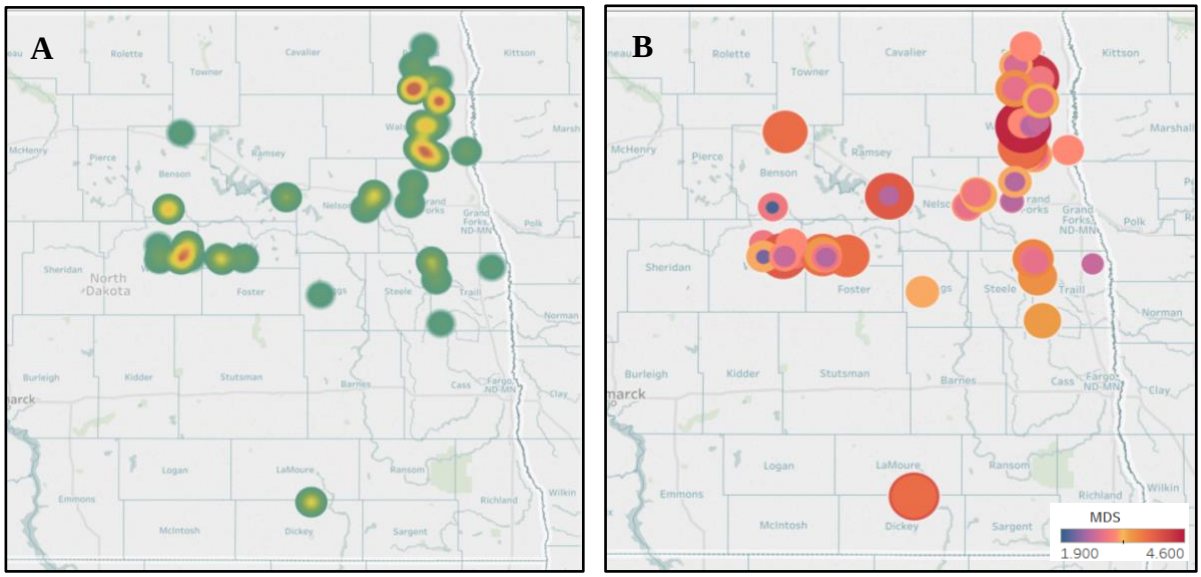

Figure 2.3. Sample collection density map during 2015 and 2016 (A). Magnitude plot representing Mean Disease Severity (MDS) of common bean rust during both years in North Dakota. Size and color represents low to high MDS ranging from blue (1.9) to red (4.6).

In 2015, 62 samples displaying pustules characteristic of *U. appendiculatus* infection were collected from 21 fields (Table 2.4). In 2016, 56 samples with *U. appendiculatus* signs and rust symptoms were collected from 38 fields. A total of 63 and 57 single pustule isolates were generated from these 59 common bean fields in North Dakota in 2015 and 2016, respectively.

In 2015, 11 rust races were identified using the standard differential set (12 lines). The number of unique races per county varied from one in Nelson County to five in Walsh County (Table 2.4). Approximately 70% of isolates from 2015 were identified as race 20-3, virulent on the *Ur-6, Ur-7, Ur-3* genes and unknown gene in Montcalm (Table 2.5; Figure 2.4 and 2.5). Race 28-3 was identified in 6% of isolates, virulent on *Ur-6, Ur-7, Ur-3, Ur-9, Ur-12,* and unknown gene in Montcalm. Nine additional races were identified in less than 5% of isolates (Table 2.5; Figure 2.4).

In 2016, 14 *U. apendiculatus* races were phenotypically described (Table 2.6; Figure 2.4). The number of unique races per county varied from one in Benson, Dickey, Giggs and Nelson Counties to four in Walsh and Wells Counties (Table 2.4). Nearly 50% of isolates were 20-3. Rust race 21-3 was identified in 15% of isolates, virulent on *Ur-6, Ur-7, Ur-3, Ur-4,* and unknown gene in Montcalm (Table 2.6; Figure 2.4). Race 29-3 was identified in 9% of the isolates and is virulent on *Ur-6, Ur-7, Ur-3, Ur-4, Ur-9, Ur-12,* and unknown gene from Montcalm. Eleven additional races were identified in less than 4% of isolates (Table 2.6; Figure 2.4).

Among the *U. appendiculatus* races identified in North Dakota in 2015 and 2016, race 29-33 is most virulent, overcoming all genes except *Ur-13* and *Ur-11* (Table 2.6). Race 29-33 was only identified in one isolate collected in 2016. Across all races identified, 97.5% overcome *Ur-3* from Aurora, the resistance gene introgressed into varieties widely grown in North Dakota (Figure 2.5). Other genes were overcome by more than 98% of isolates including *Ur-7* from GN1140, *Ur-*

6 from Golden Gate Wax (GGW) and unknown gene from Montcalm (Figure 2.6). No isolate were virulent on the Middle American *Ur-11* gene from PI181996 or the unknown gene from PI260408.

Table 2.4. Number of unique *Uromyces appendiculatus* races and total of number of isolates and fields per North Dakota county

	Total fields		Total isolates		Total unique races	
County	2015	2016	2015	2016	2015	2016
Benson	1	1	7	1	3	1
Dickey	0	1	0	1	0	1
Eddy	2	2	5	4	2	3
Griggs	0	1	0	1	0	1
Grand Forks	2	2	4	2	2	2
Nelson	1	2	1	4	1	1
Pembina	5	2	17	3	6	3
Ramsey	0	2	0	3	0	3
Traill	0	5	0	8	0	2
Walsh	4	5	17	16	8	6
Wells	3	3	12	4	3	4

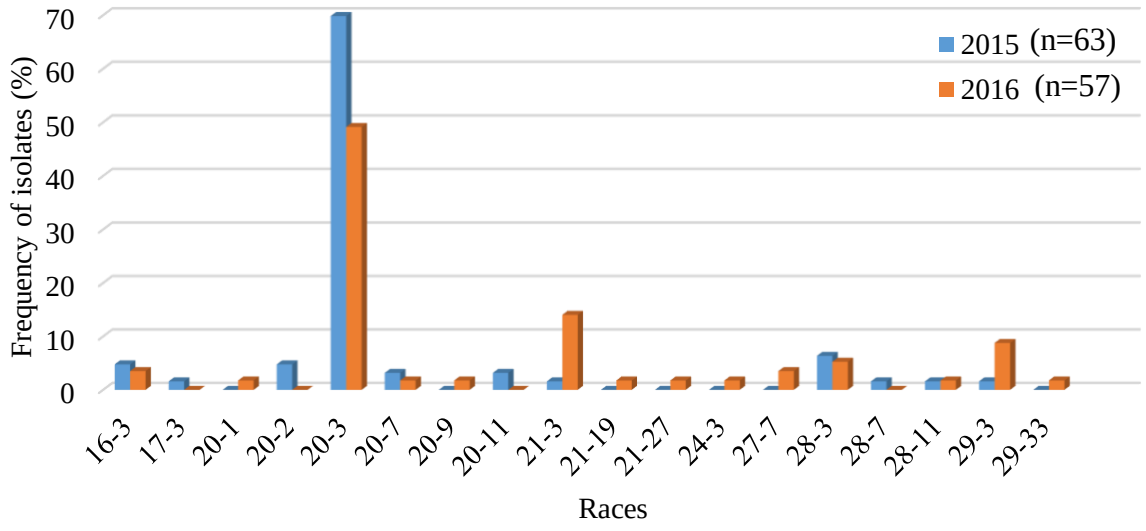

Figure 2.4. Frequency of isolates (%) of each race identified in 2015 and 2016. Sixty-three and 57 single pustule isolates were characterized from 2015 and 2016, respectively.

Table 2.5. Virulence phenotype and mean disease severity (MDS) of 63 *Uromyces appendiculatus* single pustule isolates collected from common beans fields in North Dakota in 2015.

Single pustule isolate[a]	Race ID[b]	Differential line[c]												MDS[d]
		EG	RP	M	PC50	GGW	PI18	GN1140	Aurora	Mx309	Mx235	CNC	PI96	
UP15-1-2-SP3	17-3	5,6	1	3,4	2	5	1	5,6	6,5,4	1	1	1	1	2.8
UP15-1-4-SP1	20-3	1	1	4	3	5,4	3	6	4,5,4	1	3,2	1	1	2.8
UP15-1-4-SP6	20-3	2	1	5,6,4	1	5,6	1	6	4,3	1	3,2	1	1	2.7
UP15-1-4-SP2	28-11	2	1	4	4,3	5,6	2,3	6	4	2,3,4	4,3,2	3,2	1	3.2
UP15-1-5-SP1	20-3	2	1	5,6,4	1	4,5,3	3	6	6,5	1	3,2	1	1	2.9
UP15-1-5-SP6	20-3	2	1	6,5	2	5,4	3,2	6	6	1	3,2	1	1	3.1
UP15-1-5-SP3	16-3	2	2	3,4	3,4	5	3,4	6,5	6,5	3,2	3,2	2,3	1	3.3
UP15-1-5-SP5	20-3	3,2	1	4,5	1	6,5	2	6	3,4	1	1	1	1	2.6
UP15-1-6-SP1	20-3	2	1	5,3,4	2	5,4	3,4	6	6	1	3,2	1	1	3.0
UP15-1-6-SP3	20-3	1	1	4,3	1	5,4	1	5,4	5,6	1	2	1	1	2.4
UP15-1-6-SP6	20-3	2	1	4,5,3	1	5,4	2,3	6,5	4,5,3	1	2	1	1	2.6
UP15-2-1-SP3	20-11	2	1	5,6	2	5,6	1	5	5,6	1	4,3	1	1	2.9
UP15-2-1-SP9	29-3	6,4	2,3	4,3	4,5	6	3,2	6	5,6,4	1	1	1	1	3.4
UP15-2-1-SP6	16-3	1	1	3	1	5,4	1	6	4	1	1	1	1	2.2
UP15-2-1-SP4	20-3	2	1	5,4,3	2,3	4	1	6	5,4	1	2	1	1	2.7
UP15-2-2-SP3	20-3	2	1	5.4	2	4	1	6	5,4	1	1	1	1	2.6
UP15-2-2-SP5	20-3	2	2,3	6	2,3	5,6	3,2	6	6	2,3	3,2	1	1	3.4
UP15-2-2-SP6	20-3	2	1	6	2	6,5	1	6,5	6	1	1	1	1	2.9
UP15-2-3-SP1	21-3	4,3	2,3	4,5	3	5,6	2,3	6	5,4	2,3	3,2	1	1	3.3
UP15-2-3-SP2	20-3	2	1	6,5	2	6,5	1	6	6	1	3,2	1	1	3.0
UP15-2-3-SP4	20-7	2	1	6,5	2	6,5	1	6	6	5,6	1	1	1	3.2
UP15-2-5-SP2	20-3	2	1	4,3	3,2	5,4	3	6,5	5	2	3,2	2	1	3.1
UP15-2-5-SP4	20-3	2	1	5,6	2	6,5	1	6	5,6	1	2,3	3,2	1	3.0
UP15-3-3-SP1	20-3	2	1	5,4	2	6	1	6	6	1	1	1	1	2.8

(*continued on next page*)

Table 2.5. Virulence phenotype and mean disease severity (MDS) of 63 *Uromyces appendiculatus* single pustule isolates collected from common beans fields in North Dakota in 2015 (continued).

Single pustule isolate[a]	Race ID[b]	Differential line[c]												MDS[d]
		EG	RP	M	PC50	GGW	PI18	GN1140	Aurora	Mx309	Mx235	CNC	PI96	
UP15-4-2-SP1	20-3	2	1	4,3	2	6	1	6	6	1	1	1	1	2.7
UP15-4-2-SP3	20-3	2	1	6,5	2	5,6	1	6,5	6	1	1	1	1	2.8
UP15-4-2-SP2	20-2	1	1	4	1	4	1	3,2	4	1	1	1	1	2.0
UP15-6-1-SP1	20-3	1	1	6	1	5,6	1	6	4	1	1	1	1	2.5
UP15-6-1-SP2	20-3	2	1	5,4	2	6,5	3,4	6	6,5	2,3	3,2	1	1	3.2
UP15-28-SP2	28-3	1	1	5,4,3	4	5	1	6	5,6,4,3	1	2,3	1	1	2.8
UP15-28-SP3	20-3	1	1	5,4,3	2	5,4	2,3	6	6	1	3	3,2	1	3.0
UP15-29-SP6	28-7	2	2	5,4	5,4	5,6	-[e]	4,5	4,3	5,4	2,1	1	1	3.3
UP15-30-2-SP2	28-3	1	2,3	4,3,5	3,4,5	4,5,3	3,2	6,5	5,4	1	3,4	1	1	2.9
UP15-30-2-SP	20-3	2	1	4,3,5	2	6,5	1	6	6,5	1	3,2	3,2	1	2.9
UP15-31-2-SP4	28-3	1	1	5	4	5,4	-	6,5	4,5	1	1	1	1	2.8
UP15-34-1-SP	20-3	2	1	4,3	3,2,4	6,5	2,3	6	5,4	2,3	3	1	1	3.0
UP15-35-2-SP1	20-3	2	1	6,5	2	6,5	3	6	6,5	2,3	2,3	1	1	3.2
UP15-35-2B-SP1	20-3	2	1	4,5	1	5,6	3	6	6,5	1	1	1	1	2.8
UP15-36-SP2	16-3	3	1	3,4	1	4	1	4,5	5,4	1	1	1	1	2.3
UP15-2W-1-SP2	20-3	2	1	6,5,3	2	5,6	1	6	4,5	1	1	1	1	2.7
UP15-2W-2-SP3	20-3	2	1	5,6	3,4	5,4,6	3,2	5,4	5,6	1	3,4	1	1	3.0
UP15-2W-2-SP6	20-3	1	1	5,4	2	6	3,2	6	5,4,6	1	1	1	1	2.8
UP15-2W-4-SP1	20-3	2	1	5,4	2	5,4	2	6,5	5,4,6	1	2,3	1	1	2.8
UP15-2W-4-SP2	20-3	2	1	5,4,6	2	6,5	1	6	5,6	1	2,3	1	1	2.8
UP15-2W-4-SP3	20-3	2	1	5	2	6,5	1	6	6	1	3,2	1	1	3.0
UP15-4W-1-SP5	20-2	2	1	4,3	2	6	1	2,3	5	1	2,3	1	1	2.4
UP15-4W-1-SP8	20-3	2	1	6,5	2	6	3,2	6,5	6	2,3	3,2	1	1	3.2
UP15-7W-1-SP6	20-2	2,3	1	4,3	2,3	5,4,6	-	4,5	1	1	1	1	1	2.2

(*continued on next page*)

Table 2.5. Virulence phenotype and mean disease severity (MDS) of 63 *Uromyces appendiculatus* single pustule isolates collected from common beans fields in North Dakota in 2015 (continued).

Single pustule isolate[a]	Race ID[b]	Differential line[c]												MDS[d]
		EG	RP	M	PC50	GGW	PI18	GN1140	Aurora	Mx309	Mx235	CNC	PI96	
UP15-7W-2-SP4	20-3	2	2	5	2,3	6	3,2	6	6,5,4	1	1	1	1	3.0
UP15-7W-3-SP2	28-3	1	1	4,5	3,4	5,4	2,3	6	6,5	1	3,4	1	1	3.0
UP15-7w-4-SP3	20-3	2	1	6	2	5,6	3,4	5,6,4	6	2,3	2,3	1	1	3.2
UP15-7W-4-SP7	20-3	2	3	6,5	2	6	3,2	5,4	4,5	1	1	1	1	3.1
UP15-7W-4-SP6	20-3	2	1	4,5	1	6,4	1	6	5,4	1	2,3	1	1	2.4
UP15-7W-4-SP8	20-3	1	1	4,3	1	4,3,2	1	6,5	5,4	1	1	3	1	2.4
UP15-8W-1-SP2	20-11	2	2,3	4	3,4	4,3	2,3	6,5	4,3	1	4,3,4	3	1	3.0
UP15-8w-3-SP2	20-3	2	1	6,5	2	6	1	6	4,3	1	1	1	1	2.7
UP15-8W-3-SP3	20-3	2	1	5,6	2	6	1	6,5	6,5	1	1	1	1	2.8
UP15-8W-3-SP5	20-3	2	1	5,4	2	5,4,6	3	6,5	5	1	2,3	1	1	2.9
UP15-9W-3-SP2	20-3	2	1	4,5	2	6,5	3	6	6,5	1	2,3	1	1	3.0
UP15-9W-3-SP5	20-3	2	1	5,4,6	2	5	4,5	6,5	5,6	1	1	1	1	2.9
UP15-10W-2-SP1	20-3	2	2	5,3	2	5,4	1	6,5	4,5	1	1	1	1	2.7
UP15-10W-2-SP4	20-3	2	1	6	2	6	3,	6	4,6	2,3	2,3	1	1	3.1
UP15-10W-3-SP3	20-7	1	1	6	1	6,5	1	6	6	5,4	1	1	1	3.0

[a] UP= refers to *Uromyces appendiculatus* sample with collection year, second #= fields, F= fields, w=west, and third # = sample from field

[b] Virulence phenotype based on binary value attributed to virulence on 12 differential lines.

[c] Reaction grades 1= No visible symptoms; 2= Necrotic spots without sporulation; 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0.3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0.3-0.49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0.5-0.8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= Reaction 6 with few type 5; 6= Uredinia 0.8-1.2 mm in diameter.

[c] Differential lines: EG = Early Gallatin, RP = Redland Pioneer, M = Montcalm, GGW = Golden Gate Wax, Pi18 = PI260418, Mx = Mexico, CNC = Compuesto Negro Chimaltengo, PI96 = PI181996.

[d] MDS= Mean Disease Severity, calculated by averaging virulence phenotype across all differential lines.

[e] (-)= missing data.

Table 2.6. Virulence phenotype and mean disease severity (MDS) of 57 *Uromyces appendiculatus* single pustule isolates collected from common beans fields in North Dakota in 2016.

Single pustule isolate[a]	Race ID[b]	Differential line[c]												MDS[d]
		EG	RP	M	PC50	GGW	PI18	GN1140	Aurora	Mx309	Mx235	CNC	PI96	
UP16-1-2-SP1	21-27	4,3	3,2	4	3	6,5	1	6	6	2,3	4,3	4,3	1	3.6
UP16-3-1-SP1	21-3	5	2	4,5	2	4	1	5	4	1	1	1	1	2.7
UP16-3-2-SP1	20-3	2	1	5,6	2	6,5	-[e]	6,5,4	6	1	-	1	1	3.2
UP16-3-2-SP2	21-19	4,3	1	5,6	3	5,6	-	6,5,4	6	3,2,4	-	4,3	1	3.8
UP16-5-3-SP1	20-3	2	1	5,6	2	5,6	-	5,6	4	3,2,1	1	2,3	1	3.0
UP16-6-1-SP1	29-33	4,5	1	5	5,4,3	6,5	-	6	6	6	4	4,3	1	4.4
UP16-8-1-SP1	24-3	3,2	1	3	4	5,4	-	4,5	4,5	1	3,2	1	1	2.8
UP16-8-2-SP1	21-3	5,4	3	4,5	3,2	6	2,3	6	6	2,3	2,3	3,2	1	3.7
UP16-9-2-SP3	20-9	2	1	5,4	2	5,4	-	4,5	4,3,2	3,2	4,3	3,2	1	3.0
UP16-9-2-SP5	20-3	2	1	4,3	3,4	5,4	1	6	6	1	3	3,2	1	3.0
UP16-10-1-SP1	27-7	2	1	4,3	4,3	4,3	-	6	6	4	3,2	1	1	3.2
UP16-10-2-SP4	27-7	5,4	4,3	4,3,2	6,3	6	-	6	6	5,6	3	3,2	1	4.4
UP16-10-3-SP1	20-3	3,4	1	4,3	2	6,4,5	-	6	5,6,4	1	1	1	1	2.6
UP16-12-1-SP2	21-3	5,4	1	5,6	2	6,5	3,4	6	6	2,3	3,2	2,3	1	3.6
UP16-12-1-SP3	21-3	4,3	3,2	5,4	2	6	-	6	6	2,3	3,4	3,4	1	3.8
UP16-12-1-SP4	21-3	4,5	3,4	5,4	3,2,4	5,4	-	6	4,3,5	3,4	3,2	3	1	3.6
UP16-12-2-SP1	29-3	5,6	1	6,5	4,3	5,6	-	6	6,5	1	1	1	1	3.4
UP16-13-2-SP1	29-3	5,4	1	5,4	1	6	-	6,5	5,6	1	1	1	1	3.0
UP16-13-3-SP1	21-3	1	1	5,4	1	6,5	-	6,5	6,5,4	1	1	1	1	2.7
UP16-13-3-SP2	20-3	5,4	1	6,5	4,3	5,4	-	6	6	1	3,2	3,2	1	3.6
UP16-13-3-SP3	20-1	2	1	5,4	2	6,5	-	6	1	1	1	1	1	2.5
UP16-14-SP1	20-3	3,2	3,2	6,5	1	5	-	6	5,4,6	1	1	2,3	1	3.1
UP16-14-1-SP7	20-3	1	1	5,4	1	5,6	3,2	6	6	1	1	1	1	2.7
UP16-15-1-SP1	21-3	4,3	2,3	5,6	2	4,5	-	4,5	6	3,2	3,4	2,3	1	3.5

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Table 2.6. Virulence phenotype and mean disease severity (MDS) of 57 *Uromyces appendiculatus* single pustule isolates collected from common beans fields in North Dakota in 2016 (continued).

