RUST RESISTANCE IN THE GUATEMALAN CLIMBING BEAN GERMPLASM

COLLECTION

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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

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ABSTRACT

Common bean is the main source of protein, fiber, and iron for Guatemalan's poorest households; however, bean rust can cause up to 100% yield losses. There is limited information about bean rust in Guatemala, especially at mid-altitude highlands. During 2015, 23 bean rust samples were collected in the Western Guatemalan Highlands and 17 isolates where characterized, as a result, races 63-1 and 31-1 were identified. Those were used to evaluate rust resistance by using 372 Guatemalan climbing beans. In total, 82% of accessions were resistant to race 63-1, 86% to race 31-1, and 90% to race 20-3. Based on GWAS results (78,754 SNPs) the Pv02 (38.13 Mb-38.22Mb) and Pv04 (379 kb) regions were associated with resistance to race 20-3. The Pv10 (10.71-10.68 Mb and 11.09 Mb) and Pv04 (1.42 MB) regions control resistance to race 63-1. And, the Pv04 (39.28 Mb) and Pv02 (35.92 Mb) control resistance to race 31-1.

ACKNOWLEDGEMENTS

DEDICATION

To my parents Luz Dominguez and Benjamin Montejo

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INTRODUCTION

Common bean *(Phaseolus vulgaris* L.*)* is one of the main crops in the world as a protein source and is the main food legume among all pulses in tropical Latin America and sub-Saharan Africa (Akibode and Maredia, 2011). In Guatemala, common beans are one of the most inexpensive sources of protein, fiber, and iron for low-income households. Climbing beans are cultivated in mid-altitude highlands and information about their genetic potential is limited. In addition, climbing beans have received less breeding efforts compared with bush types (Osorno and Mcclean, 2013). The population at this region suffer of critical malnutrition problems, where approximately 50% of the children under 5 years are undernourished. Thus, is one of the most affected areas in the world in the nutritional aspect (Osorno et al., 2013; CIA World Fact Book, 2016). Beans are one of the most important crops at this region and an important source of nutrients. Hence, food security can be enhanced by improving climbing bean productivity. Unfortunately, abiotic and biotic stresses can cause high yield losses. Among biotic stresses, bean rust is one of the most severe fungal diseases. The causal agent of bean rust, *Uromyces appendiculatus* (Pers) Unger, is considered one of the critical pathogens due its abundant virulence diversity. More than 300 rust races have been reported by using the set of 19 differential cultivars with known resistance genes (Araya et al., 2004). Since bean rust can reduce bean seed production, host resistance is the most effective management. In addition, it causes no negative environmental impact and has lower costs compared to other methods such as chemical control. Small-farmers have a limited production budget and therefore, cannot afford chemicals. Also, more than one half of the climbing bean area is planted in an intercropping system (maize-bean-squash), making chemical application more difficult (Aldana, Pers. comm., 2015).

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Approximately 24 rust resistance genes (*Ur*) have been formally named in common bean (Liebenberg and Pretorius, 2010). High levels of virulence can be observed in the tropics and subtropics but also in temperate environments (Schwartz and Pastor-Corrales, 1989; Souza et al., 2008). When early bean rust infection occurs, farmers can experience up to 100% yield losses causing a significant negative impact in the food chain (Schwartz and Pastor-Corrales, 1989).

Identification of bean rust isolates has changed through the time (Liebenberg and Pretorius, 2010). The first system proposed the use of 20 bean differential cultivars with known resistance genes (Stavely et al., 1983). Then, the Mountaineer White Half Runner cultivar was eliminated due to genes duplication (*Ur-4*) and ended up in 19 (Stavely and Steadman, 1992). A value was assigned to each accession, and the values of the susceptible accessions were added to the sum. However, a binary system was established later (Habgood, 1970) and the number differential lines were reduced to 12 (Steadman et al., 2002). Categorization of genotypes into resistant or susceptible based on reaction grade and uredinium size, which are in scale of 1 to 6 (Stavely, 1984; Mmbaga et al., 1996). (Stavely, 1984; Mmbaga et al., 1996). In 1979, a rust diversity study was conducted at the central part of Guatemala on bush type beans by using the old nomenclature (Stavely et al., 1983). As a result, races 20, 24 and 30, were identified which were previously reported in Mexico. Also, race 17, of Brazilian origin, was identified. (Anzueto, 1979). More recently in 2014, another survey of bean rust diversity was conducted in 12 bean producing fields at the mid-altitude highlands of Guatemala (Dardon et al., 2016). As a result, six virulence patterns were identified across the 12 locations. Among those six patterns, two isolates caused a susceptible reaction to only one accession of Andean origin. One isolate was avirulent to all differentials with resistance genes of Mesoamerican origin. The rest of isolates were virulent to different genes of Andean origin, and most of the ones from Mesoamerican. It

demonstrated the high levels of diversity of bean rust in fields. However, there are still several locations that needed to be surveyed in order to have a better understanding of rust virulence diversity in the country, especially at mid-altitude highlands due to its broad range of climate, topography, and diversity of bean genotypes both naturally and cultivated by farmers.

Many resistance genes have been identified and tagged within the common bean genome sequence (Schmutz et al., 2014). However, most of those genes have been found using bush type beans and bi-parental populations. Currently, genomic techniques such as Wide-Genome Association Studies (GWAS) allow to identify genomic regions associated with traits of interest. GWAS use non-parental accessions such as wild or germplasm collections, taking advantage of broader allelic diversity (Zhu et al, 2008). Many GWAS in beans for abiotic and biotic stresses resistance/tolerance, and yield components have been done lately (Agarwal, 2014; Cichy et al., 2015; Kamfwa et al., 2015; Ghising, 2016; Moghaddam et al., 2016; Perseguini et al., 2016; Vasquez, 2016). Therefore, GWAS has proven to be a powerful tool to find potential genomic regions on climbing beans germplasm associated with genetic resistance/tolerance to limiting production factors.

A germplasm collection of climbing beans was assembled and kept at the Agricultural Institute of Sciences and Technology in Guatemala (ICTA in Spanish). Based on preliminary results of principal components analysis, population structure and the phylogenic tree, the Guatemalan climbing beans are a separate race of beans, representing a new source of genetic diversity in the Mesoamerican gene pool (Tobar et al., 2017). Visual scores for several diseases have been taken under field conditions and natural disease pressure, but with no clear knowledge of which races were present during these field evaluations. Therefore, no formal study under controlled conditions have evaluated rust resistance in this collection yet. This project is an

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attempt to evaluate this germplasm collection of climbing beans from ICTA, Guatemala with the goal of finding sources of resistance.

Consequently, objectives of this projects were: 1) To assess the race diversity of bean rust from the Western Highlands in Guatemala; 2) to evaluate the reaction of the Guatemalan climbing bean germplasm to rust races from Guatemala and race 20-3 identified at North Dakota; 3) and to identify genomic regions associated with rust resistance using GWAS.

These resistant germplasm accessions will be useful as prospective parents to improve bean cultivars, in order to reduce seed yield losses in Guatemala as well as other countries/regions with bean rust problems.