Single pustule isolate[a]	Race ID[b]	Differential line[c]												MDS[d]
		EG	RP	M	PC50	GGW	PI18	GN1140	Aurora	Mx309	Mx235	CNC	PI96	
UP16-16-1-SP1	16-3	4,3	1	3,4	2,3	5	1	6,5	5	1	2,3	1	1	2.8
UP16-16-2-SP1	20-3	2	1	5,4	2	6,5	-[e]	6,5	6,5	1	1	1	1	2.9
UP16-16-2-SP2	20-7	1	1	5,4,3	2	5,4	-	-	6,5	4,3,5	3	2,3	1	3.3
UP16-16-2-SP3	20-3	2	1	6,4	2	6,5	-	6	4,3	1	1	1	1	2.8
UP16-16-3-SP1	21-3	4,3	1	4,3,5	3,4	4,5	-	5	6	2,3	3,2	1	1	3.2
UP16-17-1-SP1	29-11	3,2,4	1	5,4	3,4	5,6	1	6	6	3,2	4,3	3,2	1	3.3
UP16-17-1-SP2	20-3	2	1	4	2	6,5		5,6	6,5	1	1	1	1	2.8
UP16-17-2-SP1	29-3	6,5	3,2	5,4	5	6	-	6	6	2,3	3,2	1	1	4.0
UP16-17-2-SP2	20-3	3,4	1	5,4	2	5,6	-	6	5,4,3	1	3	1	1	3.1
UP16-17-2-SP3	20-3	2	1	6	2	6	-	6	6	1	1	1	1	3.1
UP16-F5-SP2	20-3	1	1	4,3	1	5,4	-	6,5,4	4,5,3	1	1	1	1	2.4
UP16-F12-SP2	20-3	2	1	4,3	2	5,6	-	6	4,3	1	1	1	1	2.6
UP16-F12-SP3	20-3	2	1	5,4	1	5,4		6	6,5,4	1	1	1	1	2.8
UP16-F14-SP1	20-3	2,3	2,3	6,5	2	5,6	-	6	4,5	1	3,2	1	1	3.1
UP16-F14-SP2	20-3	2,3	1	6	1	6	-	6	6,5	1	3	1	1	3.2
UP16-F15-SP1	20-3	3,4	1	4,5	3	5	-	5,4	5	1	1	1	1	2.8
UP16-F15-SP3	20-3	2	2	6,5	2	6		6	6	1	1	1	1	3.2
UP16-F16-SP1	20-3	3,2	1	4,3	1	5,4	3	6,5	4,5	1	3	1	1	2.7
UP16-F16-SP2	28-3	3,4	1,3	5,4	5,4	6	3	6	6	1	3	3,2	1	3.8
UP16-F16-SP3	20-3	2	1	6	2	5,6	-	6,5	6,5	1	1	1	1	3.0
UP16-F18-SP1	20-3	3,4	3,2	4,3	1	5,4	-	5,4	5,4	3,2	3,2	3,2	1	3.1
UP16-F18-SP2	20-3	2	1	6,5	2	5,4,6	-	6	5,4	1	1	1	1	2.8
UP16-F19-SP2	20-3	2	1	6	2	6	3	6	6	2,3	3,2	2,3	1	3.5
UP16-F19-SP3	28-3	2,3	2,3	5,6	5,4	6,5	-	6	6	3,2	2,3	2,3	1	3.8

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Table 2.6. Virulence phenotype and mean disease severity (MDS) of 57 *Uromyces appendiculatus* single pustule isolates collected from common beans fields in North Dakota in 2016 (continued).

Single pustule isolate[a]	Race ID[b]	Differential line[c]												MDS[d]
		EG	RP	M	PC50	GGW	PI18	GN1140	Aurora	Mx309	Mx235	CNC	PI96	
UP16-F20-SP1	20-3	1	1	6,5	2	6	-[e]	6	6	1	1	1	1	2.8
UP16-F22-SP1	28-3	2	2,3	4,5	4,3	5	-	6	5,4	1	1	1	1	2.9
UP16-F22-SP7	29-3	4,3	1	5,4	4,3	6,5	4	6	6	3,4	3,4	3,2	1	3.3
UP16-F23-SP1	16-3	2	2	3,4	1	5,4	3,4	6	5,4	1	3,4	1	1	2.8
UP16-F27-SP1	28-11	3,2	1	4,5	2	4	1	5,6	4	1	3	1	1	3.2
UP16-F27-SP2	20-3	3,4	1	4	4	5,4	-	6	6	3,4	4,3	3,2	1	3.3
UP16-F31-SP1	20-3	2	1	5,4	2	6	3	6	6	2,3	3,4	3,2	1	2.8
UP16-F38-SP1	29-3	5,4	1	4,5	4	5,6	-	6	4,3	1	1	1	1	4.0
Race 31-1 (53)	31-1	4	5,4,6	6,5	4	6	-	6,5	2,3	1	1	1	1	3.1
UP1991-SP2	20-3	1	2	5	2,3	6	2,3	6	6	2,3	3	3,2	1	3.1
UP1991-SP3	20-3	2	1	4,5	2	5	3,2	6	5,6	1	3	1	1	2.4
UP1993-SP1	29-3	6,5	2,3,	6,5	4,5	6,5	-	6	6	2	2,3	2	1	2.6
UP1995-SP1	21-3	5,4	3,4	4,5	3,4	5,4	-	5,4	5,6	1	3,4	1	1	2.8
UP1996-SP2	20-3	1	1	5,4	1	5,6	-	6	6	2,3	2,3	3,2	1	3.1
UP2007-SP2	20-3	2	1	5,6	2	6	2	6	6	2,3	3,2	3,2	1	3.2
UP2007-SP4	20-3	2	1	5,6	2	6,5	-	6	6	3,4	3,4	3,4	1	2.8
PR1-SP1	20-3	2	1	5,6	2	6	2,3	6	6	1	2,3	3,2	1	3.2

[a] UP= refers to *Uromyces appendiculatus* sample with collection year, second #= fields, F= fields, w=west, and third # = sample from field. Race 31-1 (53)= Maryland. PR= Puerto Rico

[b] Virulence phenotype based on binary value attributed to virulence on 12 differential lines.

[c] Reaction grades 1= No visible symptoms; 2= Necrotic spots without sporulation; 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0.3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0.3-0.49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0.5-0.8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= Reaction 6 with few type 5; 6= Uredinia 0.8-1.2 mm in diameter.

[c] Differential lines: EG = Early Gallatin, RP = Redland Pioneer, M = Montcalm, GGW = Golden Gate Wax, Pi18 = PI260418, Mx = Mexico, CNC = Compuesto Negro Chimaltengo, PI96 = PI181996.

[d] MDS= Mean Disease Severity, calculated by averaging virulence phenotype across all differential lines.

[e] (-)= missing data.

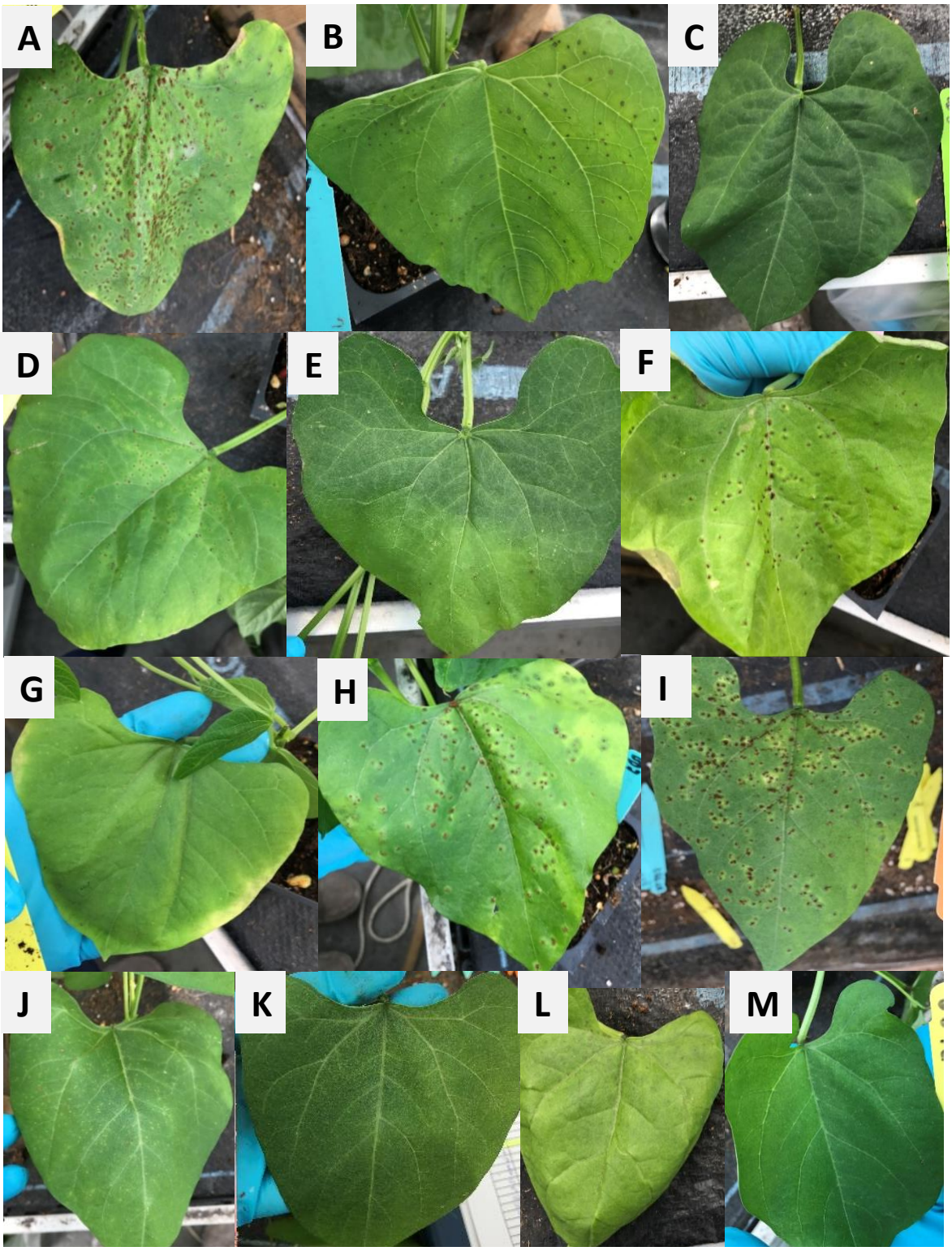

Figure 2.5. Phenotype of race 20-3. Virulent on susceptible check PI14 (A). Hypersensitive response on Early Gallatin (B) and PC-50 (E). Avirulent on Redland Pioneer (C), PI260418 (G), Mexico 309 (J), Mexico 235 (K), CNC (L), and PI181994 (M).Virulent on Montcalm (D), GGW (F), GN1140 (H) and Aurora (I).

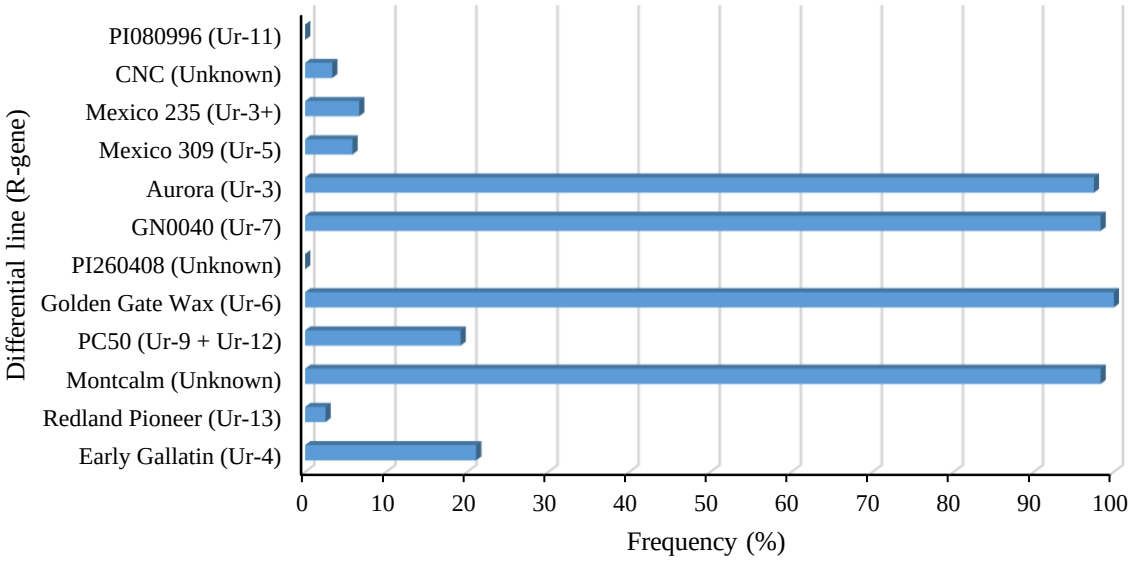

Figure 2.6. Virulence frequency across 12 differential lines of 119 single pustule *Uromyces appendiculatus* isolates. The gene associated with each differential line is denoted in parentheses.

2.4. Discussion

This research determined that in 2015 rust prevalence was higher than in 2016; on the contrary rust severity was higher in 2016 than in 2015. In 2015, 3% of the total amount of hectares planted were not harvested; while 10% of hectares were not harvested in 2016 due to unspecified reasons (NASS, 2019). Coincidentally, this data agrees with the higher rust severity observed in 2016. However, we are unable to correlate the losses to the severity of rust. In 2015, up to 7% of hectares planted in Walsh County were not harvested. Our survey determined up to 90% of rust prevalence in Walsh County in 2015. In 2016, up to 21% of hectares planted in Pembina County; 20% in Walsh; and 11% in Ramsey and Wells counties were not harvested (NASS, 2019). Based on our survey Pembina County reached 70% of prevalence and 10% severity; while Ramsey reached 80% prevalence and 70% severity. Walsh County reached 60% of prevalence and 40% of rust severity. Benson County had one of the highest rust prevalence and severity during both years.

In Benson county 15,054 ha of common bean were planted in 2016, where 2% were not harvested (NASS, 2019). Rust is still highly prevalent on major common bean producing counties.

In this research, 18 races were identified during this survey, being 20-3 the most frequent. Based on reports of aecial and pycnial sexual stages of bean rust, usually on volunteer bean plants in North Dakota. Hence, sexual recombination might be the primarily source of diversity in the *U. appendiculatus* population in North Dakota. Although, bean rust inoculum has not been proven to be windblown from other bean growing states or countries, we unable to totally exclude this possibility. Among these 18 races, *U. appendiculatus* race 29-3 has been reported in Kenya and Brazil, along with 21-3 in the latter (Arunga et al., 2012; Nyang et al., 2016). Races similar to those identified in North Dakota including 20-21, 28-1, 29-21 have been reported in Honduras; however, none of the races reported share the exact virulence phenotype as those characterized in North Dakota during this research (Acevedo et al., 2013). Other rusts such as wheat stripe rust spread via the 'Puccinia pathway' which moves pathogen races from southern states northward (Kolmer 2001). A study suggested that coffee rust urediniospores spread from Angola via wind currents, responsible for the appearance of the disease in Brazil (Bowden et al., 1971). However, unique 20-3 race has only been reported in North Dakota, windblown of new races could come from other bean growing areas.

In this research, 97.5% of *U. appendiculatus* isolates collected in North Dakota were virulent on the *Ur-3* gene, with the exception of one single pustule isolate collected in 2016 characterized as race 20-1. Virulence on *Ur-3* gene have been previously reported in North Dakota and Michigan where races 20-3 and 31-1 were identified, respectively (Markell et al., 2009; Wright et al., 2009). Isolates virulent on *Ur-3* have been reported from other bean growing regions around the world such as Honduras, Dominican Republic and South Africa (Jochua et al., 2008).

Analyzing previously reported *U. appendiculatus* races in North Dakota and races reported in this study we can speculate over changes in the *U. appendiculatus* population over time. No details of frequency or prevalence of the characterized races was included in the 2002 report from North Dakota. Hence, this discussion is based on speculations. Also, due to the discrepancies between the previous bean differential set and the current, we can only discuss the seven genes from lines that are present on both. In 2002, five *U. appendiculatus* races 52, 54, 69, 70, and 71 were reported in North Dakota, none were virulent on resistant genes *Ur-3*, *Ur-3+*, *Ur-5*, and *Ur-CNC* (Gross and Venette, 2002). Currently, 97.5% of isolates were virulent on *Ur-3*, while 6.7% were virulent on *Ur-3+*, 5.8% on *Ur-5*, and 3.3% on *Ur-CNC*. All races reported in 2002 were virulent on *Ur-6* from Golden Gate Wax (GGW). Race 52 was virulent on *Ur-4* (Early Gallatin); current races 21-3, 21-19, 29-27 and 27-7 are virulent on *Ur-4* gene, about 21.1% of all isolates characterized. In contrast, races 69, 70 and 71 were virulent on resistant gene *Ur-13* from Redland Pioneer and *Ur-6* from GGW. In this research, only race 27-7 is virulent on *Ur-13*, representing 1.8% of the total isolates. Race 54 was the only race virulent on the *Ur-6* gene from GGW based on the new differential set; currently 100% of isolates are virulent on this gene.

Genes of Middle American origin, such as *Ur-11*, convey resistance to most rust races currently reported except Honduran race, 22-52 (108) (Acevedo et al., 2013; Hurtado-Gonzales et al., 2017b). The NDSU dry bean breeding program released a new line, ND-Falcon that possess the *Ur-11* gene and is resistant to the most frequent *U. appendiculatus* races in the state (Osorno, 2019; Chapter 3). A new source of resistance had been reported, Andean landrace G19833 (Chaucha Chuga), with a wider resistance spectrum than any other characterize resistance gene (Hurtado-Gonzales, et al., 2017a). The gene from G19833 is resistant to race 22-52 (108), which is virulent on *Ur-11* (Hurtado-Gonzales et al., 2017a).

The discovery of new single dominant resistant genes are promising; however, it has some limitations. The use of a uniquely single dominant gene can create selection for that resistant gene, as previously observed with the *Ur-3* gene. Because single resistance genes introgressed into cultivars have been repeatedly overcome by the pathogen, gene pyramiding and integrating quantitative trait loci are now recommended over single gene resistance (Acevedo et al., 2013; Souza et al., 2008; Liebenberg and Pretorious, 2010).While these approaches are both more time and labor intensive than incorporating single genes, this effort is warranted due to the losses incurred common ben rust worldwide.

2.5. Conclusions

Uromyces appendiculatus, the causal agent of dry bean rust, is a limitation to dry bean production worldwide. Based on surveys conducted in 2015 and 2016 in North Dakota, rust reached 50% to 80% severity in some fields, and up to 100% incidence. Across both years, race 20-3, previously reported in the state in 2008, was the most prevalent race characterized. In 2015 and 2016 11 and 14 *U. appendiculatus* races were identified, respectively. Thus, studies are recommended to determine the genetic diversity. Based on the characterization of various *U. appendiculatus* races, we can speculate that sexual recombination is taking place across North Dakota. Race 29-33, found on 2016 is virulent on all differential genes but *Ur-11*, limiting possible sources of resistance. Lines containing resistance gene *Ur-11* displayed an immune response to all of the races characterized, portraying an excellent gene for breeding programs. Based on this results we suggest a different approach to rust resistance breeding, one that includes gene pyramiding of multiple existing and/or new resistant genes, or quantitative trait loci (QTL) horizontal resistance.

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3. SCREENING FOR RESISTANCE TO BEAN RUST (*UROMYCES APPENDICULATUS)* AND IDENTIFYING GENOMIC REGIONS ASSOCIATED WITH RESISTANCE TO RACES PREVALENT IN NORTH DAKOTA

3.1. Introduction

North Dakota is the primary common bean producing state in the US, where bean rust caused by *Uromyces appendiculatus* (Pers.:Pers.) Unger has a history of damage and represents a risk of future epidemics and economical losses. Historically, bean rust epidemics had been reported repeatedly during the 1970's, 1980's and 1990's in North Dakota. The last rust epidemic reported in the state occurred in 1996 and resulted in 16% seed yield losses and $12 million USD in economic losses (Venette et al., 1998).

U. appendiculatus is an autoecious, macrocyclic, biotrophic fungus. Diversity in this fungus is based on race characterization following a gene for gene interaction with host *Phaseolus vulgaris* L. Common bean has two major gene pools, Middle American and Andean. However, resistant genes from both origins have been pyramided across gene pools. Correspondingly, *U. appendiculatus* races can be characterized as Middle American, Andean, or a mixture (Araya et al., 2004).

Multiple bean differential sets have been created to race characterize *U. appendiculatus* isolates based on virulence reaction to rust resistance genes. Based on the original differential set of 19 bean lines, five pathogen races were characterized in North Dakota in the early 1990's. The races identified, 52, 54, 69, 70, and 71, were virulent on known resistance genes *Ur-6*, *Ur-6+*, *Ur-4* and *Ur-13* (Gross and Venette, 2002; Stavely et al., 1983). These races were also virulent on other unknown resistance genes from bean lines US3, CSW643, Pinto650, KW765, KW780 and KW814. No *U. appendiculatus* races reported in North Dakota from 1996 to 2000 were virulent

on resistance gene *Ur-3* (Gross and Venette, 2002). The *Ur-3* gene has been incorporated into germplasm developed at North Dakota, Michigan and Nebraska since the 1980's (Adams et al., 1986). Pinto beans such as Lariat (2007), Stampede (2007), and ND-307 (2008) were marketed as rust resistant, due to the incorporation of *Ur-3* gene (Osorno et al., 2009; 2010). Navy bean varieties such as Norstar (1991) and Avalanche (2008) (Grafton et al., 1993; Osorno et al., 2011); along with black bean Eclipse (2004) were resistant to rust races present at that time in the state (Osorno et al., 2009). In 2008, a new race, 20-3, was reported in North Dakota, resulting in susceptibility of 27 commonly grown varieties (Markell et al., 2009). Race 20-3 is virulent on *Ur-3*, *Ur-6*, *Ur-7*, and an unknown gene from Montcalm based on the new rust differential set containing 12 common bean lines (Markell et al., 2009; Steadman et al., 2002). The appearance of this new race presented a new threat of dry bean rust epidemics in North Dakota. All varieties currently grown in North Dakota are susceptible to race 20-3, and other *U. appendiculatus* races that overcome *Ur-3* (Knodel et al., 2019). While epidemics have not been observed in recent years, growers have suffered economic losses, even with the application of fungicides. Rust prevalence in North Dakota has increased over the last eight growing seasons ranging from 13% in 2013 to 40% of hectares reported in 2016 (Knodel et al., 2014, 2015, 2016, 2017). More recently, rust prevalence in 2017 and 2018 were reported at 37.1% and 15.7%, respectively (Knodel et al., 2018, 2019).

Urediniospores of *U. appendiculatus* have been reported to survive North Dakota winters; including a period of several months with no living host tissue and temperatures below freezing (Gross and Venette, 2001). In addition, evidence of sexual reproduction, pycnia and aecia, have been reported in the state, although this is believed to be uncommon in the field (Venette et al., 1978). Eighteen *U. appendiculatus* races in North Dakota were characterized in 2015 and 2016

(Chapter 2). More than 98% of isolates were virulent on resistance genes *Ur-6*, and the unknown gene from Montcalm of Andean origin. Approximately 20% of isolates were virulent on Andean genes *Ur-4* and *Ur-9*; while 2% were virulent on *Ur-13*. More than 97% of isolates were virulent on Middle American genes *Ur-3* and *Ur-7*. While 6% of isolates were virulent on *Ur-3+*; 5.8% on *Ur-5*, and 3.3% on *Ur-CNC* (Chapter 2).

Phaseolus vulgaris genetic maps have been used to localize major rust resistant genes (Kelly et al., 2003; Miklas et al., 2002). *Uromyces appendiculatus* resistance gene *Ur-9* was mapped to chromosome Pv01; *Ur-5*, *Ur-14*, and *Ur-Dorado* were mapped to Pv04; *Ur-4* was mapped to Pv06; *Ur-12* was mapped to Pv07; and *Ur-13* was mapped to Pv08 (Miklas et al., 2006). *Ur-12* confers adult plant resistance (APR), an specific type of partial resistance expressed as small pustules on leaves grown beyond the third trifoliolate stage (Jung et al., 1998). Six resistant genes have been mapped to Pv11 including *Ur-3, Ur-3+, Ur-6, Ur-6+, Ur-7, Ur-Dorado53*, and *Ur-11*. The locations of genes *Ur-1, Ur-2, Ur-8, Ur-10, Ur-Montcalm, Ur-CNC, Ur-15, Ur-G19833*, and *Ur-G40022* have not been determined (Miklas et al., 2006; Pastor-Corrales and Steadman, 2015). Fine mapping localized the widely used *Ur-3* on Pv11 from 46.96 to 47.01 Mb, about 45.6kb sized genomic region (Hurtado-Gonzalez et al., 2017b). However, fine mapping for many of resistant genes remain largely unknown.