LITERATURE REVIEW

The Importance of Common Bean

Grain legumes have high protein content and are worldwide consumed (Broughton et al., 2003). In some countries of the Sub Saharan Africa region, legumes provide more than 10% of daily total caloric consumption (Akibode and Maredia, 2011). However, common bean (*Phaseolus vulgaris* L.) is the most important among them (Broughton et al., 2003). Common bean represents 46% of total world legume production, and its production growth 1.9% annually (Akibode and Maredia, 2011). Also, common beans improve soil fertility by nitrogen fixation that is then metabolized into seed protein. (Graham and Vance, 2003). In addition to its high quality protein content, beans provide complex carbohydrates, fiber, vitamins, and minerals (iron), making beans a complete food (Geil and Anderson, 1994). Hence, beans play an important role in food security, especially in developing countries in Africa, Latin America, and Asia, where prices of animal sources of protein are not accessible for the poorest population segment (Gepts, 2004). Common bean provide approximately 50% of the daily protein requirement in many Sub-Saharan Africa regions (Broughton et al., 2003). Worldwide, an average of 26.67 million ha of dry beans were harvested between 2008 to 2010, producing an average of 18.82 million of tons (Akibode and Maredia, 2011). The top producing regions are Latin America and Caribbean, South and South East Asia, and The Sub Saharan Africa Region (Nedumaran et al., 2015). Within the Latin America region, Brazil and Mexico are the largest producers. In Africa, Uganda, Kenya, and Rwanda are some of the principal producers (CGIAR, 2013). The fourth largest producing country is U.S., where more than 0.6 million ha of dry beans were harvested during 2016 (USDA-NASS, 2016). In 2014, North Dakota was ranked as the top dry bean producer state within the U.S (31% of total U.S. production), followed by Michigan

(15%), Nebraska (13%), Minnesota (9%) and Idaho (9%). Main bean market classes produced in U.S. are: pinto (most predominant class), navy, black, great northern, light red kidney, lima, pink, and small red, (USDBC, 2016).

Dry bean consumption may vary across regions, being the economic factor one of the most determinants (Leterme and Muñoz, 2002). Bean production is mainly a smallholder crop in developing countries (Latin America and Africa) (CGIAR, 2017). This crop provides 65% of the total protein consumed and 32% of the energy in regions of Eastern Africa and Latin America. One of the main advantages is its low cost compared with animal source of proteins. In addition, its high iron content helps in the fight of malnutrition (Petry et al., 2015). Despite all the benefits of dry beans, low seed yield averages of 644 kg ha^{-1} are reported in developing countries versus an average of 1632 kg ha^{-1} in developed countries (Akibode and Maredia, 2011). U.S. has the highest seed yield worldwide; for example in 2016, seed yield was approximately 2000 kg ha^{-1} (USDA-NASS, 2016). Poor soil conditions and lack of agronomic inputs strongly affect bean production in developing countries in contrast with developed countries (Namugwanya et al., 2014). Also, diseases, insect pests, and drought may decrease seed yield quantity and quality (Schwartz and Pastor-Corrales, 1989).

Bean Genetic Diversity

Common bean belongs to the Leguminosae (Fabaceae) family. Other members of the family include lentils (*Lens culinaris* M.); pea (*Pisum sativum* L.); Chickpea (*Cicer arietium* L.); Faba bean (*Vicia faba* L.); cowpea (*Vigna unguiculata* L.); pigeonpea (*Cajanus cajan* L.) among others; all of them except common bean have the center of origin in the Old World (Zohary and Hopf, 2000).

The Phaseolus genus includes the domesticated species: Common bean, scarlet runner bean (*P. coccineus* L.), year-long bean (*P. dumosus* Syn. *P. polyanthus*), tepary bean *(P. acutifolius* A. Gray) and lima bean (*P. lunatus* L.)* (Osorno and McClean, 2014). Nucleotide diversity studies suggest that *P. vulgaris* was originated in Mesoamerica, from where the wild beans were extended to South America, establishing two domestications events, known as the two bean gene pools (Bitocchi et al., 2012). However, evolutionary studies indicated that each gene pool experienced its particular domestication events, and higher genetic diversity was found at the Mesoamerican gene pool. (Mamidi et al, 2010; Bitocchi et al., 2012). Those domestication events created variation at many traits (seed and leaf size, seed coat color, growth habit, etc.) (Mcclean et al., 2002), and lead to differentiation into subpopulations (Schmutz et al., 2014) that have been divided based on its morphological features and adaptation response to establishment regions (Singh et al., 1991). Each race has specific phenotypic, agronomic, biochemical, and genetic features, and they differ among them at specific loci in allele frequency (Blair et al., 2007).

The Mesoamerican gene pool is subdivided in Durango, Jalisco, and Mesoamerica races while Nueva Granada, Chile, and Peru belongs to the Andean gene pool (Singh et al., 1991). Mesoamerica race include navy, black, and some small red beans while Durango race include pinto, great northern, small red, and pink (Mensack et al., 2010). Within the Andean gene pool, Nueva Granada race include kidney beans and bush cranberry, Peru race include yellow beans such as Canario and Mayocoba, and Chile race include vine cranberry beans and others exclusive of this race, such as Tortola and Coscorron (Kelly, 2010).

Relevance of Climbing Beans in Latin America

Climbing beans can belong to *P. vulgaris*, *P. coccineus,* or *P. dumosus*. Those beans have growth habit IV (indeterminate) and usually seed size is bigger than bush type beans (Singh et al., 1991). Flowering patterns differ from bush type beans, since climbing beans flower ascendingly by node groups. Therefore, flowering period is much longer than in bush type beans. In addition, depending on climate conditions, climbing bean production cycle can last up to 7 months (L.F. Aldana, Pers. comm., 2015). Brougthon et al. (2003) mentioned that Latin America and Africa produce more than 30% of common beans in the world, the majority produced by small-scale and subsistence farmers where standard common bean production is on farms of less than 10 ha. For example, during 2012-2013, the Guatemalan production of black beans was approximately 2,194 MT. However, this production was not enough to satisfy local demand and 10,980 MT had to be imported at a cost of US$ 7,320,830, indicating that dry bean productivity must be increased to create availability of low cost sources of protein to the Guatemalan population (Azurdia, 2014). In addition, climate change might negatively affect food security mostly in developing countries, including dry bean production, based on statistical crop models and climate projections for 2030. Those changes will increase abiotic (drought, heat, flooding) and biotic stresses (virus, fungal diseases, and pests) (Beebe et al., 2011).

Guatemala is located in Central America, its population is about 16 million people and approximately 2.5 million of people were undernourished in 2015 (FAO, 2016). Approximately 50% of Guatemalan children under age five present chronically malnourishing (CIA World Fact Book, 2016), and half of the population is under poverty (World Bank, 2016). According to the Guatemalan Ministry of Agriculture, per capita consumption of bean is approximately 13.3 kg per year. Maize (*Zea mays* L.) and dry beans are the main source of calories and protein for

Guatemalan poorest households, but this amount is not enough to achieve an acceptable level of nutrition (Osorno et al., 2013).

De Young et al., (2016) conducted a survey in the Guatemalan highlands and results indicate that bean is one of the two most important crops produced in this region. This survey also found that households provide beans to their children an average of three days a week. Approximately 66% of the total beans are planted by smallholders on hillsides but only produce 53% of the national bean production. On the other side, large-scale farmers plant 28% of the production in lowlands and around 15% are planted in valleys (Osorno and Mcclean, 2013). On hillsides, farmers plant both climbing and bush bean types while bush type is predominant in the lowlands. Even though seed yield of climbing beans can be twice as bush type beans (Wortmann and Alen, 1994), breeding efforts have been more focused on bush types because they have a shorter season and requires less labor. If snap/green bean breeding is excluded, there are only two institutions currently working with climbing beans around the world (CIAT and ICTA).