The breakdown of *Ur-3* resistance and the epidemic potential of common bean rust, have made finding new sources of resistance a priority to common bean breeding programs. Most recent breeding efforts at North Dakota State University successfully incorporated the *Ur-11* gene into ND-Falcon (J. Osorno, personal communication). Thus, this research aims to identify and map new potential sources of resistance to *U. appendiculatus* among diversity panels and advanced breeding lines in the NDSU breeding program.

3.2. Materials and methods

3.2.1. Pathogen collection

Common bean fields were surveyed during the 2015 and 2016 growing seasons in the main growing counties of North Dakota. Isolates were collected and race typed as previously described (Chapter 2). The most commonly observed race, 20-3, across both years was selected for germplasm screening; along with race 29-3 and 27-7.

3.2.2. Germplasm evaluations

Advanced Breeding Lines (ABL-194 lines) from the NDSU dry bean breeding program, and a subset from the Middle American diversity panel (MDP) that included genotypes from both the Mesoamerican race (103), Durango race (187), and part of the Andean diversity panel (ADP-49) were screened for resistance. One isolate from each of three *U. appendiculatus* races (20-3, 29-3, and 27-7) were used to screen lines in this study. Isolate UP15-4-2-SP1, race 20-3; isolate UP15-2-1-SP9, race 29-3, were both collected in 2015; and isolate UP16-10-1-SP1, race 27-7, was collected in 2016. Race 20-3 is virulent on the *Ur-3, Ur-6, Ur-7,* and unknown resistance gene from Montcalm. Race 29-3 is virulent on the same genes as race 20-3 plus *Ur-4* from Early Gallatin and *Ur-9* and *Ur-12* from PC50. Race 27-7 is virulent on *Ur-3, Ur-4, Ur-5, Ur-6, Ur-7, Ur-9, Ur-12* and *Ur-13*. In contrast to 20-3 and 29-3, this race is not virulent to unknown gene from Montcalm.

ABL and lines from the Mesoamerican bean race were screened with all three races 20-3, 29-3 and 27-7. The Durango race and Andean Diversity Panel (ADP) were screened with race 20-3 and 27-7. Line PI14 (Singh, University of Idaho) was used as the susceptible check in all evaluations. Evaluations of each market class also included commonly grown varieties as agronomic checks. ABL include black (45 lines), great northern (20), navy (24), pinto, including

slow darkening (37), and small red (18) market classes from Middle America background; and light red kidney (20), and dark red kidney (30) market classes from Andean background. Greenhouse inoculations to determine the reaction of these lines to *U. appendiculatus* followed the protocol described in chapter 2 for race determination on the differential set. Pustule measurement (diameter) was averaged across 10 plants and converted to a reaction type 1 to 6, where a disease reaction of ≥4,3 was considered a susceptible reaction (Table 3.1) (Stavely et al. 2002).

Table 3.1. Disease scale used to determine reaction of the advanced breeding lines and Mesoamerican Diversity panel.

Reaction type[a]	Symptom description[b]	Score[c]	Rust reaction
1	No visible symptom	1.1	Resistant
2	Necrotic spots without sporulation	2.1	Resistant
2,3	Reaction 2 with few type 3	2.4	Resistant
3,2	Reaction 3 with few type 2	2.7	Resistant
3	Uredinia <0.3 mm in diameter	3.1	Resistant
3,4	Reaction 3 with few type 4	3.4	Resistant
4,3	Reaction 4 with few type 3	3.7	Susceptible
4	Uredinia 0.3-0.49 mm in diameter	4.1	Susceptible
4,5	Reaction 4 with few type 5	4.4	Susceptible
5,4	Reaction 5 with few type 4	4.7	Susceptible
5	Uredinia 0.5-0.8 mm in diameter	5.1	Susceptible
5,6	Reaction 5 with few type 6	5.4	Susceptible
6,5	Reaction 6 with few type 5	5.7	Susceptible
6	Uredinia 0.8- 1.2 mm in diameter	6.1	Susceptible

[a] Reaction type based on correspondent pustule diameter measured using a comparator.
[b] Modified from Stavely et al., 2002.
[c] Quantitative disease score based on Mmbaga et al. 1996.

3.2.3. Genome wide association studies

Single nucleotide polymorphism (SNP) data from 131 breeding lines of Middle American background from the ABL from the NDSU dry bean breeding program were used to conduct genome wide association studies (GWAS) (Simmons et al., 2019; Simmons, personal communication). In addition, 286 lines from the MDP including the Mesoamerican and Durango

bean races were used for GWAS (Oladzad et al., 2019). GWAS used phenotypic data based on the mean pustule diameter (mm) and reaction type (1 to 6 scale) (Table 3.1). Libraries from both plant populations were generated using a two enzyme (*TaqI* and *MseI*) protocol (Schroder et al., 2016) and Illumina Hi-Seq 2500 (Hudson Alpha Genome Sequencing Center, Huntsville, AL, USA).

Population structure, kinship matrix, and genome wide association mapping were conducted separately for MDP and ABL using JMP Genomics 9.0 software (SAS 2017). SNP markers with minor allele frequency less than 0.05% and more than 50% missing data were removed from both analyses. Principal component analysis (PCA) was performed to determine population structure, representing the genetic similarities among genotypes. A kinship (K) matrix was computed to represent the proportion of shared alleles for all pairwise comparisons for each population. Mixed Model analysis was conducted at p value < 0.001 (-log10 (p)=3). The p values were adjusted by calculating positive false discovery rate (pFDR) (Benjamini and Yekutieli, 2001). Marker-Trait associations were considered significant at pFDR=0.01 and 0.1 for MDP and ABL, respectively. To represent the percentage of phenotypic variation explained by the associated SNPs, adjusted R^2 values were estimated for linear regression model. Candidate genes were determined when the significant SNP was in the gene or located within a 100 Kb region from the gene. Phytozome.net database was used to identify candidate genes.

3.3. Results

3.3.1. Germplasm screening - advanced breeding lines (ABL)

Overall, 27.9% of the 194 breeding lines expressed resistance to race 20-3; likewise, 25.4% were resistant to race 29-3; and 29.5% to race 27-7 (Table 3.2). Black bean varieties Blackcat, Eclipse, Loreto, Zenith, and Zorro were highly susceptible to the three *U. appendiculatus* races evaluated here. Among black bean ABL 37.7% (17 lines) of lines were resistant to race 20-3,

31.1% (14) to race 29-3, and 51.1% (23) to race 27-7. Thirteen black bean lines were resistant to all races with reactions ranging from a hypersensitive response to small pustules. Great Northern varieties Aries, Draco, Powderhorn, and Taurus, and all great northern ABL were susceptible to all three *U. appendiculatus* races. Pinto varieties La Paz, Lariat, Monterey, SDIP-1, Vibrant, Windbreaker, and WM-2 were susceptible to all races as anticipated. Among pinto ABL, 16% (6) were resistant to race 20-3, 13% (5) to race 29-3, and 19% (7) to race 27-7. Five lines were resistant to all *U. appendiculatus* races. Navy bean varieties Ensign, Medalist and T9905 were susceptible to all three races. Among navy bean ABL, 8.3% (3) were resistant to race 20-3, 4.1% (1) to race 29-3, and 8.3% (3) to 27-7. Line ND131990 was resistant to all three *U. appendiculatus* races expressing either an immune (20-3) or hypersensitive response (29-3 and 27-7). Small red varieties Merlot, Rosetta, Ruby and Sedona were susceptible to all races. Among small red ABL only NDF140741 was resistant, displaying an immune response to race 20-3 and 27-7, and small pustules to race 29-3.

The dark red kidney variety Talon was resistant to race 20-3. Among dark red kidney ABL, 43.3% (13) were resistant to race 20-3, 20% (6) to race 29-3, and 43.3% (13) to race 27-7. Lines 1941, 1988, 2001, 2073, and 2074 were resistant to all three races with reactions including immune, hypersensitive or small pustule. Light red kidney bean variety Foxfire was resistant to races 20-3 and 27-7, but susceptible to 29-3. Among light red kidney ABL, 30% (6) were resistant to 20-3, 20% (4) to 29-3, and 35% (7) to 27-7. Lines 1922, 1995, 1998L and 2070 were resistant to all three races. Line 1996 was resistant to both 20-3 and 27-7.

Table 3.2. Reaction of advanced pinto, great northern, small red, navy, black, dark red and light red kidney bean germplasm from the North Dakota Experimental Agricultural Station breeding program to *Uromyces appendiculatus* race 20-3, 29-3 and 27-7.

Market class[a]	Line	Reaction type[b]			Market class[a]	Line	Reaction type[b]		
		20-3[c]	29-3[d]	27-7[e]			20-3[c]	29-3[d]	27-7[e]
Black					23	ND132617	4,5,3	6,5	3,2
1	Blackcat	6,5,4	6,5	5,4	24	ND132698	2,1	3,2	1
2	Eclipse	6,5,4	5,6	4,3	25	ND132700	3,4	4,3	2,3
3	Loreto	6	6,5	5,6	26	ND132719	4,5	4,3,5	3,2,4
4	Zenith	6	6,5	6,5,4	27	ND132788	5,4	6	6,5
5	Zorro	6,5	6	5,4,6	28	NDF120002	1	5,4,6	2
6	ND060769	3,4	6,5,4	4,3	29	NDF120039	5,4	6,5	6,5,4
7	ND071089	2	3,2	2,3	30	NDF120055	6,3	4,3	3,2
8	ND071244	3,2	3,4,2	2,3	31	NDF120066	2,3	3,2	3,2
9	ND071249	3,2	3,4	3,2	32	NDF120070	3,2,4	3,2	3,4
10	ND071257	2,3	3,4,2	3,2	33	NDF120117	6	6	6
11	ND081144	5,6	6,5	5,4,6	34	NDF120253	3,4	6,5	3,2
12	ND081147	5,6,4	6,5	6	35	NDF120259	2,3	3,4	1
13	ND081247	5,4	5,4	3,2	36	NDF120272	6,5	3,2	1
14	ND081295	5,4	6	5,4,3	37	NDF120276	4,5	3,2	1
15	ND081340	4,5	6,5,4	5,6	38	NDF120287	5,6	4,3	1
16	ND132375	6,5	6,5	5,4,3	39	NDF120299	6	6	6
17	ND132381	5,6	6	6,5	40	NDF120304	5,6,4	6,5	4,5
18	ND132392	4,5	5,4,3	3,4,2	41	NDF120329	6,5	6,4	4,3
19	ND132571	3,2	3,4	3,2	42	NDF120334	6,5	4,3	3,2
20	ND132581	3,2,4	3,4	3,4	43	NDF120346	4,5	5,4	4,5
21	ND132590	3,2	3,4	3,2	44	NDF120366	6,5	6,5	4,3,5
22	ND132599	2,3	3,4,2	2,3	45	NDF09304	2,3	4,3	-[f]
GN	Aries	6	6	6,5	11	ND121676	6	6	5,6
2	Draco	6	5,6	4,5,6	12	ND121686	5,6	6,5	4,3
3	Matterhorn	6,5	6	4,5,3	13	ND121692	6,4,5	5,4	4,3
4	Powderhorn	6	6,5	6	14	ND121698	6	6,5	5,4
5	Taurus	6	6	6,5	15	NDF140812	6	6,5,4	6,5
6	ND09726	6,5	5,4	4,3	16	NDF140813	6	6	6
7	ND112828	4,5	6,5	4,5,3	17	NDF140820	6	6	6,4,3
8	ND112843	5,4,6	5,4	4,5	18	NDF140831	6,4,3	6	5,4
9	ND121630	6	6	6,5,4	19	NDF140832	6,5	6	4,3,5
10	ND121660	6	6	4,5,6	20	ND12-1630	6	6	-[f]

(*continued on next page*)

Table 3.2. Reaction of advanced pinto, great northern, small red, navy, black, dark red and light red kidney bean germplasm from the North Dakota Experimental Agricultural Station breeding program to *Uromyces appendiculatus* race 20-3, 29-3 and 27-7 (continued).

Market class[a]	Line	Reaction type[b]			Market class[a]	Line	Reaction type[b]		
		20-3[c]	29-3[d]	27-7[e]			20-3[c]	29-3[d]	27-7[e]
Pinto					19	ND131502	5,4,6	6,5	6,5,4
1	La Paz	6	6,5	6,5,4	20	ND131543	5,6	5,4,6	6,5,4
2	Lariat	6	6,5	5,4	21	ND131551	6	5,6	5,4,6
3	Monterrey	6	6,5,4	4,5	22	NDF140646	6,5	5,4,3	6,5
4	Windbreaker	5,6	6,5	5,4,6	23	NDF140658	5,6	6,5,4	6,5
5	ND121229	6,5	6,5	5,6,4	24	NDF140660	6,5	5,4	6,5
6	ND121237	3,2	6,5	3,2	25	NDF140663	6	6	6,5
7	ND121315	4,5	5,4	5,4,6	26	NDF140673	6,5	6,5	6
8	ND121352	6,5	4,3,5	6,5	27	NDF140682	6	6,5	6,5
9	ND121429	6	5,4	6,5,4	28	ND131405	5	6,5	5,6
10	ND121447	2	2	2	29	ND131406	6,5	6,5	6,5
11	ND121448	2	2	2	30	ND131413	6	6	6,5
12	ND121449	2	2	2	31	NDF140505	6,5	6,5	6,5,4
13	ND121453	2	2	2	32	NDF141506	6,5	5,6	6,5
14	ND121479	6	6	6	33	NDF141507	6	6	6,5,4
15	ND121508	5	4,3	3,2	34	Palomino	6	6	5,4
16	ND121531	6	6,5	6,5	35	SDIP-1	6	6	6,5
17	ND121540	6	6,5,4	6,5,4	36	Vibrant	6	6	6,5
18	ND121560	3,2	3,2	2	37	WM-2	6	6	6,5,4
SR	Merlot	6	6	6,5,4	10	NDF140722	6,5,4	6	6
2	Rosetta	5,6,4	6,5	6,5,4	11	NDF140723	6,5	6	6
3	Ruby	5,6	5,6	5,4	12	NDF140725	5,6	6,5	4,3,5
4	Sedona	6,5,4	5,4	6	13	NDF140726	6	6,5	6,5
5	ND112929	5,4	6	6,5	14	NDF140735	5,6,4	6	6
6	ND121885	5,6	5,6	6,5	15	NDF140736	6	6	6
7	NDF140712	5,4	6,4	5,6	16	NDF140741	1	3,2	1
8	NDF140719	5,4	6	6	17	NDF140743	5,4,3	6,5	6
9	NDF140720	6,5	4,5	4,3,5	18	NDF140750	6	6,5	6,5
Navy	Ensign	6	6	6,5,4	8	ND122082	6,5	6,5	4,5,3
2	Medalist	5,6,4,3	5,6	4,3,5	9	ND122105	5,4	4,6	4,5,3
3	T9905	5	5	5,6	10	ND122114	5,4	5,6	5,4
4	ND070612	5	6	5,6	11	ND131705	3,2	6,5	4,5
5	ND122056	5,4	5,3	6,5	12	ND131762	5,6	6	6,5
6	ND122080	6	6	6,5,4	13	ND131807	3,4	6,5	4,5
7	ND122081	6	6	4,5	14	ND131905	5,4	6,5	6,5

(*continued on next page*)

Table 3.2. Reaction of advanced pinto, great northern, small red, navy, black, dark red and light red kidney bean germplasm from the North Dakota Experimental Agricultural Station breeding program to *Uromyces appendiculatus* race 20-3, 29-3 and 27-7 (continued).

Market class[a]	Line	Reaction type[b]			Market class[a]	Line	Reaction type[b]		
		20-3[c]	29-3[d]	27-7[e]			20-3[c]	29-3[d]	27-7[e]
Navy	ND131990	1	2	2	20	ND132208	5,6	6	6,5,4
16	ND132023	5,6	6,5	4,5,3	21	ND132234	6,5	5,6	4,5,6
17	ND132048	4,5	6	6,5	22	ND132254	4,3	4,3	3,4
18	ND132049	5,6	6	6,5,4	23	ND131900	6	6	6,5,4
19	ND132126	6,5	6	6,5	24	ND131717	6	6	5,6,4
DRK	Montcalm	5,4	6,5	6,5	16	2036	4,3	6,5,4	6,5
2	Redhawk	4,5	5,6	6	17	2038	4,3	3,2	4,3
3	Talon	2	6,5	5,4	18	2044	4,3	6,5,4	3,4
4	1885	5,4	4,3	5,4	19	2046	5,6	6	5,6
5	1941	2	3,2,4	2	20	2073	3,2	4,3,2	3,2
6	1988	2	3,2,4	2	21	2074	2	2,3,2	2
7	1998D	2	5,4	2	22	2077	5,4	6,4	5,4
8	2000	3,4	4,3	2,3	23	2096	6,5	4,3,5	3,4
9	2001	2	3,2	2	24	2112	5,4	6	6,5
10	2006	2	6,5,4	4,3	25	2113	5,4	6,5	5,6
11	2010	3,2	3,4,2	2,3,4	26	2114	5,4	5,6	5,6
12	2013	2	5,6	2	27	2116	4,5,3	6,5	5,4,3
13	2015	2	4,3,5	2	28	2118	5,4,3	5,4	6
14	2016	5,6	4,3	5,4	29	2131	4,3,5	6,5,4	3,4
15	2026	2	4,3,2	2	30	2133	6,5	6,5	5,6
LRK	Foxfire	2	4,3	2	11	1996	2	4,3	2
2	Pink Panther	4,5	6,5	3,4	12	1998L	2	3,2	2
3	Rosie	6,5,4	5,4	6,5	13	2030	6,5,4	4,5	4,3
4	1878	5,6	6,5,4	6,4	14	2035L	5,6	4,3,5	5,4
5	1882	6,5	5,4,6	5,4	15	2040	5,6	6,5	4,5
6	1914	4,5,6	6,5	4,3	16	2070	2	3,2	2
7	1922	2	2,3,4	2	17	2082L	4,3,2	5,6	4,5
8	1932	4,5	5,4,3	5,6	18	2102	6,5	5,6	5
9	1936	4,3,5	4,5,3	6,5,4	19	2120	5,4,3	6,5	4,3
10	1995	2	3,2,4	2	20	2121	5,6	5,4	5,4

[a] Market Class: GN=Great Northern; SR= small red; DRK= dark red kidney, LRK=light red kidney

[b] Reaction grades 1= No visible symptoms; 2= Necrotic spots without sporulation; 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0.3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0.3-0.49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0.5-0.8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= Reaction 6 with few type 5; 6= Uredinia 0.8-1.2 mm in diameter.

[c] Isolate UP15-4-2-SP1 was used to represent race 20-3

[d] Isolate UP15-2-1-SP9 was used to represent race 29-3

[e] Isolate UP16-10-1-SP1 was used to represent race 27-7

[f] (-)= missing data, line not evaluated

3.3.2. Genome wide association studies - advanced breeding lines (ABL)

Genome wide association studies (GWAS) were conducted on 131 breeding lines developed by the NDSU dry bean breeding program. Reaction types were determined for 196 breeding lines; however, only the 131 lines belonging to the Middle American gene pool, including lines from the black, great northern, navy, pinto, and small red market classes, were selected for GWAS based on genetic relatedness. Principal component analysis (PCA) was conducted to determine the structure of the population before conducting the association mapping. First principal component (PCA1) explained 23.2% of the variation; PCA2 explained 4.9%, and PCA3 explained 3.7% of variation (Figure 3.1).

GWAS was conducted using the pustule diameter (continuous; mm) and reaction type (discrete; 1-6) for each race evaluated, 20-3, 29-3 and 27-7, and genotypic data using 41,998 SNP markers (Simmons, personal communication). GWAS using reaction type (1-6) generated a total of 63 SNP markers in association with all three *U. appendiculatus* races at correction pFDR=0.1 (Table 3.3). Twenty-four significant SNP markers were observed across chromosomes Pv01, Pv03, Pv04, Pv06, Pv08, and Pv10 when evaluating with race 20-3 (Table 3.3; Figure 3.2A). In association with race 29-3, a total of 29 significant SNP on Pv04, Pv08, Pv09 and Pv10 were observed (Table 3.3; Figure 3.2B). Finally, a total of 10 significant SNP were found on Pv07, Pv10 and Pv11 in association to race 27-7 (Table 3.3; Figure 3.2C). Based on pustule diameter (mm), marker trait associations with race 20-3 and 29-3 were not significant at pFRD=0.1 (Table 3.3). However, associations with race 27-7 resulted in 12 SNP markers using pustule diameter. One

marker was observed on chromosome Pv08, ten on Pv10 resulting in four distinctive peaks, and one on Pv11.

Table 3.3. Single nucleotide polymorphism (SNP) markers from genome wide association studies (GWAS) of Advanced Breeding Lines (ABL) from the north Dakota Experiment Station common bean breeding program in association with *Uromyces appendiculatus* races 20-3, 29-3, and 27-7. Results include significant SNP markers based on pustule diameter (mm) and Reaction type (1 to 6).

Race	Type of data	Interval		Location	Peak SNP			No. Sig. SNP[c]
		Chr[a]	Genomic interval (Mb)		Position (Mb)	-Log10(P)	% Var[b]	
20-3	PD[d]	1	16508788	16508788	SNP01_16508788	3.03729	11	-
		2	15561200	15561200	SNP02_15561200	3.67791	10	-
		3	20700526	20700526	SNP03_20700526	4.77580	14	-
		4	10288209	10288209	SNP04_10288209	4.29634	15	-
		5	14241678	14241678	SNP05_14241678	3.99881	14	-
		6	12083919	12083919	SNP06_12083919	4.31326	15	-
		7	9248766	9248766	SNP07_9248766	3.46750	12	-
		8	33181674	33181674	SNP08_33181674	3.77607	13	-
		9	34165615	34165615	SNP09_34165615	3.13101	11	-
		10	7807565	7807565	SNP10_7807565	3.71888	13	-
		10	20741936	20741936	SNP10_20741936	3.69754	13	-
		11	51572039	51572039	SNP11_51572039	4.60711	16	-
		11	13262519	13262519	SNP11_13262519	3.62419	12	-
		11	26107321	26107321	SNP11_26107321	3.44046	12	-
20-3	VP[e]	1	25215993-25216065	25216065	SNP01_25216065	4.96932	15	2
		3	20700526-22954686	20700526	SNP03_20700526	5.78917	17	2
		4	10288209-45031490	10288209	SNP04_10288209	4.38876	15	3
		6	12083901-28871828	15691516	SNP06_15691516	4.64974	16	4
		8	33181674	33181674	SNP08_33181674	4.65225	16	1
		10	9486712-35436691	7807565	SNP10_7807565	4.46480	16	12
						Total		24
29-3	PD	1	49772322	49772322	SNP01_49772322	3.69582	13	-
		1	49772378	49772378	SNP01_49772378	3.69582	13	-
		3	20700526	20700526	SNP03_20700526	3.19681	9	-
		4	10288209	10288209	SNP04_10288209	4.50180	15	-
		5	5360870	5360870	SNP05_5360870	3.96882	14	-
		6	27757889	27757889	SNP06_27757889	4.26729	12	-
		7	29208521	29208521	SNP07_29208521	3.68510	13	-
		8	34013980	34013980	SNP08_34013980	3.81860	13	-

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Table 3.3. Single nucleotide polymorphism (SNP) markers from genome wide association studies (GWAS) of Advanced Breeding Lines (ABL) from the north Dakota Experiment Station common bean breeding program in association with *Uromyces appendiculatus* races 20-3, 29-3, and 27-7 (continued). Results include significant SNP markers based on pustule diameter (mm) and Reaction type (1 to 6).

Race	Type of data	Interval		Location	Peak SNP			No. Sig. SNP[c]
		Chr[a]	Genomic interval (Mb)		Position (Mb)	-Log10(P)	% Var[b]	
29-3	PD[d]	9	3216947	3216947	SNP09_3216947	3.42043	12	-
		10	2972136	2972136	SNP10_2972136	4.13145	14	-
29-3	VP[e]	4	1327300-10288231	10288209	SNP04_10288209	4.61924	16	4
		8	48229223	48229223	SNP08_48229223	4.60195	14	1
		9	3216947-3216975	3216975	SNP09_3216975	4.55655	16	3
		10	2743109-35436626	35132812	SNP10_35132812	4.51893	13	21
						Total		29
27-7	PD	8	33022348	33022348	SNP08_33022348	4.50769	13	1
		10	9063313-9223640	9223587	SNP10_9223587	5.44614	18	5
		10	20741838-20741944	20741936	SNP10_20741936	4.96246	17	3
		10	33535404-36614519	36614519	SNP10_36614519	4.91478	17	2
		11	51572039	51572039	SNP11_51572039	4.55922	15	1
						Total		12
27-7	VP	7	9599034-27278857	27278857	SNP07_27278857	4.82421	17	3
		10	9063313-36614519	9223587	SNP10_9223587	6.17740	21	6
		11	27626184	27626184	SNP11_27626184	4.55582	16	1
						Total		10

[a] Chr= Chromosome location.

[b] Percent (%) of variation.

[c] Number of significant SNP markers on the delimited region.

[d] PD=Analysis based on pustule diameter (mm).

[e] VP=Analysis based on Reaction type (1-6).

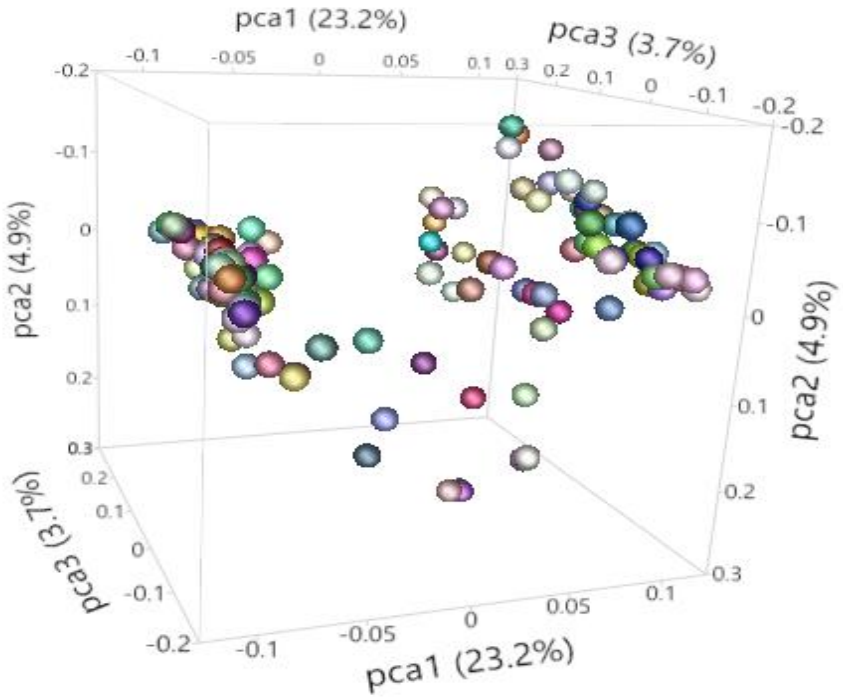

Figure 3.1. Principal component analysis (PCA) indicating the population structure of 131 advanced breeding lines (ABL) from the North Dakota Experiment Station common bean breeding program.

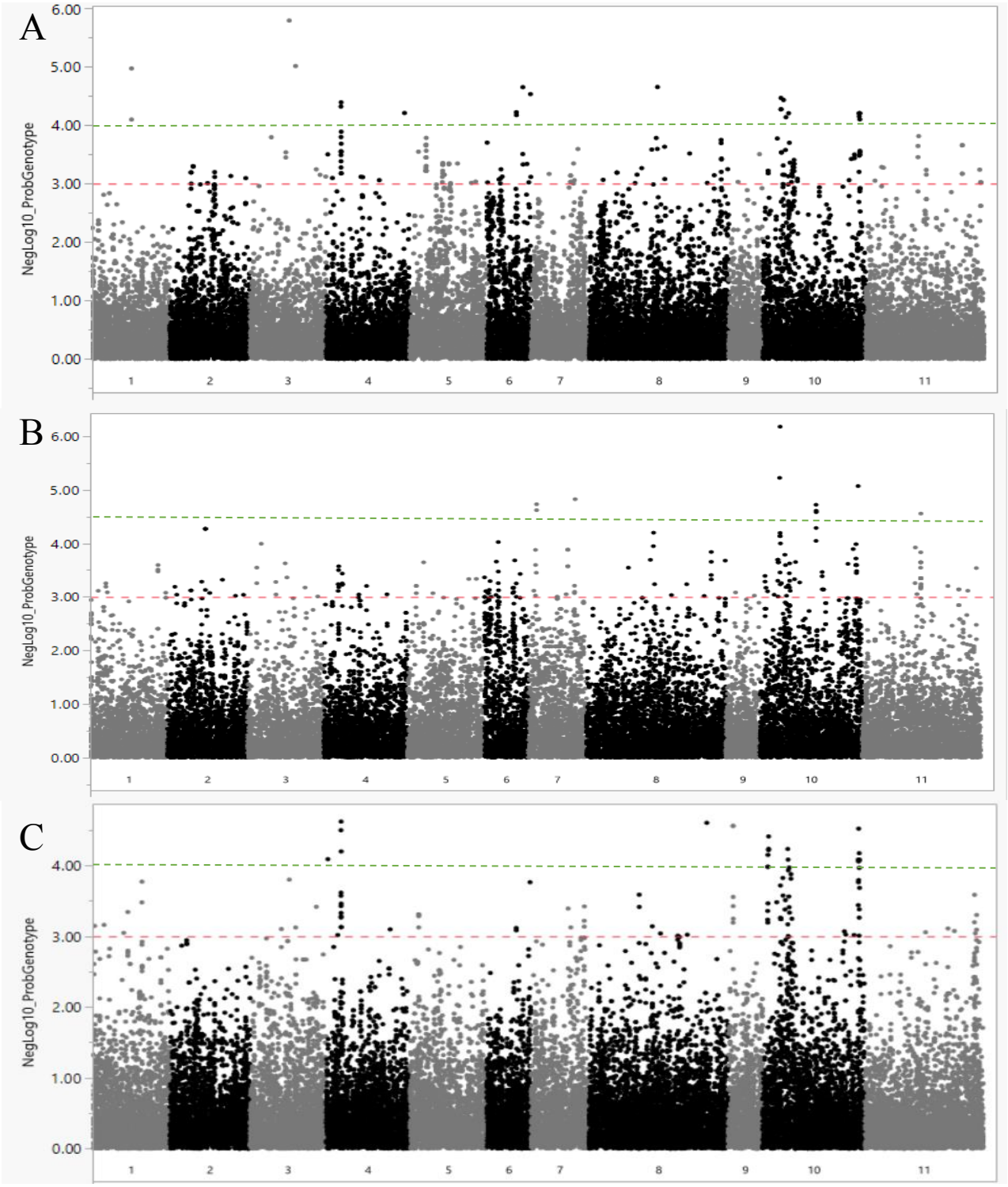

Figure 3.2. Manhattan plot showing marker trait associations on advanced breeding lines (ABL) from the Middle American gene pool reaction to race 20-3 (A), 29-3 (B), and 27-7 (C) using virulence phenotype (1 to 6). *Phaseolus vulgaris* chromosomes (1-11) are represented on x axis, a –log 10 (p) values are shown on y axis. Red line indicates threshold at –log10(p) value of 3. Green line represent pFDR=0.1 correction of significance.

3.3.3. Candidate genes in advanced breeding lines (ABL)

Significant associations were observed from reaction type on chromosomes Pv01, Pv03, Pv04, Pv06, Pv08, and Pv10 when 131 ABL were inoculated with race 20-3 (Figure 3.2). GWAS analysis using reaction type to race 29-3 revealed associations on chromosomes Pv04, Pv08, Pv09 and Pv10 (Figure 3.2). Significant marker associations were present on only three chromosomes Pv07, Pv10 and Pv11 with resistance to *U. appendiculatus* race 27-7 based on reaction type (Figure 3.2). None of the markers observed with reaction type were associated with a specific gene. However, based on pustule diameter, one marker (SNP11_51572039) located on Pv11 was directly associated with the Phvul.011G200800 gene model that encodes to an LRR-NB-ARC resistance protein in reaction to race 20-3 and 27-7.

3.3.4. Germplasm screening - middle american diversity panel (MDP)

A total of 290 lines from the Mesoamerican race (102), Durango race (188) were screened with *U. appendiculatus* race 20-3 and 27-7. In addition, lines from the Mesoamerican race were screened with race 29-3, although these evaluations were not repeated. Lines of the Mesoamerican race evaluated included, 47 black, 43 navy, three great northern, five small white, two tan, one cream, one carioca and one black mottled seed types. Lines from the Durango race included 93 pinto, 39 great northern, 29 small red, 22 pink, 2 red, one flor de mayo and one cranberry market class types.

Among lines evaluated from the Mesoamerican race, 12 were resistant to races 20-3, 25 to race 27-7 and 16 to race 29-3 (Table 3.4). Five black bean lines: 115M Black Rhino, 92BG-7, Blackjack, I9365-31, and PR 0443-151 were resistant to all races. In addition, two lines were resistant to 20-3, eight lines were resistant to 29-3, and 12 to 27-7. Great northern lines BelMiNeb-RMR-4, BelMiNeb-RMR-7, and BelMiNeb-RMR-8 were resistant to races 20-3, 29-3 and 27-7.

All three lines possess the *Ur-11* gene, among other known resistant genes (Table 3.4) (Hurtado-Gonzales et al., 2017; Pastor-Corrales, 2003). Navy bean line Swan Valley was resistant to 20-3, 29-3 and 27-7. In addition, one navy line, Newport, was resistant to 20-3 and 27-7; and two, Michelite and Neptune, were resistant to 29-3 and 27-7. Small white line Morales was resistant to races 20-3 and 27-7.

Table 3.4. Reaction type, known resistance gene(s) and release source of lines from the Mesoamerican race in reaction to *Uromyces appendiculatus* races 20-3 and 29-3 and 27-7.

Market class	Line	Reaction type[a]			Resistant gene(s)			Reference
		20-3[a]	29-3[b]	27-7[c]				
Cream	A285	2	6,5,4	5,4,6				Miklas, WA
Carioca	A-801	3,2	1	4,3				Miklas, WA
Tan	BAT477	5,4	4,3	6,5,4	*Ur-3*			Miklas, WA
Tan	SEA10	4,3	4,3	4,3				Miklas, WA
Black	115M Black Rhino	3,2,4	3,2,4	3,2				Kelly, MI
Black	92BG-7	3,2,4	3,2	3,2,4				Miklas, WA
Black	A-55	4,3,5	5,4,6	4,5	*Ur-5*	*Ur-6*	*Ur-7*	Miklas, WA
Black	AC-Black Diamond	4,3	5,4,3	4,3,5				Daniels, Canada
Black	AC-Harblack	5,4	4,3	5,6				Smith, Guelph, CAN
Black	Aifi Wuriti	6,5	1	5,6				Rosas, Honduras
Black	B05055	5,6	5,4	6,5				Kelly, MI
Black	Bandit	5,4	4,3,5	6,5				ADM, Decatur
Black	Black Knight	4,3,5	5,4,3	4,3	*Ur-3*			Griffiths/Sandsted, NY
Black	Black Magic	3,2	4,3	4,3				Kelly, MI
Black	Black Velvet	4,3	4,3	4,3,2	*Ur-3*			Kmiecik, Seminis
Black	Blackhawk	4,3	4,3	3,4				Kelly, MI
Black	Blackjack	1	1	1				Butcher, ID
Black	CDC Expresso	4,3	4,3	6,5				Bett, Saskatchewan, CAN
Black	CDC Jet	4,3	4,3,5	5,4,3				Bett, Saskatchewan, CAN
Black	CDC Nighthawk	5,4	4,3	5,4,6				Bett, Saskatchewan, CAN
Black	Condor	5,4	5,4,3	6,5	*Ur-3*			Kelly, MI
Black	Cornell 49-242	3,2	3,2	5,6				Kelly, MI
Black	Domino	4,3	2	3,2	*Ur-3*			Kelly, MI
Black	DPC-4	6,5	2,3,2	1				Beaver, PR
Black	Eclipse	5,6	1	4,3	*Ur-3*			Osorno, ND
Black	F04-2801-4-1-2	4,3	4,3,5	4,3				Porch/Urrea, PR-NE

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Table 3.4. Reaction type, known resistance gene(s) and release source of lines from the Mesoamerican race in reaction to *Uromyces appendiculatus* races 20-3 and 29-3 and 27-7 (continued).

Market class	Line	Reaction type[a]			Resistant gene(s)			Reference
		20-3[a]	29-3[b]	27-7[c]				
Black	F04-2801-4-5-1	4,3	3,2	3,4				Porch/Urrea, PR-NE
Black	F04-2801-4-6-6	4,3	4,3,5	3,4				Porch/Urrea, PR-NE
Black	Harrowhawk	5,4	4,3,5	5,4				Smith, Guelph, CAN
Black	I9365-31	2	3,2	1				Miklas, WA
Black	ICB-10	6	2,1	2	*Ur-3*			Miklas, WA
Black	ICB-3	5,4,3	4,3	5,4,3				Miklas, WA
Black	Jaguar	5,4	4,3	6	*Ur-3*			Kelly, MI
Black	Loreto	5,4	5,4	6				ADM, Decatur
Black	Midnight	4,3	4,3	5,6				Griffiths/Sandsted, NY
Black	ND021574	5,4	5,4,3	4,3				Osorno, ND
Black	ND021717	6	3,2,4	3,2	*Ur-3*			Osorno, ND
Black	OAC Gryphon	6	5,4	4,5				Smith, Guelph, CAN
Black Mottled	Orca	4,3	4,3	4,3				Miklas, WA
Black	Phantom	6	1	3,2				Kelly, MI
Black	PR 0443-151	2	2	2				Beaver, PR
Black	Puebla 152	4,3	3,2	3,2				Kelly, MI
Black	Raven	5,6	1	3,4	*Ur-3*			Kelly, MI
Black	Shania	5,4	1	2,1	*Ur-3*			ADM, Decatur
Black	Shiny Crow	4,3,5	5,4,3	5,4,6				Brick, CO
Black	T-39	4,3	4,3	3,2	*Ur-3*	*Ur-?*		Kelly, MI
Black	UCD 9634	6,5	5,4,3	5,6				Temple, Davis, CA
Black	UI-906	5,4,3	5,4,3	4,5	*Ur-3*			Temple, Davis, CA
Black	UI-911	6,5	5,4,6	4,5				Singh, Idaho
Black	Xan 176	6	5,4	4,3				Beaver, PR
Black	Zorro	5,4,3	5,4,3	5,4	*ur-3*			Kelly, MI
Great Northern	BelMiNeb-RMR-4	1	3,4	1	*Ur-11*	*Ur-4*	*Ur-6*	Pastor-Corrales, MD

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Table 3.4. Reaction type, known resistance gene(s) and release source of lines from the Mesoamerican race in reaction to *Uromyces appendiculatus* races 20-3 and 29-3 and 27-7 (continued).

Market class	Line	Reaction type[a]			Resistant gene(s)			Reference
		20-3[b]	29-3[c]	27-7[d]				
Great Northern	BelMiNeb-RMR-7	1	3,2	1	*Ur-11*	*Ur-4*	*Ur-3*	Pastor-Corrales, MD
Great Northern	BelMiNeb-RMR-8	1	1	1	*Ur-11*	*Ur-4*	*Ur-6; Ur-3*	Pastor-Corrales, MD
Small White	Hyden	5,4	4,3	6,5	*Ur-3*			Miklas, WA
Small White	Morales	3,4	4,3,5	3,2				Beaver- PR
Small White	NW-395	4,3,5	5,4,3	5,4				Miklas, WA
Navy	AC Compass	6,5	5,4,3	4,3	*Ur-3*			Smith, Guelph, CAN
Navy	AC Polaris	5,4,6	6,5	6,5				Daniels, Canada
Navy	Albion	5,4	4,3,5	-[e]				Kmiecik, Seminis
Navy	Arthur	5,4,3	4,3	4,3	*Ur-3*			Osorno, ND
Navy	Avalanche	6,5	5,4,6	6,5				Osorno, ND
Navy	Avanti	6,5,4	5,4,6	4,3				Kmiecik, Seminis
Navy	C-20	4,3	5,4	5,6	*Ur-3*			Kelly, MI
Navy	CDC Whitecap	5,4	5,4,3	5,6				Bett, Saskatchewan, CAN
Navy	Crestwood	5,4,6	5,4,6	5,4,6				Butcher, ID
Navy	Ensign	6,5	-[e]	6				ADM, Decatur
Navy	Envoy	4,5	6,5,4	4,5				Butcher, ID
Navy	F07-004-9-1	5,4	5,4,3	4,3	*Ur-3*			Porch/Urrea, PR-NE
Navy	Fleetwood	5,4	4,3	6,5,4				Smith, Guelph, CAN
Navy	Huron	5,4,3	5,4	4,5				Kelly, MI
Navy	HY 4181	5,4,3	5,4,6	5,6	*Ur-3*			Sprehe, Ontario, CAN
Navy	Laker	6	5,4,6	6,5				Kelly, MI
Navy	Lightning	6	5,4,3	6,5	*Ur-3*			Smith, Guelph, CAN
Navy	Mackinac	5,4	4,3	4,3				Kelly, MI
Navy	McHale	5,4,3	5,4,3	5,6,4				Kmiecik, Seminis
Navy	Medalist	4,3	4,3	6,5				ADM, Decatur
Navy	Michelite	6,5	3,4	3,2	*Ur-3*			Kelly, MI

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Table 3.4. Reaction type, known resistance gene(s) and release source of lines from the Mesoamerican race in reaction to *Uromyces appendiculatus* races 20-3 and 29-3 and 27-7 (continued).

Market class	Line	Reaction type[a]			Resistant gene(s)		Reference
		20-3[b]	29-3[c]	27-7[d]			
Navy	Midland	5,4,3	5,4,3	4,3			Kmiecik, Seminis
Navy	Morden 003	4,3,5	5,4	4,3			Hou, Manitoba, CAN
Navy	N05324	5,4,3	3,4	4,3	*Ur-3*		Kelly, MI
Navy	Nautica	6,5	6,5	6,5,4			Smith, Guelph, CAN
Navy	Navigator	5,6	5,4,3	4,3	*Ur-11*	*Ur-3*	ADM, Decatur
Navy	Neptune	4,3	3,2,4	1			Kelly, MI
Navy	Newport	1	4,3	2,1			Kelly, MI
Navy	Norstar	6,5	5,4	6,5			Osorno, ND
Navy	OAC Laser	6	5,4	4,3			Smith, Guelph, CAN
Navy	OAC Rex	6,5,4	5,4	5,4,6			Smith, Guelph, CAN
Navy	OAC Seaforth	5,4,3	5,4,6	6,5			Smith, Guelph, CAN
Navy	Reliant	5,6	5,4,6	5,4			Butcher, ID
Navy	Sanilac	5,4,3	5,4	6,5			Kelly, MI
Navy	Schooner	5,4	5,4,3	4,5	*Ur-3*		ADM, Decatur
Navy	Seabiskit	4,3	4,3	6,5,4			ADM, Decatur
Navy	Seafarer	5,4,6	5,4,6	4,5,3			Kelly, MI
Navy	Seahawk	6,5	5,4,6	5,4			Kelly, MI
Navy	Swan Valley	3,2	2	3,4	*Ur-3+*		Kelly, MI
Navy	T9903	6,5	4,3,5	5,6			Sphere, Ontario, CAN
Navy	T9905	5,4,3	4,3	4,3			Sphere, Ontario, CAN
Navy	USWA-50	4,3,5	4,3	5,4	*Ur-11?*		Miklas, WA
Navy	Verano	5,4	5,4,3	6,5			Beaver, PR
Navy	Vista	4,3	5,4,3	4,3	*Ur-3*		Butcher, ID
Navy	Voyager	6,5	4,3	5,6	*Ur-3*		ADM, Decatur

[a] Reaction grades 1= No visible symptoms; 2= Necrotic spots without sporulation; 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0.3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0.3-0.49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0.5-0.8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= reaction 6 with few type 5; 6= Uredinia 0.8-1.2 mm in diameter.

[b] Isolate UP15-4-2-SP1 was used to represent race 20-3
[c] Isolate UP15-2-1-SP9 was used to represent race 29-3
[d] Isolate 27-7= UP16-10-1-SP1 was used to represent race 27-7
[e] (-) = not evaluated.

Lines from the Durango race were screened with *U. appendiculatus* races 20-3 and 27-7 (Table 3.5). Overall, 34 lines were uniquely resistant to 20-3 and 27 lines were uniquely resistant to race 27-7. Twenty-five lines from the Durango race were resistant to both pathogen races. Eight out of 39 great northern lines were resistant to both races 20-3 and 27-7. In addition, two line were resistant to race 20-3. Nine out of 93 pinto lines were resistant to both 20-3 and 27-7. Four additional lines were uniquely resistant to 20-3, and one was resistant to 27-7. Two out of 22 pink lines were resistant to both races. In addition, one line was uniquely resistant to race 20-3. Five out of 29 small red lines were resistant to both races. Two additional lines were uniquely resistant to race 20-3, while one additional line was uniquely resistant to 27-7. Flor de Mayo line Desert Rose was resistant to both races. No resistance was observed in the cranberry and red lines evaluated as part of the Durango race.

Table 3.5. Reaction type, resistance gene(s) and release source of Durango race, a sub-set of the Middle American diversity panel, to *Uromyces appendiculatus* races 20-3 and 27-7.