Beans can be planted either as mono-cropping or intercropping system. Usually, bush type beans are planted as mono-cropping system and represent approximately 40% of the harvested area. The intercropping system (locally known as Milpa) represent 60% bean planted area (Michigan State University, 2005). This system involves different crop species providing food variety and many nutrients. It is one of the most relevant cultural elements of the Mesoamerican culture and it is an efficient system in terms of food security and sustainability for small holder farmers (Zizumbo-Villareal et al., 2012). The Milpa intercropping system includes climbing beans that climb around the maize stalks, both have different centers of domestication but through time, they became intercropped within same system (Bitocchi et al., 2013). The Milpa is the main crop system in the Guatemalan highlands (1500-2350 masl) for smallholder

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farmers, where maize-bean is the most common crop association and is one of the major sources of protein and calories (Francis et al., 1978; L.F. Aldana, Pers. comm., 2015). Since malnutrition problems in those areas are critical, improving climbing bean cultivars could contribute to improve nutritional levels and food security in those affected regions.

Farmers normally use two methods for planting, depending on the region. These planting methods are direct planting, which consist of planting both maize and beans simultaneously, and relay, in which the farmer plant the maize first and beans are planted a month later to reduce competition between the two crops (J.C. Villatoro, Pers. comm., 2015). Farmers at higher altitudes tend to use the direct planting system more frequently because of the shorter growing season. Generally, farmers plant in March and do harvest seven to nine months later. The Milpa system present laboriousness in disease management due to its high plant density and height which difficult spraying (J. C. Villatoro, Pers. Comm., 2015). In addition, different diseases affect both crops and chemical control is very expensive for small-farmers.

Collections of climbing bean accessions has been made across all bean production in regions in Guatemala by Gentry in 1966, Cojulum in 1970, Freytag in 1978, Rodriguez in 1982 and Debouck in 1986. The Agricultural Institute of Sciences and Technology in Guatemala (ICTA in Spanish) conserve a germplasm collection of around 600 climbing bean accessions, which belong to *P. vulgaris, P. dumosus and P. coccineus* (Orellana et al., 2006; Ponciano-Samayoa et al., 2009). Some field evaluation for disease resistance using natural pressure were reported in this Guatemalan collection, but no formal characterization of the genes present has been done. A complete genetic characterization of this germplasm collection can lead to find potential resistance genes for diseases, pests, or abiotic stresses. Some bush-type cultivars released in Guatemala such as ICTA-Super Chiva and ICTA-Hunapu have shown rust resistance

under natural pressure in the field, but it is unknown which *Ur* resistance genes are present in those cultivars. Furthermore, these potential resistance genes can be used in breeding programs to improve bean cultivars and improve food security. Gene introgression can be applied from climbing to bush beans.

Bean Rust: A Biotic Constrain

Disease pressure and distributions patterns will be altered due to climate change (Beebe et al., 2011). Diseases are considered one of the most critical factors of seed yield losses among biotic stresses. Bean diseases are caused by fungi, bacteria, nematodes, viruses and phytoplasmas. One of the most devastating groups of pathogens are rust fungi (Urediniales or Pucciniales, Basidiomycota), which cause significant economic losses by affecting a broad range of crops such as wheat (*Triticum* sp. L.), rice (*Oryza sativa* L.), common bean, etc. Urediniales have more than 120 genera and 7,800 species (Toome and Aime, 2013; Duplessis et al., 2011). Most of the species belong to the genera *Uromyces* spp. and *Puccinia* spp. (Fungorum, 2013).

Rust is an obligate biotroph pathogen that have evolved to manipulate the host metabolism to benefit and survive. Linage-specific gene families, large amount of effectors-like small protein, extensive families of aminoacids and oligopeptides membrane transporters are some of the characteristics that make rust a strong parasite (Dodds et al., 2009). In addition, the rust haustoria structure transfer effector proteins directly to the host for nutrient uptake and immune system suppression. This is one of the main features of obligate parasites (Voegele and Mendgen, 2011). The impact of rust on seed yield losses is caused by reducing the effective photosynthetic area of leaves when rust pustules and its halo appears (Lopes and Berger, 2001) and consequently, defoliation occur as a defense mechanism (Mersha and Hau, 2008).

Bean Rust Symptoms and Epidemiology

Bean rust is caused by the fungus *Uromyces appendiculatus* (Pers) Unger, an obligate biotroph that has an autoecious and macrocyclic cycle because complete its life cycle in a single host (Cooper et al., 2007; Souza et al., 2008; Kolmer et al., 2009). This disease is world-wide distributed but is more severe in humid tropical and subtropical regions (Stavely, 1984). Severe infections have been reported in Latin America and Caribbean region, Africa, Europe and North America. When early infection occur, bean rust can cause up to 100% yield losses (Schwartz and Pastor-Corrales, 1989; Schwartz et al., 2005). Its life cycle consists of five spore stages: Teliospores that will germinate to produce a basidium in which sexual recombination occur (meiosis), basidium produce basiodospores that are windblown to infect susceptible plants. Then, around 6 days after infection (depend of the environment), pycnia are developed, that produce a spermatia (male) and receptive hyphae (female) structures. Those structures sexually recombine by cross-fertilization between pycniospores, producing aecia. Aecia structures will produce aeciospores that will infect the host after their release. Then, each aeciospore produces uredinia that contain urediniospores, which will produce more urediniospores. This is a polycyclic pathogen (Fig. 1). At the end of the host life, uredinia will turn to telia to survive. Optimal conditions for this cycle are 62-85 ºF and high humidity (Agrios, 2004; Souza et al., 2008; Markell et al., 2012).
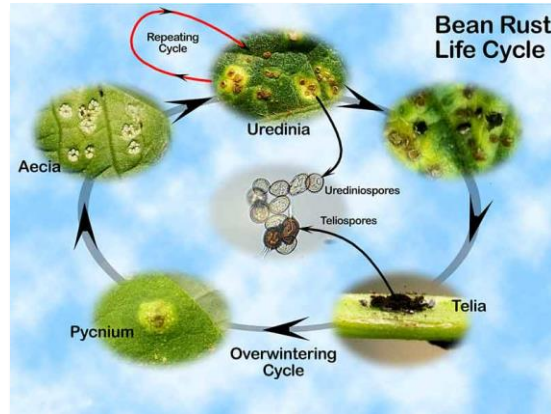
Figure 1. Bean rust life cycle
(McMillan et al., 2003)

Identification of bean rust in field or greenhouse conditions start with circular pustules

(Uredinia) that contain brown spores, sometimes surrounded by a chlorotic halo (Cooper et al.,

2007). Initially, those appear in the field by localized spots with strong symptoms, mainly at

middle leaves where they are difficult to find without field scouting. Bean rust can infect

different tissues: leaves, pods, and more unlikely, the stem (Schwartz and Pastor-Corrales, 1989).

Bean Rust Genetic Diversity

Identification of bean rust isolates has changed through the time. The first system

proposed the use of 20 differential cultivars of beans with known resistance genes (Stavely et al.,

1983). Then, a Mountaineer White Half Runner cultivar was eliminated due to genes duplication

(*Ur-4*) and ended up to 19 (Stavely and Steadman, 1992). A value was assigned to each

accession, and the values of the susceptible accessions were added to the sum. However, a binary

system was established later (Habgood, 1970) and the number differential lines were reduced to

12 (Steadman et al., 2002). Identification of bean rust races is based on the reaction that they

produce in each cultivar of the differential set (Pastor-Corrales and Stavely, 2002). The 2002

differential set is a group of 12 *P. vulgaris* accessions with specific resistance genes for rust

(Table1), which has six accessions of Mesoamerica origin and six of Andean origin; these

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accessions were selected at the Third International Rust Workshop (Steadman et al., 2002).

Categorization of genotypes into resistant or susceptible based on reaction grade and uredinium

size, which are in scale of 1 to 6 (Stavely, 1984; Mmbaga et al., 1996), where 1-3 is resistant and

4-6 is susceptible (Table 2). In order to classify or identify the races, a binary system was

proposed, were the Andean and Mesoamerican binary value of susceptible cultivars are divided

with a hyphen (Table 1). Infection type was scored 14 days after inoculation (Stavely, 1984;

Mmbaga et al., 1996).

Table 1. Common bean differential cultivars set used to characterize isolates of *U. appendiculatus*.

Differential number	Cultivar	Resistance gene	Gene pool	Binary value
1	Early Gallatain	*Ur-4*	Andean	1
2	Redlands 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*	Mesoamerica	1
8	Aurora	*Ur-3*	Mesoamerica	2
9	Mexico 309	*Ur-5*	Mesoamerica	4
10	Mexico 235	*Ur-3+*	Mesoamerica	8
11	CNC	*Unknown*	Mesoamerica	16
12	PI 181996	*Ur-11*	Mesoamerica	32

Steadman et al., 2002

Table 2. Description of infection types of *U. appendiculatus* isolates on 1-6, based on pustule diameter.

Description	Infection type	Genotype
No visible symptoms	1	Resistant
Necrotic flecks or spots without sporulation	2+	Resistant
Uredinia ≤ 300 µm in diameter	3	Resistant
Uredinia 300 – 499 µm in diameter	4	Susceptible
Uredinia 500 – 799 µm in diameter	5	Susceptible
Uredinia ≥800 µm in diameter	6	Susceptible

Stavely, 1984; Mmbaga et al., 1996

Worldwide, more than 300 rust races have been reported (by using the set of 19 differential cultivars), and new/additional races continue to be reported (Steadman et al., 2002; Liebenberg and Pretorius, 2010). Higher diversity of bean rust has been found at Central America and the Caribbean due to its co-evolutionary history with the host (Pastor-Corrales and Stavely, 2002; Pastor-Corrales and Aime, 2004). In 2004, *U. appendiculatus* isolates from Latin America were analyzed by using 50 RAPD molecular markers and two main groups and one intermediate were identified. The main groups were classified as either Andean or Mesoamerican (Araya et al., 2004). Those results suggested a pathogen interaction as a parallel evolution with the two main bean gene pools across time (Steadman et al., 2002; Araya, 2003; Pastor-Corrales and Aime, 2004).

In 2015, mobile nurseries were conducted at 12 commercial bean fields at highlands of Guatemala. The bulk collections expressed virulence phenotypes as: 13-0, 4-63, 4-39, 3-4, 22-61, 4-55, 16-63, 22-61 and 4-62. (Dardon et al., 2016). This study found that differentials carrying genes of Andean origin showed more resistance to Guatemalan *U. appendiculatus* isolates than Mesoamerican differentials. Based on the high virulence of bean rust especially in Guatemala, breeding programs confront a challenge when developing new resistant bean cultivars and therefore, new sources of rust resistance need to be identified in order to guarantee wide and durable resistance in common bean cultivars. Also, diversity studies of *U. appendiculatus* have shown that isolates from tropical and subtropical conditions are more virulent than isolates found in temperate areas (Schwartz and Pastor-Corrales, 1989; Jochua et al., 2008). The haustorial transcriptomes of *U. appendiculatus* have been sequenced and annotated and abundant transposable elements (TEs) were identified as well as secreted proteins. Some of the proteins encode for enzymes that convert nutrient sugars to storage components and are involved in

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metabolism of carbohydrates and proteins, making rust an invasive biotrophic pathogen. Also, bean rust haustoria produce effectors that affect the host (common beans) in negative manner (i.e. suppressing the immune system) (Link et al., 2014). Therefore, abundant TEs, its haustoria structure, and abundant effectors proteins play a key role on bean rust virulence and diversity. In addition, recombination can occur during sexual stages, triggering genetic changes that can lead this pathogen to quickly overcome resistance (Agrios, 2004; Cooper et al., 2007).With few exceptions, the mode of inheritance of resistance to rust is given by one or more major dominant genes as shown by numerous studies (Liebenberg and Pretorius, 2010).

By characterizing isolates of rust across different regions and at multiple times is possible to keep track changes of the pathogen populations and increase the understanding of this variable pathogen. Likewise, it will allow to design breeding strategies focus breeding efforts by regions, in addition to standardize the use of known races to identify resistant genes.

Disease Management: Host Genetic Resistance

Crop rotation is recommended to break rust life cycle over seasons. In addition, foliar fungicides are used to limit the rust infection. However, the most effective management of bean rust is host resistance because small farmers cannot afford chemical control due to its high cost. Chemical control can pollute the environment and its constant use leads to pathogen population selection, which will overcome the effectiveness of the fungicide. In addition, its application within an intercropping system is limited due to the reduced space among rows/plants.

As mentioned before, the mode of inheritance of resistance to rust is given by one or more major dominant genes as shown by numerous studies (Liebenberg and Pretorius, 2010). Nevertheless, exceptions such as some recessive genes, as well as the complementary resistance gene *Crg* (Kalavacharia et al., 2000) have been reported. The *Crg* gene has to be present for *Ur-3*

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gene expression. Many known rust resistance genes have been formally named so far: *Ur-3, Ur-4, Ur-5, Ur-6, Ur-7, Ur-9/Ur-12, Ur-11, Ur-13* and *Ur*-Ouro Negro (Liebenberg and Pretorius, 2010; Stavely, 2000). All those resistance genes have been mapped except *Ur-12* and *Ur*-Ouro Negro and are known for confer race-specific resistance (Kelly et al., 2003; Singh and Schwartz, 2010). However, a stable resistance over time to rust is uncertain because the pathogen evolves to overcome the resistance mechanism of the host. This might occur more frequently when there is a specificity between the resistant genes and the bean rust races. Therefore, some rust resistance genes need to be combined to produce a wider resistance reaction, which may take longer to be overcome (Liebenberg and Pretorius, 2010).

A tepary bean accession (G40022) have shown resistance reaction against all races evaluated so far (Pastor-Corrales et al., 2011). However*, P. vulgaris* accessions with only one source of resistance are not effective against all bean rust races through the time. Gene pyramiding has been used effectively to improve common bean cultivars with multiple rust resistance genes. This method consists in transferring more than one resistance gene into a cultivar to provide broader resistance against races of interest (Pastor-Corrales, 2003; Steadman et al., 2002).