Market class	Line	Reaction type[a]		Resistant gene(s)			Reference
		20-3 [b]	27-7 [c]				
Pinto	92US-1006	6,5	5,4				Miklas, WA
Pinto	ABCP-15	5,4	5,6				Urrea, NE
Pinto	ABCP-17	6,5	6,5	*Ur-3*			Urrea, NE
Pinto	ABCP-8	6,3	6,5	*Ur-3*	*Ur-6*		Urrea, NE
Pinto	AC Island	6,5	5,6				Daniels, Canada
Pinto	AC Ole	5,6	5,6				Hou, Manitoba, CAN
Pinto	AC Pintoba	6,5	6,5	*Ur-3/*	*Ur-4*		Hou, Manitoba, CAN
Pinto	Agassiz	6,5	6				ADM, Decatur
Pinto	Apache	1	1	*Ur-3*			Dean, ID
Pinto	Arapaho	6,5	5				Brick, CO
Pinto	Aztec	4,5	6	*Ur-3*			Kelly, MI
Pinto	Baja	6,5	5,6	*Ur-3*			ADM, Decatur
Pinto	BelDakMi-RR-5	1	1	*Ur-11*	*Ur-6+*		Pastor-Corrales, MD
Pinto	Bill Z	6,5	5,4	*ur-3*			Brick, CO
Pinto	Buckskin	6,5	6,5				ADM, Decatur
Pinto	Burke	6,5	6,5	*Ur-3*			Miklas, 1998
Pinto	Buster	4,5	6,5	*Ur-3*	*Ur-5*	*Ur-7*	Kmiecik, Seminis
Pinto	CDC Camino	6,5	6,5				Bett, Saskatchewan, CAN
Pinto	CDC Pinnacle	5,4	6,5				Bett, Saskatchewan, CAN
Pinto	CDC Pintium	5,4	6,5				Bett, Saskatchewan, CAN
Pinto	CDCWM-2	6	6				Bett, Saskatchewan, CAN
Pinto	Chase	6,5	6,5	*Ur-3*			Urrea, Nebraska
Pinto	Common Pinto	5,6	6,5				Singh, ID
Pinto	Croissant	6,5	6,5	*Ur-3*	*Ur-6*		Brick, CO

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Table 3.5. Reaction type, resistance gene(s) and release source of Durango race, a sub-set of the Middle American diversity panel, to *Uromyces appendiculatus* races 20-3 and 27-7 (continued).

Market class	Line	Reaction type[a]		Resistant gene(s)		Reference
		20-3[b]	27-7[c]			
Pinto	Durango	6	5,6	*Ur-3*		ADM, Decatur
Pinto	Fargo	6,5	6			ADM, Decatur
Pinto	Fiesta	6	5,4			Dean, ID
Pinto	Fisher	6,5	6,5			Brick, CO
Pinto	Flint	5,4	5,6			ADM, Decatur
Pinto	Focus	6	5,6			Kmiecik, Seminis
Pinto	Frontier	6	5,6	*Ur-3*		Osorno, ND
Pinto	Gala	6	6,5			Dean, Idaho
Pinto	Grand Mesa	5,6	6	*Ur-3*		Brick, CO
Pinto	GTS-900	3,2	6,5			Butcher, ID
Pinto	Hatton	5,4	6			Osorno, ND
Pinto	Holberg	6,5	6			Miklas, WA
Pinto	I06-2575-17	6	6,5			Porch/Urrea, PR-NE
Pinto	ICB-12	2,3	2,3	*Ur-3*		Miklas, WA
Pinto	IP08-2	6	6,5			Singh, ID
Pinto	Jackpot	4,3	6,5			Dean, ID
Pinto	JM-126	2,3	5,6			Miklas, WA
Pinto	Kimberly	5,6	6,5	*Ur-5*	*Ur-?*	Singh, ID
Pinto	Kodiak	5,4	6,5	*Ur-3*	*Ur-6*	Kelly, MI
Pinto	La Paz	6,5	6,5	*Ur-3*		ADM, Decatur
Pinto	Mariah	6	6,5			Kmiecik, Seminis
Pinto	Maverick	6	5,6	*Ur-3*		Osorno, ND
Pinto	MAX	6,7	5,6			Dean, ID
Pinto	Medicine Hat	6,5	6,5			Kmiecik, Seminis
Pinto	Montrose	5,6	5,6	*Ur-5*	*Ur-7*	Brick, CO
Pinto	ND040494-4	6,5	6,5	*Ur-3*		Osorno, ND

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Table 3.5. Reaction type, resistance gene(s) and release source of Durango race, a sub-set of the Middle American diversity panel, to *Uromyces appendiculatus* races 20-3 and 27-7 (continued).

Market class	Line	Reaction type[a]		Resistant gene(s)	Reference
		20-3[b]	27-7[c]		
Pinto	ND041062-1	6	2,3	*Ur-3*	Osorno, ND
Pinto	ND060197	4,3	5,4	*Ur-3*	Osorno, ND
Pinto	ND-307	5,6	6	*Ur-3*	Osorno, ND
Pinto	NE2-09-1	1	2,3		Urrea, NE
Pinto	NE2-09-10	6	6,5	*Ur-3*	Urrea, NE
Pinto	NE2-09-3	1	2	*Ur-11*	Urrea, NE
Pinto	NE2-09-4	6	5,6	*Ur-3*	Urrea, NE
Pinto	NE2-09-8	1	2		Urrea, NE
Pinto	Nodak	6,5	5,6		Miklas, WA
Pinto	NW-410	5,6	6,5		Miklas, WA
Pinto	NW-590	6,5	5,6		Miklas, WA
Pinto	Othello	6	6,5		Miklas, WA
Pinto	Ouray	6,5	5,6		Brick, CO
Pinto	P07863	5,4	5,6		Kelly, MI
Pinto	Pindak	2	2,3		Miklas, WA
Pinto	Poncho	6	6,5		ADM, Decatur
Pinto	PT7-2	5,6	6,5		Miklas, WA
Pinto	PT9-17	4,5	6		Miklas, WA
Pinto	Quincy	6	6		Miklas, WA
Pinto	Santa Fe	6,5	6	*Ur-3*	Kelly, MI
Pinto	SDPI-1	6	6		Singh, ID
Pinto	Sequoia	6	6,5	*Ur-3*	Dean, ID
Pinto	Shoshone	6	5,6		Singh, ID
Pinto	Sierra	6,5	5,6	*Ur-3*	Kelly, MI
Pinto	Sonora	6	6,5	*Ur-3*	ADM, Decatur
Pinto	Stampede	3,4	4,3	*Ur-3*	Osorno, ND

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Table 3.5. Reaction type, resistance gene(s) and release source of Durango race, a sub-set of the Middle American diversity panel, to *Uromyces appendiculatus* races 20-3 and 27-7 (continued).

Market class	Line	Reaction type[a]		Resistant gene(s)		Reference
		20-3[b]	27-7[c]			
Pinto	TARS09-RR023	5,6	5,6	*Ur-3*		Porch, TARS, PR
Pinto	TARS-VCI-4B	4,5	4,5	*Ur-3*		Miklas, MI
Pinto	UI-111	6,5	6			Singh, ID
Pinto	UI-114	6	6,5			Singh, ID
Pinto	UI-123	5,6	6,5			Singh, ID
Pinto	UI-126	6	6,5			Singh, ID
Pinto	UI-196	6	6			Singh, ID
Pinto	USPT-ANT-1	3,2	6,5	*Ur-3*		Miklas, WA
Pinto	USPT-CBB-1	3,2	2	*Ur-3*		Miklas, WA
Pinto	USPT-CBB-3	6	6,5	*Ur-3*		Miklas, WA
Pinto	USPT-CBB-5	6,5	5,6			Miklas, WA
Pinto	USPT-WM-1	3,2	3,4	*Ur-11*		Miklas, WA
Pinto	Vision	6	5,6	*Ur-3*		Kmiecik, Seminis
Pinto	Win Mor	6,5	6			Hou, Manitoba,CAN
Pinto	Windbreaker	5,4	5,6	*Ur-3*		Kmiecik, Seminis
Pink	6R-42	6	5,6			Miklas, WA
Pink	AC Early Rose	6	6,5			Daniels, Canada
Pink	CDC Rosalee	4,3	5,6			Bett, Saskatchewan, CAN
Pink	Gloria	6,5	6,5			Miklas, WA
Pink	Harold	6,5	6,5			Miklas, WA
Pink	I9365-25	2,3	3,4			Miklas, WA
Pink	I9365-5	2,3	6,5			Miklas, WA
Pink	Pink Floyd	2,3	3,2	*ur-3*	*Ur-11?*	ADM, Decatur
Pink	PK915	6	6,5			Miklas, WA
Pink	PK9-7	5,6	6,5			Miklas, WA
Pink	PR 0401-259	4,3	5,6			Beaver, PR

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Table 3.5. Reaction type, resistance gene(s) and release source of Durango race, a sub-set of the Middle American diversity panel, to *Uromyces appendiculatus* races 20-3 and 27-7 (continued).

Market class	Line	Reaction type[a]		Resistant gene(s)			Reference
		20-3[b]	27-7[c]				
Pink	ROG 312	6,5	6,5				ADM, Decatur
Pink	Roza	6,5	6				Miklas, WA
Pink	S08418	6,5	6				Kelly, MI
Pink	Sedona	6	6,5				Kelly, MI
Pink	UCD 9623	5,6	5,6	*ur-3*			Temple, Davis, CA
Pink	UI-537	6	6,5				Singh, Idaho
Pink	URS-117	5,6	6,5				Miklas, WA
Pink	USWA-61	6,5	6,5				Miklas, WA
Pink	Victor	6	6,5				Miklas, WA
Pink	Viva	6,5	6,5				Miklas, WA
Pink	Yolano	6	6,5				Temple, Davis, CA
Great Northern	ABC-Weihing	3,4	5,6	*Ur-3*	*Ur-6*		Urrea, NE
Great Northern	AC Resolute	5,4	6,5				Daniels, Canada
Great Northern	BelMiNeb 1	1	1	*Ur-11*	*Ur-4*		Urrea, NE
Great Northern	BelMiNeb 2	1	1	*Ur-11*			Urrea, NE
Great Northern	BelMiNeb 5	1	1	*Ur-11*	*Ur-4*	*Ur-6*	Urrea, NE
Great Northern	BelMiNeb-RMR-3	1	1	*Ur-11*	*Ur-4*		Pastor-Corrales, MD
Great Northern	BelMiNeb-RR-2	1	1	*Ur-11*			Pastor-Corrales, MD
Great Northern	BelNeb 2	1	1	*Ur-5*	*Ur-6*	*Ur-7*	Urrea, NE
Great Northern	BelNeb-RR-1	1	1	*Ur-5*	*Ur-6+*	*Ur-7*	Pastor-Corrales, MD
Great Northern	Beryl	5,4	6,5	*ur-3*	*Ur-7*		ADM, Decatur
Great Northern	Beryl R	1	1	*ur-3*	*Ur-11?*		ADM, Decatur
Great Northern	Bighorn	4,5	4,5				Dean, ID
Great Northern	CDC Crocus	5,4	6,5				Bett, Saskatchewan, CAN
Great Northern	Coyne	4,3	5,6	*Ur-3*	*Ur-6*		Urrea, NE
Great Northern	Emerson	6,5	6				Urrea, NE

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Table 3.5. Reaction type, resistance gene(s) and release source of Durango race, a sub-set of the Middle American diversity panel, to *Uromyces appendiculatus* races 20-3 and 27-7 (continued).

Market class	Line	Reaction type[a]		Resistant gene(s)	Reference
		20-3[b]	27-7[c]		
Great Northern	Gemini	6,5	6		Bean, Scottbluff, NE
Great Northern	GN Harris	4,5	6,5	*ur-3*	Urrea, NE
Great Northern	GN Star	6,5	6,5		Urrea, NE
Great Northern	GN9-1	6,5	6		Miklas, WA
Great Northern	GN9-4	6,5	6,5	*Ur-3*	Miklas, WA
Great Northern	Ivory	2,4	6,5		ADM, Decatur
Great Northern	JM-24	6,5	6,5		Miklas, WA
Great Northern	Marquis	4,5	6,5		ADM, Decatur
Great Northern	Matterhorn	6	6	*Ur-3*	Kelly, MI
Great Northern	NE1-09-13	6,5	6,5	*Ur-3*	Urrea, NE
Great Northern	NE1-09-19	6,5	6,5		Urrea, NE
Great Northern	NE1-09-20	6	5,6	*Ur-3*	Urrea, NE
Great Northern	NE1-09-22	6	5,6	*Ur-3*	Urrea, NE
Great Northern	NE1-09-9	6,5	5,6	*Ur-3*	Urrea, NE
Great Northern	Orion	6,5	6	*Ur-6+*	Bean, Scottbluff, NE
Great Northern	Sapphire	6	6,5		ADM, Decatur
Great Northern	Sawtooth	6	6		Singh, ID
Great Northern	Starlight	4,3	5,6	*Ur-3*	Urrea, NE
Great Northern	UI-425	6	6		Singh, ID
Great Northern	UI-59	5,6	6		Singh, ID
Great Northern	US-1140	6	5,6		Singh, ID
Great Northern	USWA-12	4,5	5,4		Miklas, WA
Great Northern	USWA-13	6	6		Miklas, WA
Great Northern	Weihing	5,6	5,4	*Ur-3*	Urrea, NE
Small Red	AC Earlired	6	5		Daniels, Canada
Small Red	AC Redbond	1	1		Daniels, Canada

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Table 3.5. Reaction type, resistance gene(s) and release source of Durango race, a sub-set of the Middle American diversity panel, to *Uromyces appendiculatus* races 20-3 and 27-7 (continued).

Market class	Line	Reaction type[a]		Resistant gene(s)	Reference
		20-3[b]	27-7[c]		
Small Red	AC Scarlet	1	4		Hou, Manitoba
Small Red	Amadeus 77	1	1		Beaver, PR
Small Red	Big Bend	6	5,6		Miklas, 1966
Small Red	CENTA Pupil	4,3	5,6		Rosas, Honduras
Small Red	Common Red Mexican	6,5	6		Singh, ID
Small Red	Coulee	5,6	5,6		Miklas, WA
Small Red	Dehoro	1	5		Rosas, Honduras
Small Red	DOR 364	6	6,5		Beaver- PR
Small Red	Ember	5,6	6,5		ADM, Decatur
Small Red	Garnet	5,4	6,5		ADM, Decatur
Small Red	IBC 301-204	-[b]	1		Rosas, Honduras
Small Red	Le Baron	5,4	6,5	*Ur-3*	Miklas, WA
Small Red	NDZ06249	6,5	6,5	*Ur-3*	Osorno, ND
Small Red	NW-63	5,4	6,5		Miklas, WA
Small Red	PR 0340-3-3-1	1	1	*Ur-11*	Beaver, PR
Small Red	Red Ryder	6	6		ADM, Decatur
Small Red	Rojo Chiquito	1	2,1	*Ur-3*	Miklas, WA
Small Red	SR7-3	5,6	6		Miklas, WA
Small Red	SR9-4	6,5	6	*Ur-3*	Miklas, WA
Small Red	TARS09-RR004	1	1		Porch, TARS, PR
Small Red	TARS09-RR007	6,5	6,5	*Ur-3*	Porch, TARS, PR
Small Red	TARS09-RR029	5,6	6,5	*Ur-3*	Porch, TARS, PR
Small Red	UI-228	6	6		Singh, ID
Small Red	UI-239	6	6,5		Singh, ID
Small Red	UI-3	6,5	6,5		Singh, ID

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Table 3.5. Reaction type, resistance gene(s) and release source of Durango race, a sub-set of the Middle American diversity panel, to *Uromyces appendiculatus* races 20-3 and 27-7 (continued).

Market class	Line	Reaction type[a]		Resistant gene(s)	Reference
		20-3[b]	27-7[c]		
Small Red	UI-37	6	6		Singh, ID
Small Red	USRM-20	5,4	6,5		Miklas, WA
Red	F07-014-22-2	6	6,5	*Ur-3*	Porch/Urrea, PR-NE
Red	F07-449-9-3	6	6,5	*Ur-3*	Porch/Urrea, PR-NE
Red	Merlot	6	6,5	*Ur-3*	Kelly, MI
Cranberry	Indeterminate Jamaica Red	-[d]	5,6		Miklas, WA
Flor de Mayo	Desert Rose	1	3		ADM, Decatur

[a] Reaction type: 1= No visible symptoms; 2= Necrotic spots without sporulation (hypersensitive); 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0.3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0.3-0.49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0.5-0.8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= Reaction 6 with few type 5; 6= Uredinia 0.8-1.2 mm in diameter.

[b] Isolate UP15-4-2-SP1 was used to represent race 20-3.

[c] Isolate UP16-10-1-SP1 was used to represent race 27-7.

[d] (-) = missing data due to insufficient seed.

3.3.5. Genome wide association studies – middle american diversity panel (MDP)

A total of 286 lines from the MDP including Mesoamerican and Durango races were combined to conduct GWAS utilizing approximately 125,744 SNP markers (Oladzad et al., 2019). PCA divided the structure of the population into two distinct groups, classifying the lines into Mesoamerican and Durango bean races. First principal component (PCA1) explained 16.9% of the variation, while the second PCA2 explained 8.4%, and the third PCA3 explained 4.2% of the variation (Figure 3.3).

GWAS was conducted using the pustule diameter (continuous; mm) for each race evaluated, 20-3 and 27-7. A separate analysis using the reaction type (discrete; 1-6) was also conducted. Manhattan plots for pustule diameter included more significant markers than reaction type at correction level pFDR= 0.01. In addition, pustule diameter explained more variation with a higher percent of cumulative variation (Table 3.6).

A total of 133 SNPs were associated to resistance using pustule diameter. From this, only 26 were observed in reaction to both races 20-3 and 27-7; three on Pv04, four on Pv06, and 19 on Pv11. In addition, 45 SNPs across chromosomes Pv01, Pv04, Pv06, Pv08, and Pv11 were associated uniquely with pustule diameter for race 20-3 (Figure 3.4). While, 88 SNPs on chromosomes Pv04, Pv06, Pv08, Pv10, and Pv11 were associated uniquely with race 27-7 (Figure 3.4). Based on reaction type (scale), a total of 46 SNP were found. From this, 11 were observed in reaction to both races, all located on Pv11. In addition, one SNP marker on Pv02 and 8 SNP on Pv11 were observed in reaction to race 20-3 (Table 3.6). Twenty-six SNPs on chromosomes Pv01 (1 SNP), Pv06 (10), and Pv11 (4) were associated with reaction type to race 27-7 (Table 3.6). Multiple resistance genes have been mapped to chromosome Pv11, including *Ur-11*. An additional analysis using pustule diameter was conducted excluding all lines known to carry the *Ur-11* gene.

Manhattan plot from this analysis resulted in the departure of the peak on Pv11 and increased the significance of previously observed a peak at the beginning of chromosome Pv04 for both *U. appendiculatus* races 20-3 and 27-7 (Figure 3.5).

Table 3.6. Single nucleotide polymorphism markers associated to disease reaction of Middle American diversity panel to *Uromyces appendiculatus* races 20-3 and 27-7. GWAS was conducted with pustule diameter and reaction type.

Race	Type of data	Interval		Location	Peak SNP			% Cum. Variation [b]	No. Sig. SNP[c]
		Chr[a]	Genomic interval (Mb)		Position (Mb)	-Log10 (P)	% Variation		
20-3	PD[d]	1	4170839	4170839	SNP01_4170839	4.9921	7.88		1
		4	524536-554762	524536	SNP04_524536	4.9425	7.80		4
		6	23832392-23859175	23832392	SNP06_23832392	5.8386	7.94	**25**	4
		8	61950226-61950299	61950226	SNP08_61950226	5.3306	8.39		6
		11	50587931-52225318	52212476	SNP11_52212476	9.5940	13.28		30
							Total		45
	VP[e]	2	46760836	46760836	SNP02_46760836	5.2362	8.33		1
		11	50587931-52225318	52212476	SNP11_52212476	9.3180	13.03	**16**	19
							Total		20
27-7	PD	4	291396-541562	541562	SNP04_541562	5.6266	8.86		6
		6	22652680-29831684	23832482	SNP06_23832392	6.9739	9.61		49
		8	60149053	60149053	SNP08_60149053	4.6048	7.31		1
		10	3776780-5463085	3776896	SNP10_3776896	5.0584	8.01	**49**	5
		11	50587960-52225318	52116681	SNP11_52116681	11.451	17.22		27
							Total		88
	VP	1	5743740	5743740	SNP01_5743740	5.3142	8.48		1
		6	22987816-29831684	22987816	SNP06_22987816	5.7887	7.97		10
		11	51052463-52225318	52212476	SNP11_52212476	9.0742	12.73	**18**	15
							Total		26

[a] Chr= Chromosome location.

[b] Percent (%) of variation.

[c] Number of significant SNP markers in the delimited region.

[d] PD=Analysis based on pustule diameter.

[e] VP=Analysis based on Reaction type

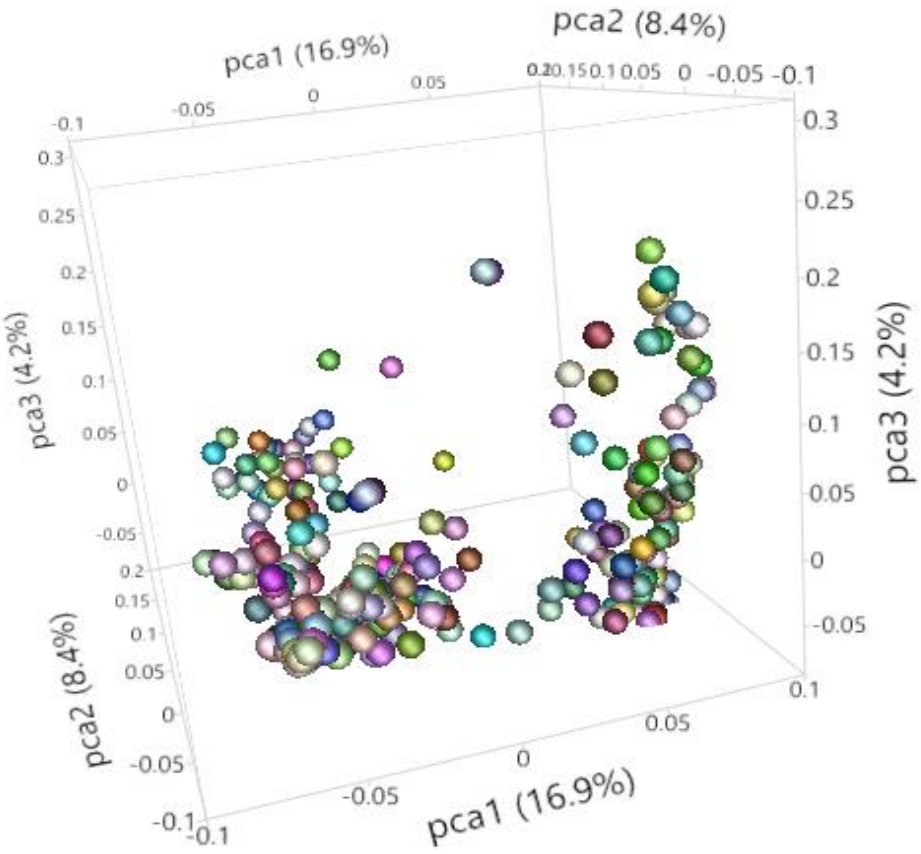

Figure 3.3. Principal component analysis (PCA) representing the population structure of 286 lines from the Middle American diversity panel.