Important germplasm lines with a unique combination of rust resistance genes have developed at U.S. with the collaboration of USDA-ARS, North Dakota State University, and Michigan State University, and University of Nebraska-Lincoln. Those lines have combinations of Middle American (*Ur-3* and *Ur-11*) and Andean (*Ur-4* and *Ur-6*) genes that confer resistance to all known *U. appendiculatus* races (Pastor-Corrales, 2006). Those bean lines have been evaluated under field conditions in the U.S., Africa, and Central America, and have been intensively used in breeding programs to develop important cultivars with rust resistance.

Despite of these gene combinations, new rust pathotypes have emerged due to its high capacity

of evolve. It has been reported that *Ur-3* is capable to confer resistance to more than 50% of the

bean rust races in storage at USDA-ARS (Pastor-Corrales, Pers. comm.) and has been used as a

source of resistance worldwide. However, in Michigan during 2007-2008, susceptible reaction

on resistant cultivars carrying *Ur-3* gene was found by an isolate identified as race 31-3 (Wright

et al., 2009). In addition, a new rust race named 20-3 was found at North Dakota which was

virulent to 27 bean genotypes of multiple market classes, some of them possessing the *Ur-3* gene

(Markell et al., 2008). Both races are virulent to *Ur-7* and *Ur-3* genes. Highlighting the

importance of broad resistance and suggesting the introduction of *Ur-11* and *Ur-5* when

pyramiding *Ur-3* in future cultivars.

Genome Wide Association Study (GWAS) in Common Bean

Molecular markers are an important tool used for Marker Assisted Selection (MAS) and

genetics studies and are becoming cheaper as science advance. Nevertheless, molecular markers

are more established for diseases screening than pests or abiotic stresses (Miklas et al., 2006). In

common bean, there are different molecular markers available: Allozymes, Isozymes,

Microsatellites (SSR), SCARs and AFLPs. Lately, with the advances in genomic techniques,

Single Nucleotide Polymorphism (SNP) are the newest marker system and have allowed to

assess high quality mapping and identification of specific genome regions (Osorno and McClean,

2014). SNPs are an impressive molecular tool because it can identify single nucleotide changes

in genomes, which can be discovered by using next-generation sequencing technologies (Ariani

et al., 2016). Moreover, these markers and sequencing advances in genomics have produced a *P.*

vulgaris reference genome sequence (~587 Mb), which has been used to improve marker's

coverage, identification of resistance genes, genetic diversity, evolution, and genome-wide association studies (Schmutz et al., 2014).

Since more genotypic information can be generated today, breeding programs are able to associate potential genomic regions controlling traits of economic importance. Quantitative trait loci (QTL) mapping can detect genomic regions (genes) associated with traits of interest through Linkage Disequilibrium (LD) in mapping populations such as: backcrosses and F2 generations of crosses between inbred lines. However, QTL mapping is time consuming because those biparental populations have to be developed, and low resolution can be obtained due to low LD (Jamann et al., 2015). Recently, an improved technique that aim for higher resolution was achieved. It has higher accuracy and different populations can be used: Genome-Wide Association Studies (GWAS), correlates polymorphism present in DNA (SNPs) and the phenotypic information of a trait of interest from a group of interest, either from a wild population or germplasm collections in order to increase variability at each locus (Rafalski et al., 2010).

Genome-wide association studies in common bean for diseases resistance, have identified genomic regions in bean associated resistance to Halo Blight, caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) (Ghising, 2016), Fusarium root rot, caused by *Fusarium oxysporum f. sp. Dianthi* (Vasquez-Guzman, 2016), anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scribner, and angular leaf spot, caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris (Singh and Schwartz, 2010; Perseguini et al., 2016b). Also, a recent study used 280 bean genotypes of the Middle American Diversity Panel, which were grown under four field US locations, and 150,000 SNPs to conduct GWAS for six important agronomic traits (Moghaddam et al., 2016). In addition, 313 new gene-based markers have been developed

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and used Genome-Wide Association to test them in the bean genome, and found important

association between some markers and seed yield components (Galeano et al., 2012). A physical

map representing the 11 pseudomolecules was generated showing candidate locations of linked

markers and resistance genes for anthracnose, rust, angular leaf spot, bean common mosaic virus,

etc. (Meziadi et al., 2016). Many other GWAS studies are reported nowadays, demonstrating its

relevance as lately, using generated information and more molecular markers.

MATERIALS AND METHODS

Rust Collection in Guatemala

Field samples of leaves with visible rust symptoms were collected from Guatemala. Fields were selected under two criteria: 1) commercial production of beans and 2) weather conditions favorable for rust infection. Those conditions were met mostly at the middle highlands, in the western part of the country. Sampling was done between October and November 2015, which are usually the last three months of the growing season. In total, 23 samples were obtained from 11 locations across three departments (political geographical division equivalent to a U.S. state) (Table 3).

Each sample consisted of two or three leaflets with visible rust symptoms. Those were individually placed into labeled envelopes (Steadman, 2015a). Later, samples were dried at room temperature with silica gel and prepared to be shipped. These steps allowed to keep rust samples viable during storage and shipping. Samples were sent to the Epidemiology and Plant Disease Resistance Laboratory of University of Nebraska, Lincoln by following the Standard Operating Protocol (Steadman, 2015b), and procedures to ship foreign samples to U.S. Closed envelopes with dry leaves in a sealable plastic bag were placed in a sturdy box. Packing material were placed around the packaged leaves to avoid leaves damage. The package contained a permit label (PPQ form 599).

Table 3. Samples of *U. appendiculatus* in beans collected at Western highlands of Guatemala

Sample ID	Location	Collection Date	Accession
G1501	La Esperanza	10/15/2015	Climbing bean ICTA Labor Ovalle
G1502	ICTA Labor Ovalle	10/14/2015	Climbing bean 1026
G1503	ICTA Labor Ovalle	10/14/2015	Climbing bean ICTA Labor Ovalle
G1504	ICTA Labor Ovalle	10/14/2015	Climbing bean ICTA Texel
G1505	ICTA Labor Ovalle	10/14/2015	Climbing bean ICTA Labor Ovalle
G1506	ICTA Labor Ovalle	10/14/2015	Guate 1074
G1507	ICTA Labor Ovalle	10/14/2015	Climbing bean, landrace
G1508	ICTA Labor Ovalle	10/14/2015	Climbing bean, Guate 257
G1509	Parramos	10/16/2015	Climbing bean, white seed, landrace
G1510	Parramos	10/16/2015	Climbing bean, black seed, landrace
G1511	Parramos	10/16/2015	Bush bean, landrace
G1512	Patzicia	10/20/2015	Bush bean, landrace
G1513	Patzicia	10/21/2015	Bush bean, landrace
G1514	Tecpan	10/21/2015	Climbing bean, landrace
G1515	Patzicia	10/21/2015	Climbing bean, landrace
G1516	Patzicia	10/16/2015	Bush bean, landrace
G1517	San Pedro Sacatepequez	11/16/2015	Climbing bean, landrace
G1518	Las Barranquitas	11/16/2015	Bush bean, landrace
G1519	Las Barranquitas	11/16/2015	Bush bean, landrace
G1520	San Pedro Sacatepequez,	11/26/2015	Climbing bean, landrace
G1521	Aldea Champollap	11/26/2015	Climbing bean, landrace
G1522	Loma Linda Sn. Pedro Sac.	11/26/2015	Climbing bean, landrace
G1523	La Esperanza	11/26/2015	Climbing bean, landrace

Geographical coordinates and altitude of locations are presented in Table A.1.

Pathogen Characterization

Since North Dakota is the largest producer of dry beans in the U.S., it was unsafe to import bean rust samples because rust spores could blow out from the greenhouses to nearby fields, survive for some time in the fields, and cause a new epidemic during the growing season. In addition, a permit for pathogen import was required. Therefore, pathogen characterization of Guatemalan field samples was executed in collaboration with J. Steadman Laboratory at the University of Nebraska, Lincoln in 2016. This procedure allowed to identify each isolate based on the binary system with the 12 differential cultivars, which is based on phenotype reaction (Stavely, 1984; Steadman et al., 2002).

Spores Increasing

Spores from each field sample were increased to have the required amount for inoculation. The urediniospores of each samples were collected from leaves by spraying them with a 0.04% Tween-20 solution and then removed with a brush. Then the spore-Tween solution was scrubbed on the primary leaves of susceptible cultivar (pinto UI-114) with the brush. Previously, pinto UI-114 was grown in the greenhouse until primary leaves were full expanded (around 6-8 days) (Fig. 2). After inoculation, each inoculated plant was placed in a mist chamber, and leaves were left to dry. When leaves were dried, the mist chamber was set up at 21 $\pm$ 1°C in cycles of 2 ½ hours on and 1 ½ off for at least 16 h with distilled H_2O, then transferred to a greenhouse into an isolation tends (made of cheesecloth) at 22 $\pm$ 2°C (Figure 3). After 10-14 days, urediniospores were harvested by shaking the infected leaves onto a piece of waxed paper or aluminum foil, then transferred into a labeled plastic micro-tube. Moisture content of collected fresh spores was reduced in a desiccator for 48 hrs. Samples were stored at –20°C until used for characterization (Steadman and McCoy, 2015).

Figure 2. Inoculation of pinto UI-114 with bean rust spore samples collected from Guatemala, for spores increasing.

Figure 3. Isolation tends for rust infected bean plants.

Single Uredinium Isolates (SUIs)

To obtain SUIs, closed single uredinia of same size in one leaf were selected, and cut off. Then, SUIs were labeled in correlation with the sample number and number of sub samples (1a, 1b, etc.). Those selected SUIs were inoculated onto a 6-day-old susceptible line (pinto UI-114) for spore increasing, to obtain the required amount of spores for the virulence test. This procedure was repeated for every uredinium size of each isolate. Urediniospores produced from each SUI were considered one *U. appendiculatus* isolate (1a, 1b, etc.) Spores of each SUIs were collected 10-14 days after inoculation (Figure 4).
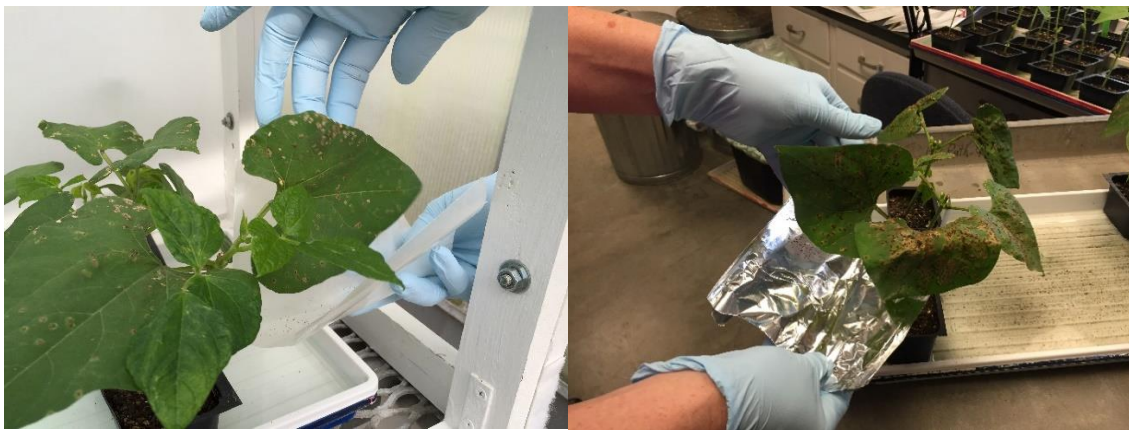
Figure 4. a) and b) Bean rust spores harvest, on susceptible genotype pinto UI-114.

Finally, the virulence characterization of each urediniospore (SUIs) was determined by evaluating the virulence phenotype on the standard set of 12 differential cultivars for bean rust (Table 1). The rust inoculum of each SUI was prepared as following: approximately 2.5 mg of urediniospores were added in a hand spray or inoculation flask, with 30 ml of a 0.004% Tween-20 solution. Since spores were stored at -20ºC, solution was mixed and left at ambient temperature for 1 hour to rehydrate. Solution was mixed again, sprayed onto plants at primary leaf stage (primary leaves full expanded) and placed in a mist chamber by 24 h of incubation under same conditions for spores increasing (Steadman and McCoy, 2015). After incubation, each differential set was transferred to the greenhouse into isolation tends to prevent cross-contamination. Infection types by differential was scored 10 to 14 days after inoculation using a hand lens with sizing diameter scale and a graphic scale 1-to-6 based on uredinium diameter (Table 2) (Stavely, 1984; Mmbaga et al., 1996). This procedure was repeated twice for each isolate to verify virulence phenotype of cultivars. Each isolate was identified based on the binary system, where each cultivar has a binary value, and its value was added if the accession has susceptible reaction to the isolate (Table 1) (Steadman et al., 2002).

Germplasm Greenhouse Evaluation

Rust isolates used for resistance evaluation were selected based on the results from *U. appendiculatus* Guatemalan isolates characterization. In addition, race 20-3 identified at North Dakota (Markell et al., 2008) was used to evaluate resistance on the germplasm collection. Isolate G1501 was used as representative for race 63-1 and isolate G1522 for race 31-1. The Plant Pathology Department, NDSU, provided North Dakota race 20-3.

The climbing bean germplasm from Guatemala contains approximately 600 accessions, where 376 belongs to *P. vulgaris,* and the rest to *P. dumosus* and *P. coccineus.* However*, P.*

dumosus and *P. coccineus* were not included because those accessions have showed high segregation in the field, and because they are a small portion of the collection. In addition, some *P. vulgaris* accessions were removed from the evaluation panel because it showed seed mixture. After removing those lines, the evaluation panel had 372 *P. vulgaris* accessions.

The germplasm evaluation for the rust isolates from Guatemala was conducted at the Plant Pathology greenhouse of the University of Nebraska, Lincoln. This evaluation was conducted during the summer of 2016. The ND20-3 isolate was evaluated at the North Dakota State University greenhouses, also during summer of 2016.

The experimental design was a randomized complete block (RCBD) with three replications. Two plants were sown as an experimental unit per accession for isolates from Guatemala and four plants per accession for the North Dakota 20-3 isolate. The difference on plants/experimental unit by isolate was due to the limited space and time at the UNL greenhouse. As done in previous bean rust studies (Acevedo et al., 2013), pinto UI-114 cultivar was used as the standard susceptible control across all experiments.

Planting and Inoculation

Labeled 24-cell insert trays were filled with sterilized peat most substrate. Bean seeds were disinfected by placing seeds into a beaker with 10% Bleach solution for 1 minute, and then rinsed off with distilled water. Then, seeds were scarified using a razor blade to achieve even germination. Then, seeds were planted into the trays (one seed/cell) and moved to the greenhouse. The susceptible check pinto UI-114 was included in each tray. Plants were ready when primary leaves were full expanded. To avoid cross contamination, each experiment (isolate) was performed at different dates.