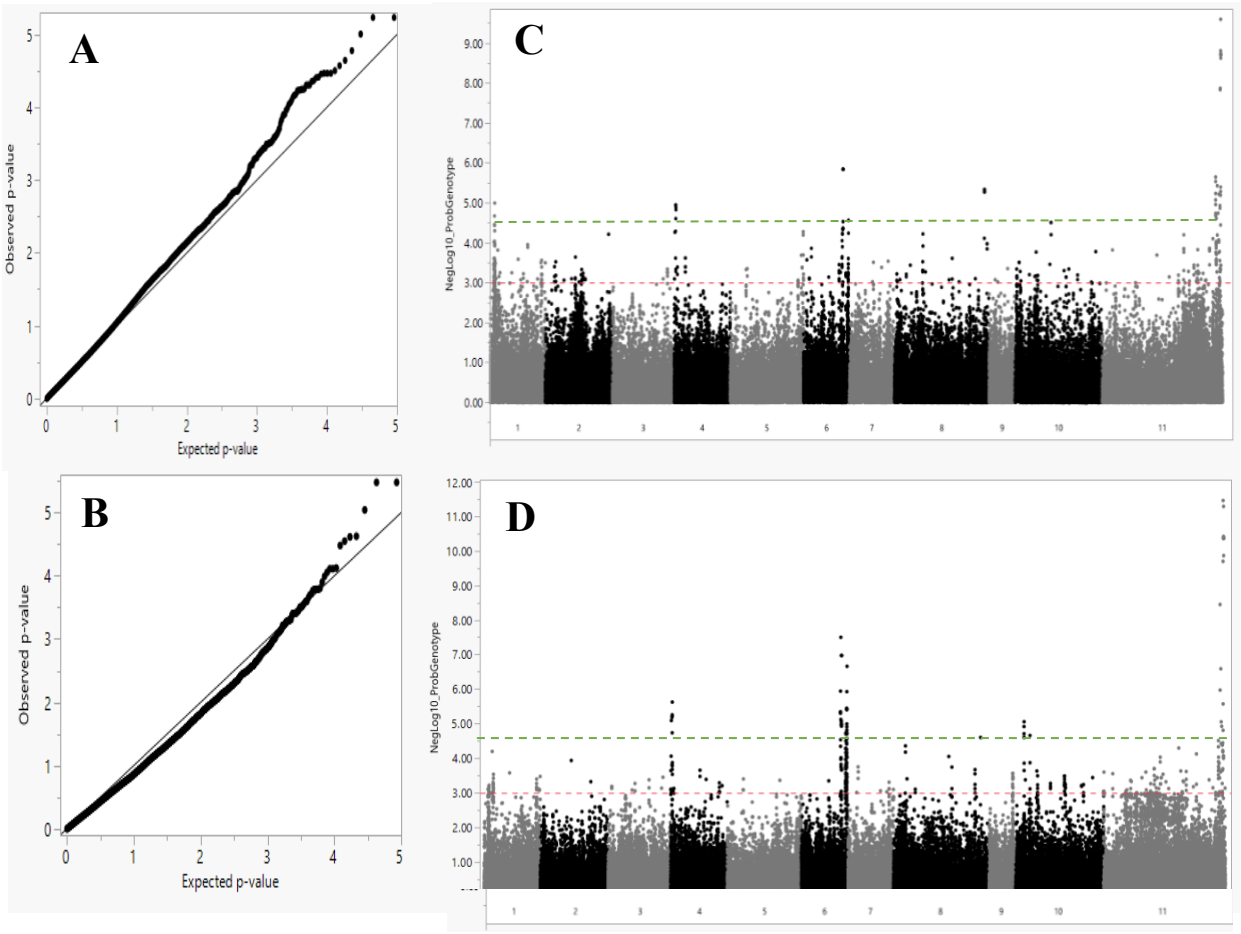

Figure 3.4. q-q plot by using mixed model A, B) and Manhattan plot for reaction of Middle American diversity Panel (MDP) C, D) including Mesoamerican and Durango races, to *U. appendiculatus* race 20-3 and 27-7, respectively, based on pustule diameter. *Phaseolus vulgaris* chromosomes (1-11) are represented on x axis, a –log 10 (p) values are shown on y axis. The red line indicates threshold at significance value of –log10(p)=3 and the green line indicates threshold at correction of pFDR=0.01.

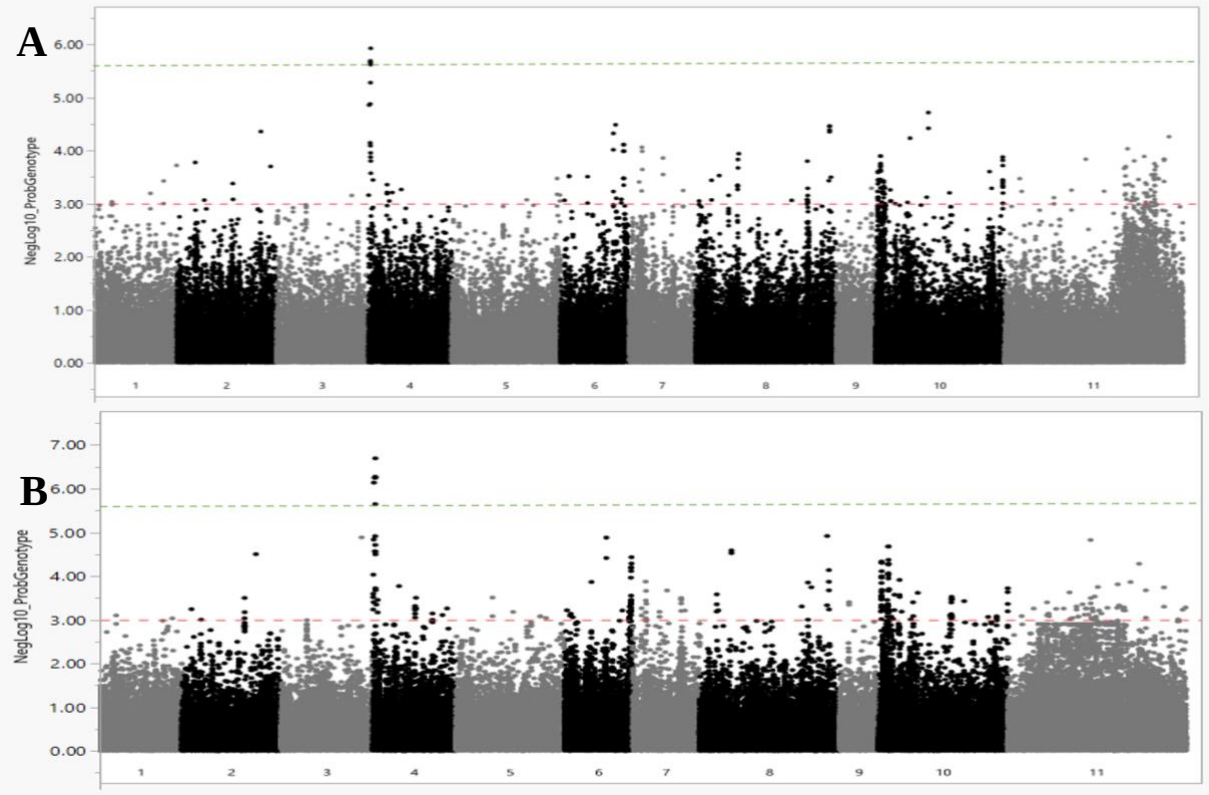

Figure 3.5. Manhattan plot for reaction of Middle American diversity Panel (MDP) including Mesoamerican and Durango races showing significant marker trait associations for pustule diameter when lines with the *Ur-11* gene were excluded. Association to *Uromyces appendiculatus* race 20-3 (A), and 27-7 (B). *Phaseolus vulgaris* chromosomes (1-11) are represented on x axis, a –log 10 (p) values are shown on y axis. The red line indicates threshold at significance value of –log10(p)=3 and the green line indicates threshold at correction of pFDR=0.01.

3.3.6. Candidate genes in middle american diversity panel (MDP)

A total of 133 SNP were found associated in reaction to race 20-3 and 27-7 using pustule diameter. From this, 26 SNP markers were shared in reaction to both races 20-3 and 27-7; three on Pv04, four on Pv06, and 19 on Pv11. Of the three SNP markers found on Pv04 one SNP marker directly associated with gene model Phvul.004G007200 that encodes to a methyl-CPG-binding domain 7. Other two markers were associated with gene model Phvul.004G007300 that encodes to a phosphate transporter 1:1. On chromosome Pv06, four markers were found associated in reaction to both races. Two of these markers were found approximately 7.5kb before gene model Phvul.006G130400 that encodes a patatin-like protein 6. The other two markers were found 3.6Kb before gene model Phvul.006G130500 a plant invertase/pectin methylesterase inhibitor superfamily protein. On chromosome Pv11, 19 SNP markers were found in association to both races located from 50,587,931 bp to 52,225,318 bp. From these, three SNP markers directly associated to gene models Phvul.011G192400, Phvul.011G201000, Phvul.011G203100, all encode to LRR and NB-ARC domain-containing disease resistance protein. Another marker directly associated with gene model Phvul.011G199600 that encodes a sulfotransferase 2A. Three markers were associated to a region approximately 4.6 Kb before the gene model Phvul.011G206450 that encodes to a nudix hydrolase homolog 16. Twelve markers were associated with uncharacterized regions.

In addition, 19 SNP markers were found in association with *U. appendiculatus* to race 20-3 producing peaks on chromosomes Pv01, Pv04, Pv06, Pv08 and Pv11 across lines from the Mesoamerican and Durango races. The significant SNP marker on Pv01 is located between two known gene models, Phvul.001G042200 and Phvul.001G042100, which encode a WRKY DNA-binding protein 40. On chromosome Pv04 and Pv06, no marker was directly associated with a

specific gene model; however, an NB-ARC domain-containing disease resistance proteins are found on Pv04 within 100Kb from the marker. Six SNP markers were observed on chromosome Pv08. All markers directly associated with the candidate gene Phvul.008G262900 that encodes a duplicated homeodomain-like superfamily protein. This gene has not been associated with any known rust resistance genes. However, 100 Kb upstream of the SNP marker is the Phvul.008G275000 gene that encodes a leucine-rich repeat protein kinase family protein.

In reaction to race 27-7, GWAS studies found additional 42 SNP markers on chromosomes Pv04, Pv06, Pv08, Pv10, and Pv11. Six SNP markers were detected within one peaks on chromosome Pv04. The highest significant marker associated with Phvul.004G007500 gene model which encodes to a protein of unknown function (DUF1421). However, downstream of this marker is the Phvul.004G007750 gene that encodes for an NB-ARC domain-containing disease resistance protein. On chromosome Pv06, one SNP marker was associated with the gene model Phvul.006G198200, a leucine-rich receptor-like protein kinase family protein. One significant SNP marker was found on chromosome Pv08 associated with gene model Phvul.008G253000 which encodes a protein phosphatase 2C family protein. On chromosome Pv10, four markers were found directly associated with gene model Phvul.010G026300 encoding the ‘target of AVRB operation1’.

3.3.7. Germplasm screening - andean diversity panel (ADP)

A subset of the ADP was also evaluated for reaction to *U. appendiculatus* races 20-3 and 27-7. These 49 lines include cranberry, white kidney, light red and dark red kidney, yellow, red mottled and mayocoba (Table 3.7). Five lines were resistant to both races, light red kidney lines Fox Fire, Red Kanner and Red Klaud displayed a hypersensitive resistant response; Mayocoba line Myasi, and yellow line Jalo-EEP558. In addition, 14 lines were resistant solely to race 20-3. Five light red kidney, three dark red kidney, three cranberry, one red mottle and two white kidney lines were resistant to race 20-3 only. Finally, were resistant to race 20-3. The remaining lines were susceptible to the *U. appendiculatus* races evaluated.

Table 3.7. Reaction type, resistance gene(s) and release source of lines from the Andean diversity panel subset to *Uromyces appendiculatus* races 20-3 and 27-7.

Market class	Line	Reaction type [a]		Reference
		20-3[b]	27-7[c]	
Light Red Kidney	Blush	5,6	4,5	Miklas, WA
Light Red Kidney	CELRK	2,3	6,5,4	Temple, Davis, CA
Light Red Kidney	Chinook 2000	3,2	5,4	Kelly, MI
Light Red Kidney	Fox Fire	2	2	ADM, Decatur
Light Red Kidney	Isabella	5,4	6	Kelly, MI
Light Red Kidney	K-42	4,5	4,3,5	Miklas, WA
Light Red Kidney	K-59	5,4	6,5	Miklas, WA
Light Red Kidney	Kardinal Kidney	4,5	4,5	Miklas, WA
Light Red Kidney	Litekid	4,5	4,5	Smith, Guelph, CAN
Light Red Kidney	Pink Panther	4,3	4,5,6	Kmiecik, Seminis
Light Red Kidney	Red Kanner	2	2	Griffiths/Sandsted, NY
Light Red Kidney	Red Kloud	2	2	Griffiths/Sandsted, NY
Light Red Kidney	Red Kote	3,4	6	Griffiths/Sandsted, NY
Light Red Kidney	Sacramento	3,4	5,4	ADM, Decatur
Light Red Kidney	VA-19	5,4	5,4,6	Miklas, WA
Light Red Kidney	Wallace 773-V98	3,4	5,6	Griffiths/Sandsted, NY
Light Red Kidney	Rosie	4,3	4,5	Osorno, ND
Dark Red Kidney	CDRK	4,5	5,4	Temple, Davis, CA
Dark Red Kidney	Charlevoix	2,1	5,6	Kelly, MI
Dark Red Kidney	Drake	3,4	6,5	Kmiecik, Seminis
Dark Red Kidney	Fiero	3,4	3,4,5	Miklas, WA

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Table 3.7. Reaction type, resistance gene(s) and release source of lines from the Andean diversity panel subset to *Uromyces appendiculatus* races 20-3 and 27-7 (continued).

Market class	Line	Reaction type [a]		Reference
		20-3[b]	27-7[c]	
Dark Red Kidney	Montcalm	6,5	6,5	Kelly, MI
Dark Red Kidney	Red Hawk	4,3	6	Kelly, MI
Dark Red Kidney	Royal Red	4,3	4,5	Miklas, WA
Dark Red Kidney	UC Nichols	4,3	5,6	Temple, Davis, CA
Dark Red Kidney	USDK-CBB-15	6,5	5,6	Miklas, WA
Dark Red Kidney	Talon	4,5	4,5	Osorno, ND
Cranberry	Bellagio	6,5	6,5	Kelly, MI
Cranberry	Capri	5,6	4,5	Kelly, MI
Cranberry	Cardinal	5,4	6	Kelly, MI
Cranberry	Crimson	3,2	6	USDA-ARS
Cranberry	Dolly	6,5	6	Kmiecik, Seminis
Cranberry	Etna	6,5	5,4	Kmiecik, Seminis
Cranberry	G-122	3,4	5,6	Miklas, WA
Cranberry	Hooter	5,6	6	Kmiecik, Seminis
Cranberry	Krimson	5,4	6	Miklas, WA
Cranberry	Michigan Improved Cran (Micran)	4,5	5,6	Kelly, MI
Cranberry	Red Rider	6,5	6,5	Smith, Guelph, CAN
Cranberry	UCD 0801	4,3	5,4,6	Temple, Davis, CA
Cranberry	USCR-9	5,6	6	Miklas, WA
Cranberry	USCR-CBB-20	3,2	6,5	Miklas, WA
Yellow	Jalo EEP558	3,2	3,4	Temple, Davis, CA
Mayocoba	Myasi	3,2	3,4	ADM, Decatur
Red Mottled	Pompadour B	1	6	Miklas, WA
White Kidney	Beluga	3,2	5,6	Kelly, MI
White Kidney	Silver Cloud	4,3	6,5	Miklas, WA
White Kidney	USWK-CBB-17	3,2	5,4	Miklas, WA

[a] Reaction grades 1= No visible symptoms; 2= Necrotic spots without sporulation; 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0.3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0.3-0.49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0.5-0.8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= Reaction 6 with few type 5; 6= Uredinia 0.8-1.2 mm in diameter.

[b] Isolate UP15-4-2-SP1 represented race 20-3.

[c] Isolate UP16-10-1-SP1 represented race 27-7.

3.4. Discussion

Resistance gene *Ur-3* had been used widely in breeding programs in North Dakota and Michigan due to the resistance response and numerous varieties had been released utilizing this resistance gene (McMillan et al., 2003; Venette et al., 1998; Wright et al., 2009). In 2008, commonly grown varieties were susceptible to *U. appendiculatus* race 20-3, virulent to *Ur-3* (Markell et al., 2009). In addition to race 20-3, this research confirmed that most released varieties are also susceptible to races 29-3 and 27-7, also found in North Dakota. Additional rust races had been reported to overcome the *Ur-3* gene in Michigan, Nebraska, Honduras, Dominican Republic and South Africa, rendering the use of *Ur-3* gene obsolete in many breeding programs (Acevedo et al., 2015; Jochua et al., 2008; Wright et al., 2009). Race characterization provided information on effective sources of resistance to prevalent *U. appendiculatus* races in North Dakota such as the use of *Ur-11* gene (Chapter 2).

Reactions of lines and varieties included in this study to screening with race 20-3, 29-3 and 27-7 were consistent with resistant genes previously reported in these lines, when available. However, many of the resistant genes carried by lines remain largely unknown. In our study, great northern lines BelMiNeb-RMR-4, -7, and -8 expressed an immune response characteristic of the *Ur-11* reaction. However, these lines include three or more rust resistant gene (Pastor-Corrales et al., 2003). Mesoamerican navy bean line Swan Valley was resistant to races 20-3, 29-3 and 27-7. This line was released in 1986 as a result of the F_5 population from 'Neb-2'/'Black Turtle Soup' cross (Adams et al., 1986). Swan Valley was resistant to all rust races characterized in 1982 (Adams et al., 1986). Recent studies had determined that parental 'Neb-2' contains the *Ur-3*+ gene (Hurtado-Gonzales et al., 2017). Our study suggests that gene *Ur-3*+ carried by Swan Valley might confer additional resistance to *U. appendiculatus* races virulent on *Ur-3*.

Among lines evaluated from the Durango race, all resistant great northern lines except BelNeb2 are reported to contain the *Ur-11* gene, corresponding with the observed immune resistant phenotype (Hurtado-Gonzales et al., 2017). BelNeb2 possess genes *Ur-5, Ur-6,* and *Ur-7*; therefore, resistance to 20-3 likely comes from the *Ur-5* gene, as 20-3 is virulent on *Ur-6* and *Ur-7* (Hurtado-Gonzales et al., 2017). Small red lines PR 0340-3-3-1 possesses *Ur-11,* while TARS09-RR004 possesses *Ur-3,* however both were resistant to both races evaluated. Pink-seeded line Pink Floyd was resistant to both races via *Ur-11*. Pinto line USPT-WM-1 is known to possess the *Ur-11* gene; while lines ICB-12 and USPT-CBB-1 possess the *Ur-3* gene, all were resistant to both races (Hurtado-Gonzales et al., 2017). Lines possessing only the *Ur-3* gene but still expressing resistance carry an additional gene (or genes) conferring resistance.

GWAS analysis in ABL and MDP identified several marker trait associations on multiple chromosomes. Candidate genes that encodes WRKY transcription factors, LRR, and NBS-ARC kinases were the most common. NBS-LRR are the largest and most important disease resistance genes. Some NBS genes also have a TIR (Toll/Interleukin-1 Receptor) region. NBS-LRR are part of the NB-ARC domain for its presence in APAF-1 (apoptotic protease-activating factor-1). In this study LRR, NBS-LRR and NB-ARC were the most common candidate genes linked to SNP markers on chromosomes Pv01, Pv02, Pv04, Pv06, Pv08, and Pv11. NBS-LRR have a key role in disease resistance against fungal and bacterial pathogens on common bean, including the pathogens that cause anthracnose and common bacterial blight (CBB) (Wu et al., 2017). NBS-LRR genes associated with disease resistance have been mapped on all *P. vulgaris* chromosomes except for Pv08 (Wu et al., 2017). However, NBS-LRR related to anthracnose and CBB resistance are not located in the same chromosomal regions as the rust resistant genes, with two exceptions. The region from 45.75 to 47.55 Mb on Pv10 that confers resistance to CBB is similar to genomic

region to the target of AvrB operon-1 (Phvul.010G026300) gene we found for rust resistance. The second exception is the cluster 21 (45.75 - 47.55Mb) on chromosome Pv11 that also confers resistance to anthracnose.

We observed peaks on chromosome Pv01, Pv06, Pv08 and Pv10 near gene models that encode to WRKY factors. Peaks on Pv10 were in reaction of ABL to races 29-3 and 27-7. Peaks on Pv06 and Pv08 in MDP were observed in response to race 27-7. The WRKY family of proteins are highly involved in the response to abiotic and biotic stimuli (Satapathy et al., 2018). WRKY40 has DNA-binding transcription factor activity, binding to proteins related to defense response to fungi and bacteria (Birkendihl et al., 2016; Xu et al., 2006). In soybean, reaction to rust is highly mediated by WRKY transcription factors including WRKY-40, -36 and -45 from *Rpp-2* gene mediated resistance (Pandey et al., 2011). Silencing of WRKY transcription factors on soybean resulted in loss of resistance to soybean rust (Pandey et al., 2011).

The peak on Pv01 was observed in the MDP in reaction to 20-3. *Ur-9* has been mapped on chromosome Pv01 (Jung et al., 1998; Park et al., 1999). Fine mapping of *Ur-9* has not been conducted; therefore, we can only speculate on the relationship between this gene and the significant peak observed in this study. The significant SNP on Pv01 was observed in the MDP in reaction to race 20-3, and was absent on race 27-7 (virulent on *Ur-9*), this supports the hypothesis that our SNP marker may be associated with *Ur-9*.

A peak observed on Pv04 was located at the interval 524,536 - 554,762bp. Multiple *U. appendiculatus* resistant genes including *Ur-5* and *Ur-14* have been mapped on chromosome Pv04 (Miklas et al., 2006). High resolution mapping located *Ur-14* at 1,230,785bp on chromosome Pv04 (Valentini et al., 2017). Thus, we conclude that the significant SNP observed during this study is likely not associated with *Ur-14*. *Ur-5* is overcome by race 27-7, and we observed peaks when

inoculating with both *U. appendiculatus* races 20-3 and 27-7. Therefore, we conclude that the peak we observed is not *Ur-5*. The anthracnose resistant gene *Co-3/Phg-3* gene is located in the interval 483,427 - 575,259bp on Pv04. Our peak was located in this region in our GWAS in response to races 20-3 and 27-7, and may be the same gene encoding to NB-ARC receptors (Valentini et al., 2017; Zuiderveen et al., 2015). This finding is in agreement with previous studies that determined some dual resistance, where the same genes are involved in resistance to anthracnose and rust (Valentini et al., 2017).