Inoculum of each rust isolate was prepared by adding 5 mg of urediniospores into an inoculation flask with 60 ml of 4% Tween-20 solution. Solution was mixed before used. Plants with full expanded primary leaves were inoculated and moved into a humidity chamber as previously described (Acevedo et al., 2013; Steadman and McCoy, 2015). After 16-21 hours, plants were moved to a different room in the greenhouse for symptoms development. Plants were watered every day but without spraying leaves. Infection type of genotypes were scored 10-14 days after inoculation using the standard 1-6 scale (Stavely, 1984; Mmbaga et al., 1996). Accessions with infection types of 1 to 3 were considered resistant. Accessions with infection types of 4 to 6 were considered susceptible. Also, accessions with more than one infection type, were scored by annotating first the most prevalent reaction, followed by the next one, coma separated (i.e. 4, 5). Temperature conditions in the greenhouse during evaluation were around 70-85 °F and high humidity (Fig. A.1).

Data Analysis

First, infection types of all accessions on the scale of 1 to 6 were transformed to a quantitative score because more than one infection type pattern (uredinium size) were observed on some accessions. It consisted of giving a quantitative value based on the reaction type of each accession (Table 4) (Mmbaga et al., 1996). Infection type values were analyzed using the PROC MIXED procedure in SAS V.9.3. Replications were considered as random and genotypes as fixed effects. Each race was analyzed by separate. Quantitative scores of each accession across every replicate were averaged by calculating the Least Squares Means (LSM) by using SAS V.9.3 software. Genotypes used for this study are mostly landraces and some still showed variability. Then, if phenotypic variation was observed within the same accession (i.e. stem color, leaf size), those accessions were removed from the analyses to reduce error. In addition,

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accessions that could not be categorized as susceptible or resistant because presented extreme scores for infection type (i.e. 1, 1, 1, 5, 4, 1) were also removed for data analysis.

Table 4. Quantitative scores for 1 to 6 infection types to *U. appendiculatus.*

Quantitative score	Infection type	Symptoms	Genotype
1.1	1	No visible symptoms	Resistant
2.1	2	Necrotic lesions	Resistant
2.4	2,3	Reaction type with few type 3	Resistant
3.1	3	Uredinia <0.3 mm diameter	Resistant
2.7	3,2	Reaction type 3 with few type 2	Resistant
3.4	3,4	Reaction 3 with few type 4	Susceptible
4.1	4	Uredinia 0.3-0.49 mm diameter	Susceptible
3.7	4,3	Reaction 4 with few type 3	Susceptible
4.4	4,5	Reaction 4 with few type 5	Susceptible
5.1	5	Uredinia 0.5-0.8 mm diameter	Susceptible
4.7	5,4	Reaction 5 with few type 4	Susceptible
5.4	5,6	Reaction 5 with few type 6	Susceptible
5.7	6,5	Reaction 6 with few type 5	Susceptible
6.1	6	Uredinia 0.8-1.2 mm diameter	Susceptible

According to Mmbaga et al., 1996

Genome Wide Association Analysis

Genotyping

A total of 369 climbing bean accessions of *P. vulgaris* were genotyped using an improved GBS methodology reported by (Schröder et al., 2016) which produces approximately 150,000 SNPs. However, five accessions of 372 were not genotyped for rust resistance and therefore, these were eliminated from the GWAS analyses. SNP calling was conducted using GATK software by aligning the sequences to the reference genome G19833 (*P. vulgaris*) (Schmutz et al., 2014). SNP markers were filtered to remove those SNP with >50% of missing data and then were imputed by using fastPHASE. Then, markers were filtered for >50% of heterozygosity and for minor allele frequency of 5% (MAF0.05). After filtering, 78,754 SNPs

remained for further studies. The genotypic information was generated by the NDSU Bean

Genomics and Bioinformatics laboratory (Tobar et al., 2017).

GWAS

Genome-Wide Association Studies has been done with continuous or either binary data,

the method vary depend on the trait measured (VanRaden, 2008; Gao et al., 2016; Moghaddam

et al., 2016; Vikram et al., 2017). For this study, GWAS analysis were performed separately by

each rust race. SNPs marker-rust resistance association was performed using two R functions 1)

GAPIT V.3.2.2 (Lipka et al., 2012) and 2) GenABEL V. 1.8 (Aulchenko et al., 2007). Data for

GWAS with GAPIT function were the Leas Square Means (LSM) of the quantitative scores.

Multiple models were tested: 1) Null general linear model (Naïve), 2) General linear model with

fixed effects to control population structure, 3) Univariate unified mixed linear model to control

for relatedness and 4) A mixed model controlling for population structure and relatedness

(Mamidi et al., 2011). Population structure was evaluated in the model with Principal

Components Analysis (PCA), generating a correlation matrix in R software. Relatedness was

accounted by using EMMA algorithm and the mixed model controlling for population structure

and relatedness used PCA+EMMA. Those models help reducing the number of false positives

(Zhang et al., 2010). Based on the matrix, six principal components were used to control ~25%

of the variance. To control relatedness, a kinship matrix was (*k*) generated with GAPIT V.3.2.2.

The binary system consisted on grouping accessions into two categories based on

infection type (IT): a) 0 =Resistant accessions: Include all accessions with IT of 1 to 3; and b) 1

=Susceptible: Include all accessions with IT of 4 to 6. In addition, those accessions that showed

phenotypic variation were removed as is described on Data Analysis section. GenABEL package

implements linear, logistic regression to find relation between SNPs and the trait of interest in

binary system. Model evaluation included: PCA to account for population structure, EMMA to account for relatedness, and a mixed model (PCA+EMMA). The quantile-quantile plot was generated for each model by corresponding R functions, as well as the Manhattan plots. The best model was selected based on the mean squared difference (MSD) (Mamidi et al., 2011). Significant SNP markers ($P<0.001$) were selected from the best model (Mamidi et al., 2014).

Potential candidate genes were searched on the second version of the bean genome annotation (Schmutz et al., 2014), 50kb upstream and downstream from the location of significant SNPs at each GWAS by race (Moghaddam et al., 2016). The annotation data is available at https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris. However, if the significant SNP felt within the position of an annotated gene, then it was considered as candidate gene.

RESULTS

Pathogen Characterization

In total, four of 23 of *U. appendiculatus* isolates from Guatemala could not be increased. Those four samples had dark teliospores, which did not infect the susceptible check UI-114 for spores increasing.   Infection types of isolates are presented in Table 5. Among those 17 isolates successfully characterized, two races were identified: 63-1 and 31-1. Race 63-1 has not been previously reported. Race 31-1, known in the old system as race 53, has been previously reported and used to identify *Ur-3* (Hurtado-Gonzales, et al., 2017).

Both bean rust races identified in this study were present across the three departments in the Western Guatemalan highlands (Quetzaltenango, Chimaltenango, and San Marcos) (Table 3 and 5). Race 63-1 was virulent to all the differential cultivars containing rust resistance genes of Andean origin. It was also virulent to GN1140 (*Ur-7*) of Middle America origin (Table 6). Race 31-1 was virulent to all the differentials cultivars of Andean origin except PI 260418 (*Ur-unknown*). It was also virulent to GN1140 (*Ur-7*) of Mesoamerica origin (Table 6).