Gene *Ur-4* has been mapped on chromosome Pv06, however no specific location has been determined. We observed markers on chromosome Pv06 associated in reaction to race 20-3 and 27-7 in the MDP. Race 27-7 is virulent on *Ur-4*, while race 20-3 is avirulent, thus, we conclude that the peak observed on Pv06 is not *Ur-4*. Our SNP markers are likely tagging a new resistance gene or QTL.

A peak was observed on Pv08 during this study in reaction to race 20-3 located at 61.9Mb, distinct from the peak in reaction to 27-7 located at 60.1Mb. Rust resistant gene *Ur-13* has been mapped at the end of the chromosome Pv08 (Miklas et al., 2006). Chromosome Pv08 is about 63Mb long. Rust resistant gene *Ur-13* is overcome by race 27-7, but not 20-3. Therefore we can speculate that *Ur-13* might be located around 61.9Mb on Pv08, associated here in response to race 20-3. The peak in reaction to *U. appendiculatus* race 20-3 encodes for a duplicated homeodomain-like superfamily protein, while the peak in response to 27-7 encodes to a protein phosphatase 2C family protein.

Multiple markers were associated to kinase-like proteins on chromosome Pv10 of ABL in response to race 20-3 and 27-7; and chromosomes Pv06 and Pv08 on MDP in response to race 20-3 and 27-7, respectively. Among other things, kinases play a role in plant defense signal

transduction cascades and as a receptor-like kinase (RLK) (Bent, 1996; Goff and Ramonell, 2007; Tang et al., 2017). RLK have been identified acting in both broad-spectrum and elicitor-initiated dominant resistance genes in race-specific defense response (Goff and Ramonell, 2007). RLK are considered pattern recognition receptors as the first line of defense against pathogens (Tang et al., 2017). Fine mapping of the bean rust resistance gene *Ur-3* (Pv11) found a cluster of genes that included serine/threonine kinases (Hurtado-Gonzalez et al., 2017). However, since all the races used for screening overcome the *Ur-3* gene, we can conclude that kinases play an important role in disease response in more rust resistant genes than *Ur-3*.

Chromosome Pv11 includes multiple rust resistant genes *Ur-3, Ur-3+, Ur-6, Ur-6+, Ur-7, Ur-Dorado53* and *Ur-11* (Hurtado-Gonzales et al., 2017; Miklas et al., 2006; Pastor-Corrales and Liebenberg, 2010). Two markers at 52.22 Mb were associated with an area 4kb before a NUDIX (nucleoside diphosphates linked to some moiety X, hydrolases) hydrolase homolog 16 at the end of chromosome Pv11. This region might be part of the *Ur-11* gene. NUDIX had been observed to work as a negative regulator of disease resistance, where NUDIX mutants have increased ROS, cell death and increase expression of pathogenesis-related genes (Ogawa et al., 2008; Ge and Xia, 2008). NUDIX hydrolases can be produced in multiple cell organelles and work as 'housekeeping' enzymes to maintain cell homeostasis (Ogawa et al., 2008; Ge and Xia, 2008). NUDIX hydrolase genes were expressed in a compatible interaction during gene expression studies of soybean rust (Pandey et al., 2011; Tremblay et al., 2010).

We found a clear peak on chromosome Pv11 located at approximately 52.11 Mb. Fine mapping of the *Ur-3* gene has localized it on chromosome Pv11 at the 46.96 to 47.01 Mb position and about 46.5kb in size (Hurtado-Gonzales et al., 2017). Additionally, the races used for screening all overcame *Ur*-3; therefore, the NBS-LRR gene found on Pv11 does not correspond to the *Ur-3*

gene. GWAS conducted in this study excluding all lines known to possess the *Ur-11* gene resulted in disappearance of the Pv11 peak, indicating that the major peak is likely *Ur-11*. Gene *Ur-11* has not been previously mapped to a specific region on Pv11. Here we narrowed down the location of *Ur-11* from 50.5 to 52.2 Mb on Pv11.

Resistance gene *Ur-11* from line PI181996 is being targeted by many breeding programs, where only Honduran *U. appendiculatus* race 22-52 (108) is virulent to this gene (Acevedo et al, 2013; Liebenberg and Pretorius, 2010; Pastor-Corrales and Steadman, 2015; Wright et al., 2009). Most recently, a new pinto bean variety ND Falcon, was released by the ND common bean breeding program, containing the *Ur-11* gene, and therefore, is resistant to all races of *U. appendiculatus* currently present in North Dakota (Chapter 2; Osorno personal communication). However, the use of a single dominant resistant gene is not recommended because pressure on the pathogen results in the selection of resistant individuals, as occurred with the *Ur-3* gene in North Dakota and elsewhere (McMillan et al. 2003; Markell et al., 2009). Gene pyramiding currently is the most recommended tool to reduce resistance gene failure, but it is challenging and very time-intensive (Acevedo et al., 2013; McMillan et al., 2003; Pastor-Corrales and Steadman, 2015).

3.5. Conclusions

Bean rust caused by *Uromyces appendiculatus* is a yield limiting disease worldwide and in North Dakota. Previous sources of resistance based on the single dominant resistance gene *Ur-3* had been overcome by race 20-3, reported for the first time in 2008 in North Dakota. New potential sources of resistance were found by screening germplasm including ABL from NDSU bean breeding program and the MDP to three *U. appendiculatus* races. GWAS on the MDP narrowed the location of the highly efficient *Ur-11* gene on chromosome Pv11, at approximately 50.5Mb to 52.2Mb. We found SNP's significantly associated with rust resistance on chromosome Pv01,

Pv02, Pv04, Pv06, Pv08, Pv10 and Pv11 that hasn't been reported previously. Most candidate genes tagged by SNP markers encode for NBS-LRR kind of receptors for disease resistance, among others. However, two regions, one in Pv10 and other in Pv11 were associated with resistant genes to the common bacterial blight and anthracnose pathogens, respectively. The comprehensive approach utilized in the current studies will guide breeders and geneticists by providing sources of resistance to the most virulent and prominent pathogen races and functional candidate genes for resistance to *U. appendiculatus*. New approaches for developing resistant varieties include focusing on the use of gene pyramiding, resistance by gene silencing and the use of quantitative trait loci (QTL).

3.6. Literature cited

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4. GENOTYPING *UROMYCES APPENDICULATUS* ISOLATES FROM NORTH DAKOTA

4.1. Introduction

Uromyces appendiculatus (Pers:Pers), the causal agent of common bean rust, is a biotrophic, autoecious, macrocyclic fungus. The fungus is able to infect all aerial parts of the plant (Venette and Lamey, 1995). Bean rust can cause losses up to 80% if it develops early in the season (Schwartz, et al., 1984). As a biotroph, *Uromyces appendiculatus*, has a very close interaction with its host. Common bean, *Phaseolus vulgaris* L., has one center of origin in Middle America and two centers of domestication, Mesoamerica, near present day southern Mexico, and the Andean mountains of Peru (Liebenberg and Pretorius, 2010).The center of origin for *U. appendiculatus* was determined to be Honduras, as the location of highest pathogen virulence diversity (Acevedo et al., 2013). Pathogen population genetics suggest that *U. appendiculatus* has coevolved with the host, resulting in major genetic groups (Acevedo et al., 2005; Hurtado-Gonzales et al., 2018; Wright et al., 2008). The pathogen clustered in distinctive groups as a Middle American race, an Andean race and a combination of Middle American and Andean races based on random amplification of polymorphic DNA (RAPD) analysis using parsimony and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) methods (Araya et al, 2004; Sandlin et al, 1999). Two pathogen gene pools, Mesoamerican and Andean, were proposed in recent studies based on Simple Sequence Repeat (SSR) markers (UPGMA method) evaluating 46 races of *U. appendiculatus* (Hurtado-Gonzales et al., 2018). True Andean races are virulent on resistance genes of Andean origin, and avirulent on genes of Middle America origin, and vice-versa. True Andean *U. appendiculatus* races were unable to produce teliospores under greenhouse conditions; and therefore, are evolutionarily deferred from teliospore producing races (Lu and Groth, 1987). The

determination of the center of diversity of the pathogen and the aggrupation of races accordingly to the center of domestications of the host supports the theory of coevolution of the host:pathogen (Acevedo et al., 2005; Wright et al., 2008).

High levels of diversity where observed when studying isolates from multiple countries, where some races were unique to a specific country (Jochua et al., 2008). Isolates from tropical and subtropical regions had higher virulence and a greater number of races were present when compared to temperate regions (Jochua et al., 2008). Local stimuli such as cultivated varieties and cultural practices can influence the virulence composition within and among pathogen populations (Alleyne et al., 2008). Therefore, genetic differences in race composition may be present, even at a local level. *Uromyces appendiculatus* belongs to the phylum Basidiomycota and is dikaryotic (n+n) for the majority of its lifecycle. The whole genome of *U. appendiculatus* is currently not available but has been estimated at 560Mb, roughly seven times larger than *Puccinia graminis* (94Mb) (Eilam et al., 1992; Puthoff et al., 2008), making genetic studies difficult to perform and interpret. *De novo* genome assembly of races 5-0 and 31-1 resulted in genome sizes of 587.6 and 546.7 Mb, respectively, confirming previous estimates (Hurtado-Gonzales et al., 2018). However, this genome assembly is not publicly available. In comparison to the host of this pathogen, *P. vulgaris* is a diploid with 11 chromosomes (2n=22) with a genome size of 450 to 650 Mb (Gepts, 1999).

Little is known about *U. appendiculatus* population structure in North Dakota, the primary common bean producing state in the United States. The last race characterization was conducted in 2008 (Markell, et al., 2009). Urediniospores and teliospores have been observed to overwinter in the state (Gross and Venette, 2001; Venette et al., 1978). Evidence of sexual reproduction, aecia, has been observed in North Dakota, although this is uncommon in the field (Gross and Venette,

2001). We aim to study the structure of the *U. appendiculatus* population in North Dakota using a genotyping approach.

4.2. Materials and methods

4.2.1. Pathogen collection

Common bean fields were surveyed during the 2015 and 2016 growing seasons in the main growing regions of North Dakota. Samples were collected and race typed as described on chapter 2. Among the 128 single pustule *U. appendiculatus* isolates characterized using the current bean differential set (12 lines), data from 86 isolates characterized into 22 races were utilized in this study (Table 4.1) (Mmbaga et al., 1996; Stavely et al., 2002; Steadman et al., 2002). . Twenty-five isolates were collected from North Dakota in 2015 and 49 on 2016. Seven isolates were obtained from a historical rust collection at North Dakota State University. Three were obtained from United Stated Department of Agriculture, Agricultural Research Station at Beltsville, MD courtesy of Dr. Marcial Pastor-Corrales. In addition, one isolate was received from Michigan (courtesy of Dr. James Kelly) and one from Puerto Rico (courtesy of Dr. James Beaver). The 74 isolate subpopulation from North Dakota represented the 128 isolates in terms of virulence phenotype. Fifty percent of isolates used were identified as race 20-3, the most commonly race found in North Dakota in 2015 and 2016 (Figure 4.1). Thirteen percent of isolates used were identified as race 21-3, 7% as race 29-3 and 5% as 28-3. One to two isolates were included from the remaining races.

Table 4.1. Virulence phenotype and race of *Uromyces appendiculatus* isolates based on reaction on 12 lines in the common bean differential set used for genotype by sequencing.

		Differential line[d]												
Isolate ID[a]	Race[c]	EG	RP	M	PC50	GGW	PI2	GN11	Aurora	Mx309	Mx235	CNC	PI18	Origin[e]
MI-22-2	22-2	1	6	6	1	6	1	1	6	1	1	1	1	MI
PR-1-SP1	20-3	1	1	5,6	1	6	2,3	6	6	1	2,3	3,2	1	PR
RACE 47[b]	15-3	4,3	6	5,4	3,5	1	- [f]	6,5	6,5	1	1	1	1	FL
RACE 53[b]	31-3	4	5,6	6,5	4	6	-	6,5	2	1	1	1	1	MD
RACE 67[b]	31-22	6	6	6	6	6	1	6	5,6	1	1	1	1	FL
UP15-1-2-SP3	17-3	5,6	1	3,4	1	5	1	5,6	6,5	1	1	1	1	ND
UP15-1-4-SP2	28-11	1	1	4	4,3	5,6	2,3	6	4	2,3	4,3	3,2	1	ND
UP15-1-4-SP6	20-3	1	1	5,6	1	5,6	1	6	4,3	1	3,2	1	1	ND
UP15-1-5-SP1	20-3	1	1	5,6	1	4,5	3	6	6,5	1	3,2	1	1	ND
UP15-1-5-SP3	16-3	1	2,3	3,4	3,4	5	3,4	6,5	6,5	3,2	3,2	2,3	1	ND
UP15-1-6-SP6	20-3	1	1	4,5	1	5,4	2,3	6,5	4,5	1	2	1	1	ND
UP15-2-1-SP3	20-11	1	1	5,6	1	5,6	1	5	5,6	1	4,3	1	1	ND
UP15-2-1-SP9	29-3	6,4	2,3	4,3	4,5	6	3,2	6	5,6	1	1	1	1	ND
UP15-2-2-SP6	16-3	1	1	6	1	6,5	1	6,5	6	1	1	1	1	ND
UP15-2-3-SP1	21-3	4,3	2,3	4,5	3	5,6	2,3	6	5,4	2,3	3,2	1	1	ND
UP15-2-5-SP4	20-3	1	1	5,6	1	6,5	1	6	5,6	1	2,3	3,2	1	ND
UP15-4-2-SP1	20-3	1	1	6,5	1	5,6	1	6,5	6	1	1	1	1	ND
UP15-6-1-SP2	20-3	1	1	5,4	1	6,5	3,4	6	6,5	2,3	3,2	1	1	ND
UP15-2W-2-SP3	20-3	1	1	5,6	3,4	5,6	3,2	5,4	5,6	1	3,4	1	1	ND
UP15-2W-4-SP3	20-3	1	1	5,6	1	6,5	1	6	5,6	1	2,3	1	1	ND
UP15-4W-1-SP5	20-2	1	1	4,3	1	6	1	2	5	1	2	1	1	ND
UP15-7W-2-SP4	20-3	1	1	5	H	6	3,2	6	6,5	1	1	1	1	ND
UP15-7W-4-SP3	20-3	1	1	6	1	5,6	3,4	5,6	6	2	2,3	1	1	ND
UP15-8W-3-SP5	20-3	1	1	5,4	1	5,6	3	6,5	5	1	2	1	1	ND
UP15-9W-3-SP5	20-3	1	1	5,6	1	5	4,5	6,5	5,6	1	1	1	1	ND
UP15-10W-2-SP4	20-3	1	1	6	1	6	3	6	4,6	2,3	2,3	1	1	ND
UP15-28-SP2	28-3	1	1	5,4	4	5	1	6	5,6	1	2,3	1	1	ND
UP15-28-SP3	20-3	1	1	5,4	1	5,4	2	6	6	1	3	3,2	1	ND
UP15-34-1-SP	20-3	1	1	4,3	3,2	6,5	2,3	6	5,4	2,3	3	1	1	ND
UP15-35-2-SP1	20-3	1	1	6,5	1	6,5	3	6	6,5	2,3	2,3	1	1	ND
UP16-1-2-SP1	21-27	4,3	3,2	4	3,1	6,5	1	6	6	2,3	4,3	4,3	1	ND

(*continued on next page*)

Table 4.1. Virulence phenotype and race of *Uromyces appendiculatus* isolates based on reaction on 12 lines in the common bean differential set used for genotype by sequencing (continued).

		Differential line[c]												
Isolate ID[a]	Race[b]	EG	RP	M	PC50	GGW	PI2	GN11	Aurora	Mx309	Mx235	CNC	PI18	Origin[d]
UP16-3-1-SP1	21-3	5	1	4,5	2	4	1	5	4	1	1	1	1	ND
UP16-3-2-SP1	21-19	4,3	1	5,6	3	5,6	-	6,5	6	3,2	-	4,3	1	ND
UP16-5-3-SP1	20-3	1	1	5,6	1	5,6	-	5,6	4	3,2	1	2,3	1	ND
UP16-6-1-SP1	29-33	4,5	1	5	5,4	6,5	-	6	6	6	4	4,3	1	ND
UP16-8-1-SP1	24-3	3,2	1	3	4,1	5,4	-	4,5	4,5	1	3,2	1	1	ND
UP16-8-2-SP1	21-3	5,4	3	4,5	3,2	6	2,3	6	6	2	2	3,2	1	ND
UP16-9-2-SP3	20-9	1	1	5,4	1	5,4	-	4,5	4,3	3,2	4,3	3,2	1	ND
UP16-9-2-SP5	20-3	2	1	4,3	3,4	5,4	1	6	6	1	3	3,2	1	ND
UP16-10-1-SP3	27-7	5,4	4,3	4,3	6,3	6	3,2	6	6	5,6	3	3,2	1	ND
UP16-10-2-SP4	27-7	5,4	4,3	4,2	6,3	6	-	6	6	5,6	3	3,2	1	ND
UP16-10-3-SP1	20-3	3,4	1	4,3	2	6,4	3,4	6	5,6	1	1	1	1	ND
UP16-12-1-SP2	21-3	5,4	1	5,6	2	6,5	4,3	6	6	2,3	3,2	2,3	1	ND
UP16-12-1-SP3	21-3	4,3	3,2	5,4	2	6	-	6	6	2,3	3,4	3,4	1	ND
UP16-12-1-SP4	21-3	4,5	3,4	5,4	3,2	5,4	-	6	4,3	3,4	3,2	3	1	ND
UP16-12-2-SP1	29-3	5,6	1	6,5	4,3	5,6	-	6	6,5	1	1	1	1	ND
UP16-13-2-SP1	29-3	5,4	1	6,5	4,3	5,4	-	6	6	1	3,2	3,2	1	ND
UP16-13-3-SP1	20-3	1	1	5,4	1	6,5	-	6,5	6,5	1	1	1	1	ND
UP16-13-3-SP1	21-3	5,4	1	5,4	1	6	-	6,5	5,6	1	1	1	1	ND
UP16-13-3-SP3	20-1	2	1	5,4	2	6,5	-	6	1	1	1	1	1	ND
UP16-14-1-SP7	20-3	1	1	5,4	1	5,6	3,2	6	6	1	1	1	1	ND
UP16-15-1-SP1	21-3	4,3	2,3	5,6	1	4,5	-	4,5	6	3,2	3,4	2,3	1	ND
UP16-16-1-SP1	21-3	4,3	1	3,4	2	5	1	6,5	5	1	2	1	1	ND
UP16-16-2-SP1	20-3	2	1	5,4	2	6,5	-	6,5	6,5	1	1	1	1	ND
UP16-16-2-SP2	20-7	1	1	5,4	1	5,4	3,2	-	6,5	4,5	3	2,3	1	ND
UP16-16-3-SP1	21-3	4,3	1	4,3	3,4	4,5	-	5	6	2,3	3,2	1	1	ND
UP16-17-1-SP1	29-11	4,3	1	5,4	4,3	5,6	1	6	6	3,2	4,3	3,2	1	ND
UP16-17-2-SP1	29-3	6,5	3,2	5,4	5	6	-	6	6	2	3,2	1	1	ND
UP16-17-2-SP2	20-3	3,4	1	5,4	1	5,6	3,4	6	5,4	1	3	1	1	ND
UP16-17-2-SP3	20-3	2	1	6	1	6	-	6	6	1	1	1	1	ND
UP16-F5-SP2	20-3	1	1	4,3	1	5,4	3,2	6,5	4,5	1	1	1	1	ND
UP16-F14-SP1	20-3	2,3	2,3	6,5	1	5,6	6	4,5	1	1	3,2	1	1	ND

(*continued on next page*)

Table 4.1. Virulence phenotype and race of *Uromyces appendiculatus* isolates based on reaction on 12 lines in the common bean differential set used for genotype by sequencing (continued).

		Differential line[c]												
Isolate ID[a]	Race[b]	EG	RP	M	PC50	GGW	PI2	GN11	Aurora	Mx309	Mx235	CNC	PI18	Origin[d]
UP16-F14-SP2	20-3	2,3	2,3	6,5	1	5,6	6	4,5	1	1	3,2	1	1	ND
UP16-F15-SP1	20-3	3,4	1	4,5	3	5	-	5,4	5	1	1	1	1	ND
UP16-F15-SP3	20-3	2	2,3	6,5	2	6	-	6	6	1	1	1	1	ND
UP16-F16-SP1	20-3	3,2	1	4,3	1	5,4	3	6,5	4,5	1	3	1	1	ND
UP16-F16-SP2	28-3	3,4	2,3	5,4	5,4	6	3	6	6	1	3	3,2	1	ND
UP16-F16-SP3	20-3	2	1	6	2	5,6	3,2	6,5	6,5	1	1	1	1	ND
UP16-F18-SP1	20-3	3,4	3,2	4,3	1	5,4	-	5,4	5,4	3,2	3,2	3,2	1	ND
UP16-F18-SP1	20-3	3,4	3,2	4,3	1	5,4	-	5,4	5,4	3,2	3,2	3,2	1	ND
UP16-F19-SP2	20-3	2	1	6	2	6	3	6	6	2,3	3,2	2,3	1	ND
UP16-F19-SP3	28-3	2	2,3	5,6	5,4	6,5	-	6	6	3,2	2,3	2,3	1	ND
UP16-F20-SP1	20-3	1	1	6,5	1	6	3,2	6	6	1	1	1	1	ND
UP16-F22-SP1	28-3	2	2,3	4,5	4,3	5	-	6	5,4	1	1	1	1	ND
UP16-F22-SP7	29-3	4,3	1	5,4	4,3	6,5	4	6	6	3,4	3,4	3,2	1	ND
UP16-F23-SP1	16-3	2	2	3,4	1	5,4	3,4	6	5,4	1	3,4	1	1	ND
UP16-F27-SP1	28-11	3,4	1	4	4	5,4	3,4	6	6	3,4	4,3	3,2	1	ND
UP16-F27-SP2	20-3	1	1	4,5	2	4	1	5,6	4	1	3	1	1	ND
UP16-F31-SP1	20-3	2	1	5,4	2	6	3	6	6	2,3	3,4	3,2	1	ND
UP1991-SP2	20-3	1	2,3	5	2,3	6	2,3	6	6	2,3	3	3,2	1	ND
UP1991-SP3	20-3	2	1	4,5	2	5	3,2	6	5,6	1	3	1	1	ND
UP1993-SP1	29-3	6,5	2,1	6,5	4,5	6,5	-	6	6	2,3	2,3	2,3	1	ND
UP1995-SP1	21-3	5,4	3,4	4,5	3,4	5,4	-	5,4	5,6	1	3,4	1	1	ND
UP1996-SP2	20-3	1	1	5,4	1	5,6	-	6	6	3,2	2,3	2,3	1	ND
UP2007-SP2	20-3	2	1	5,6	2	6	2,3	6	6	2,3	3,2	3,2	1	ND
UP2007-SP4	20-3	2	1	5,6	2	6,5	-	6	6	3,4	3,4	3,4	1	ND

[a] *Uromyces appendiculatus* naming format for isolates collected in North Dakota in 2015 and 2016. UP=*Uromyces appendiculatus* followed by collection year 15=2015, 16=2016; field and sample number (w = west), SP= single pustule isolate number

[b] Race = Race nomenclature based on previous bean differential set

[c] Reaction grades 1= No visible symptoms; 2= Necrotic spots without sporulation (hypersensitive response); 2,3= Reaction 2 with few type 3; 3,2= reaction type 3 with few type 2; 3= Uredinia <0,3 mm in diameter; 3,4= reaction 3 with few type 4; 4,3= Reaction 4 with few type 3; 4= Uredinia 0,3-0,49 mm in diameter; 4,5= Reaction 4 with few type 5; 5,4= Reaction 5 with few type 6; 5= Uredinia 0,5-0,8 mm in diameter; 5,6= Reaction 5 with few type 6; 6,5= Reaction 6 with few type 5; 6= Uredinia 0,8-1,2 mm in diameter, (-)= missing data.

[d] Differential lines: EG= Early Gallatin, RP= Redland Pioneer, M= Montcalm, GGW= Golden Gate Wax, PI2= PI260418, GN11=Gn1140, Mx= Mexico, CNC= Compuesto Negro Chimaltengo, PI18= PI181996.
[e] MI = Michigan (courtesy J. Kelly), PR = Puerto Rico (courtesy J. Beaver), MD = Maryland (courtesy M. Pastor-Corrales), FL = Florida (courtesy M. Pastor-Corrales), ND = North Dakota
[f] (-)= data not available

4.2.2. DNA extraction

Uromyces appendiculatus urediniospores were generated under greenhouse conditions and collected as previously described (chapter 2). DNA was extracted from urediniospores from single pustule isolates using the OmniPrep^{TM} (G-BioScience, Cat. No. 786-399) extraction kit. Urediniospores were frozen with liquid nitrogen and ground before following the protocol detailed by the manufacturers with minor adjustments. A pre-protocol step was added to the DNA extraction method to optimize extraction. Five-hundred microliters of Genomic Lysis Buffer and 5µL of Proteinase K solution were added to each sample tube. Samples were vortexed for 3 min, incubated in a water bath at 60ºC for 10 min, and vortexed for 3 min. The remaining of the manufacture's protocol was followed with no modifications. Samples were incubated at 60ºC for 2 h with periodic inverting of tubes and cooled to room temperature. Two-hundred microliters of chloroform was added and the solution was mixed by inverting several times. Samples were centrifuged for 10 min at 14,000×g. and the upper phase was carefully pipetted into a clean microcentrifuge tube. Fifty microliters of DNA stripping solution was added to the upper phase, the solution was inverted multiple times to mix and incubated for 5 to 10 min at 60ºC. On-hundred microliters of Precipitation solution was added and the tube was inverted. Additional aliquots of 50µL of Precipitation solution were added as needed until a white precipitate was observed. Samples were centrifuged at 14,000×g for 5 min. The supernatant was transferred to a clean tube and 500µL of isopropanol was added. Sample tubes were carefully inverted 10 times to precipitate DNA, centrifuged at 14,000×g for 5 min and the supernatant was carefully removed via pipetting. Seven-hundred microliters of 70% ethanol was added to wash the pellet, followed by centrifuge at 14,000×g for 1 min. Ethanol was decanted and samples were set on an absorbent surface to dry for 5 to 10 min. Rehydration was complete after 15 min incubation in 70µL of TE buffer at room

temperature. LongLife™ RNase was added to samples, 1µL per tube. DNA quantity was measured using the Qubit® 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA) and DNA was stored at -20ºC.

4.2.3. DNA sequencing

DNA preparation was conducted using New England Biolab (NEB) *Hhal* and *Apek1* enzymes to cleave DNA for ligation of adapters designed for sites. Total reactions of 50µl consisted of 40µl of genomic DNA (0.01-0.5 ng/µl), 5µl of 10× NEB3.1, 0.2µl of *HhaI* (R0139) (400 U/mL), 0.2µl of *ApeKI* (R0643) (400 U/mL), and 4.6µl of molecular grade water. Reaction was incubated 2 h at 37°C and 2 h at 75°C.

Nonligated genomic DNA was removed by cleaning samples using Sera-Mag SpeedBeads (GE Healthcare 24152105050250) followed by the addition of 1.7× volumes (85µl) of Sera-Mag beads to each 50µl sample. The samples were mixed well by pipetting, incubated at room temperature for 5 min, placed on a magnetic stand for 2 min, or until clear, and washed twice with 200µl of freshly prepared 80% EtOH (ethyl alcohol). Beads were air dried for 5 to 15 min (over-drying this will cause poor recovery) and 10µl Ligation mix was added directly to the beads.

Adapters were ligated to fragmented DNA using T4 DNA ligase (NEB M0202). Reactions of 10µl consisted of 1µl of 10× T4 Buffer, 0.5µl of T4 ligase (1-2 µM), 1µl of Barcode Adapter (5µM), 1µl of universal Barcode Adapter (5µM), and 6.5µl of molecular grade water were added to the beads. Sample solutions were vortexed lightly to re-suspend, incubated at 16°C overnight and heat inactivated at 65°C for 10 min.

Samples were cleaned using the Sera-Mag SpeedBeads procedure as described above and diluted in 10µl water. DNA concentration was determined using Qubit 2.0 High Sensitivity Flurometer. Equal amounts of samples (weight:weight) where combined and sized to a solid band at 275 bp on an electrophoresis gel, using Blue Pippen DNA size selector. Sized samples were

amplified using NEBNext High-Fidelity 2× PCR Master Mix; with 10µl of sample (0.01-0.5 ng/µl), 2µl of NEB Ion primers (premix concentration), 13µl molecular water, 25µl of 2× PCR Master Mix. PCR protocol was run at 98ºC for 30 sec initial denaturation, 98ºC for 10 sec, 58ºC for 30 sec, 65ºC for 30 sec, repeated 10×, with a final extension at 65ºC 5 min and held at 4ºC. Following amplification, samples were cleaned using the Sera-Mag SpeedBeads procedure, diluted into 20µl in molecular grade water, and concentration was determined using a Qubit 2.0 High Sensitivity Fluorometer. Samples were diluted to 80pM for ION CHEF Chip preparation. Amplified samples were sequenced by restriction site associated DNA sequencing genotyping by sequencing (RAD-GBS) on ION 540 chips using the ION S5.

4.2.4. Bioinformatics

Sequencing reads generated with the ION S5 were uploaded to CLC genomics workbench v8.0 software (QIAGEN) to perform quality assessment (using default parameters) and trim end reads. The 5' and 3' end of each sequencing read were trimmed by 22 nucleotides to remove the PCR adapters (Richards et al., 2016). The trimmed reads were utilized for downstream analysis (*de novo* assembly and variant calling). Isolate UP15-6-1-SP2, identified as race 20-3, was used as the *U. appendiculatus* model for *de novo* assembly using the publicly available software SPAdes (v. 3.13.0) (Bankevich et al., 2012). This isolate provided the highest number of high quality sequences and represented the most frequent virulence phenotype characterized. The sequencing reads were corrected using the "careful" option that utilizes IonHammer (an error correction tool for Ion Torrent data) embedded in the software. The reads were *de novo* assembled using default options and an initial k-mer length parameter of 21, 33, 55, 77 and reassembled using options '-restart-from k77 -k 21, 33, 55, 77, 99, 127 -mismatch-correction' to reduce assembly errors and minimal read coverage across the genome.

The quality trimmed reads were mapped to the *de novo* assemble reference sequence of UP15-6-1-SP2 using the Burrows–Wheeler Aligner maximal exact match (BWA-MEM) algorithm (Li, 2013). Variants [(Single Nucleotide Polymorphisms (SNPs) and Insertion/Deletion (INDELs)] were called using GATK HaplotypeCaller tools in ERC GVCF mode with the parameters suggested for genomic sequences (Van der Auwera et al., 2013). The individual g.vcf files were combined using GATK GentoypeGVCFs tool to generate final VCF flies containing variants from all samples used for sequencing. Each individual variant call/ sample was filtered to select for the variants with genotype quality greater than 10 and read depth greater than 4 using Vcftools (Danecek et al., 2011). Variants with minor allele frequency (MAF) < 5% and missing data > 40% were removed from the analysis. The allele frequency of each variant per sample were corrected for heterozygous calls using a custom visual basic script to generate a final VCF file for subsequent analysis (Sharma Poudel et al., 2018).

4.2.5. Population genetics analysis

The filtered and corrected VCF file was converted into R-packages poppr (Kamvar et al., 2014; 2015) and adgenet (Jombart et al., 2008) readable format using an R-package vcfR (Knaus and Grünwald, 2017). A distance tree was constructed using UPGMA/Neighbor-joining algorithm with 100 bootstrapping. The structure/variance in the population based on location and years were inferred using principal component analysis (PCA) and discriminant analysis of principal component (DAPC).

4.3. Results

Eighty-six *U. appendiculatus* isolates representing 22races were characterized and used for GBS. Because the genome of *U. appendiculatus* is not publically available, a total of 53.6M reads were generated for *U. appendiculatus* isolate UP15-6-1-SP2 and used for *de novo* assembly. The

Restriction site Associated DNA Sequencing (RAD-Seq) data were assembled to 5691 contigs that accounted for 1.99MB of bean rust genome, with an N50 contig size of 1.12KB. Sequencing 86 single pustule isolates of *U. appendiculatus* resulted in 428.1M single end reads (average of 4.5 M S.D. ± 8.6M reads/samples) with an average read length of 174.1bp and 102,098 biallelic raw variants. Initially, variants with missing data > 80% were removed to obtain 22,772 variants. These 22,772 variants were used to filter isolates with < 90% genotypic data. This resulted in 71 samples for downstream analysis with 6,279 variants and missing data < 40%.

Nineteen PCA were required to explain 50% of the variance based on eigenvalues, where PCA1 only explained 5.87% of the variation (Figure 4.1). No principal component explained virulence phenotype. No clustering was observed based on collection year or location (Figure 4.2).

Results from the neighbor joining tree across all 86 *U. appendiculatus* isolates indicate that up to 2.5% of loci were different based on genetic distance (Figure 4.3). Phylogenetic analysis placed isolates of race 20-3 in all major branches, and no distinctive clades were formed based on phenotypic race.

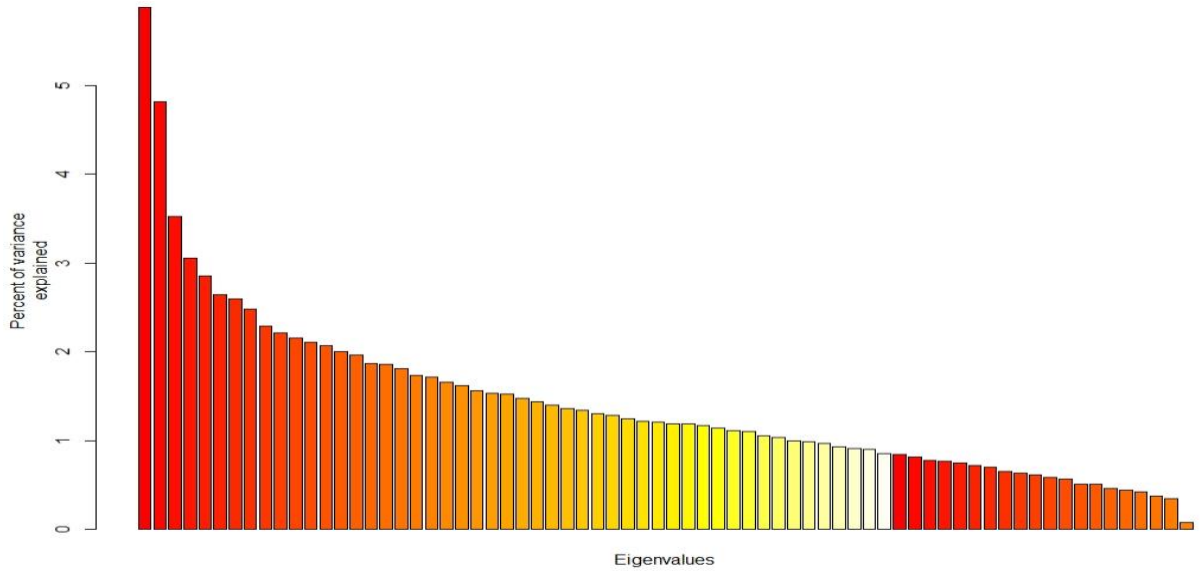

Figure 4.1. Histogram representing percent of variance explained (y-axis) by Principal Component Analysis (PCA) on x-axis.

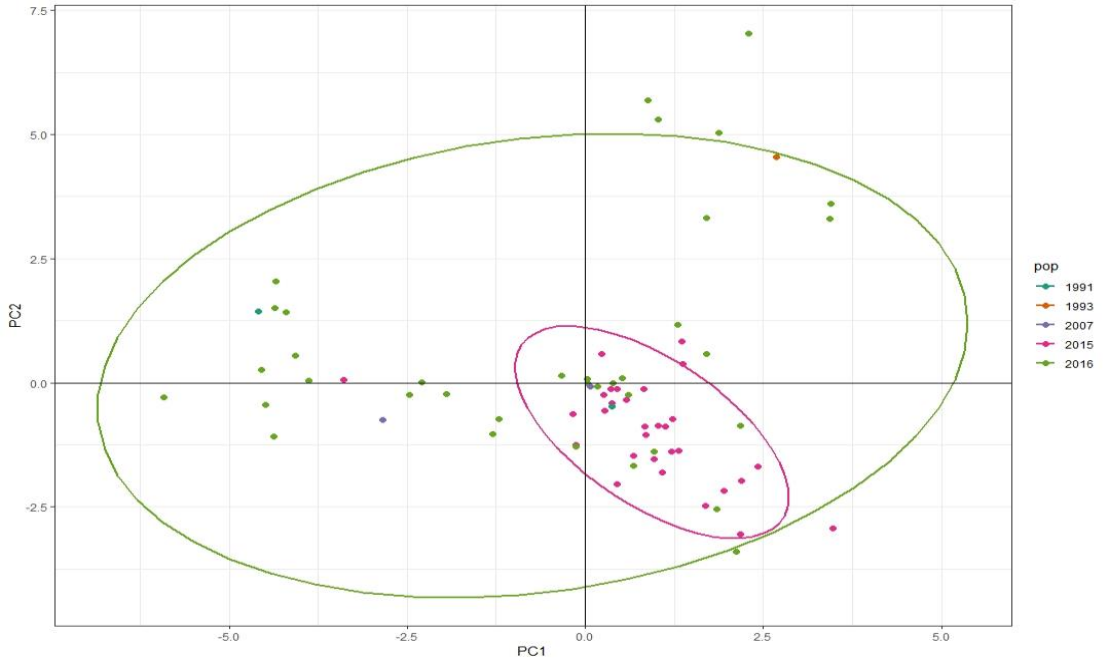

Figure 4.2. Principal component analysis of *Uromyces appendiculatus* single pustule isolates from North Dakota, Michigan, Maryland and Puerto Rico across multiple years.

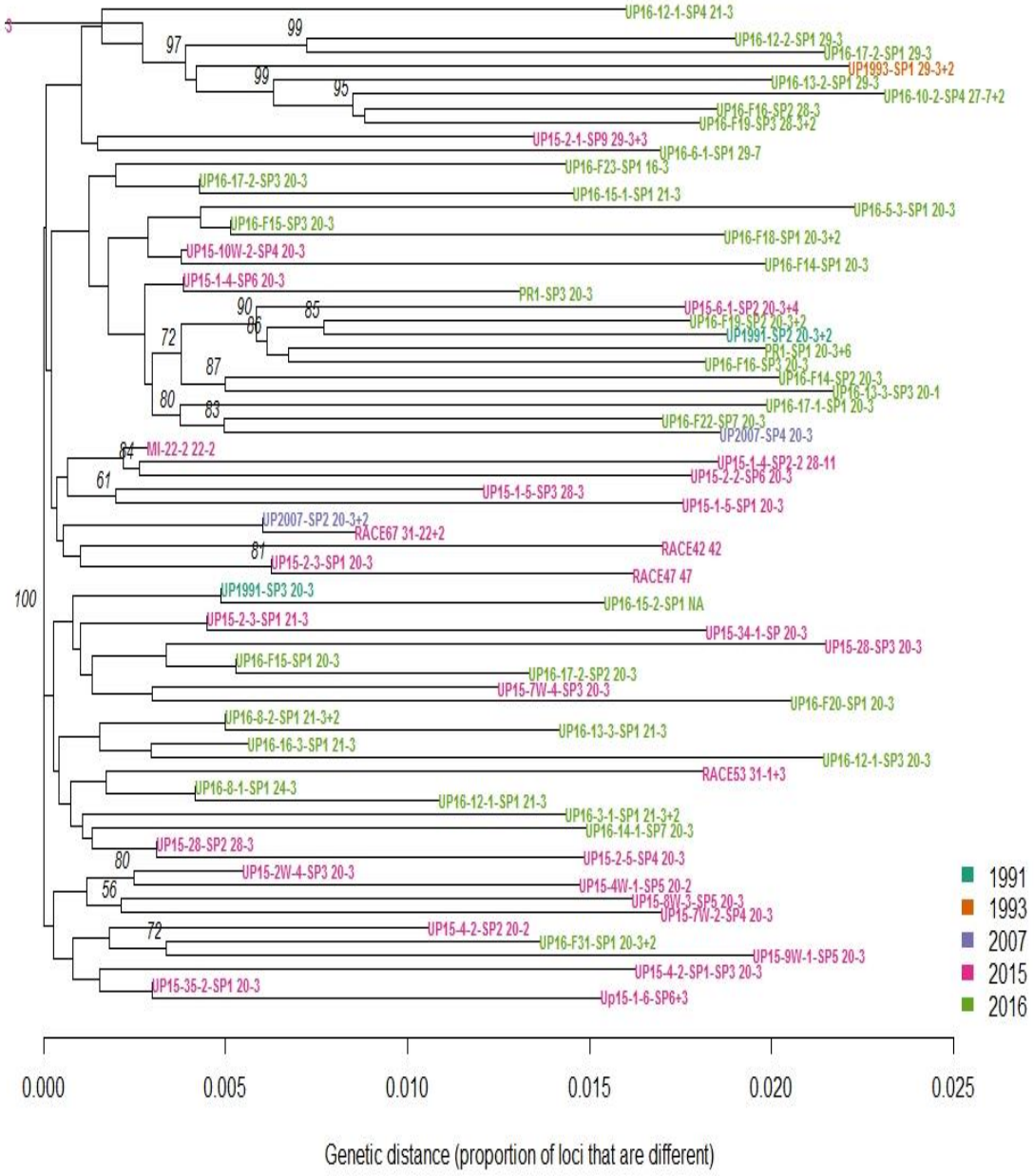

Figure 4.3. Neighbor joining dendrogram based on genetic distances of *Uromyces appendiculatus* single pustule isolates from North Dakota, Michigan, Florida, Maryland and Puerto Rico across multiple years.

4.4. Discussion

Results from this study substantiate previous findings that the North Dakota *U. appendiculatus* population is highly diverse and sexually reproducing as previously reported in the state (Venette et al., 1978). Principal component analysis was unable to explain the variation, suggesting lack of genetic structure in the population due to high levels of diversity. No clustering was observed based on year or location. Again indicating that great diversity is present in the *U. appendiculatus* population in North Dakota. Phylogenetic analysis was unable to correlate the polymorphic markers with the virulence phenotype (race). Therefore, we hypothesize that potential avirulence genes are independently assorted as would be expected in a population with high recombination. This suggests that there is no correlation between genotype and phenotype, meaning that multilocus genes do not correlate with race type. This suggests that the North Dakota *U. appendiculatus* population is sexually reproducing in panmixia.

The present study has some limitations. The genome size of *U. appendiculatus* is very large, resulting in low sequencing coverage and subsequent difficulties in interpreting the results. More accurate and conclusive results will likely require genotyping on a much larger scale. The lack of a reference genome also presents certain limitations, particularly in identifying avirulence candidate genes. Optimal results are dependent upon a high-quality reference genome. Even though sampling was made in multiple counties across North Dakota, this can still be considered a local population given the narrow geographic area covered and its environmental homogeneity. Hence, using more isolates from other geographic locations or across time would make future studies more robust.

The current research has provided further evidence of sexual recombination in the *U. appendiculatus* population in North Dakota. Race characterization based solely on virulence

phenotype is useful to classify races and to devise the best genetic approaches for resistance breeding. However, minor genetic changes can accumulate over time and may be difficult to perceive by phenotypic characterization only. Sexual recombination is likely playing a major role in the emergence of new *U. appendiculatus* races by accumulating or pyramiding existing genes. Genes that are less frequent can become more prevalent in a population by sexual recombination and mutations.

Aecial and pycnial sexual stages of *U. appendiculatus* have been identified in North Dakota (Venette et al., 1978), Colorado (McMillan et al., 2003; Schwartz et al., 1990; 1999), Oregon (Zaumeyer et al., 1957), New York (Jones, 1960) and Nebraska (Schwartz et al., 1994). While sexual recombination is an important source of diversity, other factors also may be playing important roles including windblown spread of spores from other areas. Such is the case of other rusts like wheat stripe rust spread via the 'Puccinia pathway' which details the spread of primary inoculum and rust races from southern to northern states (Kolmer, 2001). *U. appendiculatus* race 22-2, 22-3 and 31-3 have been reported in Michigan, race 63-19 was reported in Puerto Rico (Pastor-Corrales et al., 2010; Vega et al., 2009; Wright et al., 2009). Even though Michigan has similar virulence races in their *U. appendiculatus* reports, it has been demonstrated that they are not the same as the race 20-3 race reported in North Dakota (Pastor-Corrales et al., 2010). Therefore, wind-blown spores from other growing regions are likely not contributing to race complexity in North Dakota.

While sexual recombination is taking place in the *U. appendiculatus* populations in North Dakota, and elsewhere, asexual reproduction and heterokaryosis of asexual urediniospores might be playing a role in genetic diversity (Souza et al., 2008). While sexually reproducing populations are typically very diverse, asexually reproducing populations have been observed to be very

diverse as well. This is particularly evident in Andean races of *U. appendiculatus*, which fail to produce teliospores (Groth et al., 1995; Liebenberg and Pretorius, 2010). Population studies of *Puccinia striiformis* f,sp. *tritici* (wheat yellow rust) also have determined that high levels of diversity are present in asexual populations where an alternate host is not present (Bahri et al., 2009).

4.5. Conclusions

Uromyces appendiculatus is the causal agent of common bean rust. This disease has reached epidemic levels worldwide and in North Dakota, the primary common bean producing state in United States. Studies of *U. appendiculatus* in North Dakota have concluded that the pathogen is sexually reproducing, contributing to great diversity in the population. In the current studies, no correlation was observed between genotype and phenotype, suggesting that there are minor changes in virulence that are unable to be tracked based solely on phenotype, or that additional data are needed, particularly genomic data. Further studies are recommended to gain a greater understanding of the genomic regions important in the *P. vulgaris*:*Uromyces appendiculatus* interaction.

4.6. Literature cited

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