Table 5. Infection types for the standard set of 12 differential bean cultivars to *U. appendiculatus* isolates collected from Guatemala, and races based on the binary system at the seedling stage[d]

Differential cultivar/ resistance gene	*Uromyces appendicutus* isolates																
	G1501	G1502	G1503	G1504	G1505	G1506	G1507	G1508	G1509	G1510	G1511	G1515	G1516	G1517	G1518	G1519	G1522
Early Gallatin/ Ur-4	6	6	5	5	5	5	4	5,6	6	5	5	4,5,6	5	5,6	4,5	4,5	5,4
Redlands Pioneer/ Ur-13	6	6	5	5	6,5	5,6	4,5	2,5	6	5,6	5	4,5	4	5	4,5	4,5	5,4
Montcalm/ Ur-unknown	5,6	6	4,5	5,6	5,6	5,6	4,5	5,6,	6,5	5	5	4,5	5	5	4	5,6	5
PC 50/ Ur-9, Ur-12	5,6	6	6,5	5,6	5,6	5,6	5,4	6,5	6,5	5	6,5	5,6	5	5,6	4,5	4,5	5,6
Golden Gate Wax/ Ur-6	6,5	6	5,4	6	5,6	5,6	4,5	5,4,	6	5	6,5	5,6	5,4	6,5	5,6	5,6	5
PI 260418/ Ur-unknown	5	4	3	4	3,2+	3,2+	5,4	4	4	4,5	3,2+	4,5	4,3	2+,3	3,4	2,3	3,2+
GN 1140/ Ur-7	6	6	6	6	6,5	6,5	6,5	5	6	6,5	6,5	5,6	6	6,5	5,6	6	6,5
Aurora/ Ur-3	2+	2+	2+,3	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
Mex 309/ Ur-5	2,3	3	3	3	2+,3	2+	3	2+	3	2+	2+	2	3	3,2+	2	2+	2+,3
Mex 235/ Ur-3+	2+	2+	2+	2	2+	2+	2+	2+	2+	2+	2+	1	2+	2+	1	1	2+
CNC/ Ur-unknown	2+	1	2+	3	2+	2+	3,2+	2+	2	2+	2+	2	3	2+	2	2	2+
PI 181996/ Ur-11	2+	1	1	1	2+	1	2+	1	1	1	1	1	1	2+	1	1	1
Race	**63-1**	**63-1**	**31-1**	**63-1**	**31-1**	**31-1**	**63-1**	**63-1**	**63-1**	**63-1**	**31-1**	**63-1**	**63-1**	**31-1**	**63-1**	**31-1**	**31-1**

[d]ITs are based on the 0 to 6 scale from Stavely, 1984; Mmbaga et al., 1996; Steadman et al., 2002. Races are classified based on binary values of each differential cultivars as described on Table 1 (Steadman et al., 2002).

Table 6. Virulence phenotype of bean rust races 63-1, 31-1 and 20-3* to *P. vulgaris* differential cultivars for rust resistance.

Differential Cultivar	Rust Resistance Gene	Gene Pool	Virulence Phenotype		
			63-1	31-1	20-3*
Early Gallatin	*Ur-4*	Andean	Virulent	Virulent	Avirulent
Redlands Pioneer	*Ur-13*	Andean	Virulent	Virulent	Avirulent
Montcalm	*Ur-Unknown*	Andean	Virulent	Virulent	Virulent
PC 50	*Ur-9, Ur-12*	Andean	Virulent	Virulent	Avirulent
Golden Gate Wax	*Ur-6*	Andean	Virulent	Virulent	Virulent
PI 260418	*Ur-Unknown*	Andean	Virulent	Avirulent	Avirulent
GN 1140	*Ur-7*	Mesoamerica	Virulent	Virulent	Virulent
Aurora	*Ur-3*	Mesoamerica	Avirulent	Avirulent	Virulent
Mex 309	*Ur-5*	Mesoamerica	Avirulent	Avirulent	Avirulent
Mex 235	*Ur-3+*	Mesoamerica	Avirulent	Avirulent	Avirulent
CNC	*Ur-Unknown*	Mesoamerica	Avirulent	Avirulent	Avirulent
PI 181996	*Ur-11*	Mesoamerica	Avirulent	Avirulent	Avirulent

*Race 20-3: Characterization data of race 20-3 previously reported by Markell et al. (2008).

Germplasm Greenhouse Evaluation

In total, 372 climbing beans accessions were evaluated for rust resistance under greenhouse conditions. Isolate G1501 for race 63-1, isolate G1522 for race 31-1, and race 20-3 were used for virulence tests. There were significant differences ($p<0.001$) among diseases reactions of accessions with races 20-3, 63-1, and 31-1 (Table 7). Rust symptoms in susceptible accessions were well defined (Fig. A.2). However, many accessions presented differences of infection type scores within accessions and phenotypic differences in stem color (green or purple). Then, those accessions were tested again to find out if there were inoculation and/or environment conditions problems (escapes). However, the accessions kept showing different infection type scores (i.e. 1, 1, 6, 1) which did not allow accurate classification as resistant or susceptible. Since the accessions used for virulence phenotype evaluation are landraces from a germplasm collection (Ponciano-Samayoa et al., 2009), segregation or seed mixture is expected in many accessions. Therefore, accessions with variable infection types were removed for further genomic analysis.

In total, 215 of 372 accessions showed consistent IT scores to race 63-1 (Table A.2). For race 31-1, 185 of 372 accessions showed consistent IT scores (Table A. 3). Similarly, 209 accessions showed consistent IT scores to race 20-3 (Table A.4). Maximum IT score for susceptible genotypes was 6 (scale 1-6) and 1 for resistant genotypes. Table 8 describes the percentage of consistent resistant and susceptible genotypes. The susceptible check pinto UI 114 had IT score of 6 across all replications and races, suggesting that disease pressure was high (Table A.2, A.3, and A.4).

Table 7. Analysis of Variance with fixed effects for rust diseases reactions in 372 climbing bean accessions.

Rust race	Source of Variation	df	F-Value	$P > F$
20-3	Genotype	371	9.06	<.0001
63-1	Genotype	371	3.32	<.0001
31-1	Genotype	371	3.16	<.0001

The majority of genotypes showed resistant reaction to race 63-1, 31-1 and 20-3.

Therefore, there are many options of accessions that can be chosen as prospective parents for

breeding programs. Especially the ones with the lowest IT scores (1 and 2+) across replications,

because they were highly resistant (Table A.2, A.3, and A.4).

Table 8. Percentage of resistant and susceptible genotypes by race, maximum and minimum infection type scores.

Race	% Resistant genotypes	% Susceptible genotypes	Max IT	Min IT
63-1	82	18	6	1
31-1	86	14	6	1
20-3	90	10	6	1

Genome –Wide Association Studies

After removing accessions with possible seed mixture or data inconsistencies, less than

372 accessions remained for GWAS studies as is showed in Tables A.2, A.3, and A.4, to reduce

error in the analysis. The best GWAS model was selected using Mean Square Deviations (MSD).

MSD values were almost identical across all models (Table 9). However, results with GenABEL

were selected since most refined data was obtained with binary system due to a solid definition

of categories. In addition, GenABEL include established threshold/significance cutoff lines for

resulting graphs which allows easier identification of significant associations.

Table 9. Mean Square Deviation (MSD) of each GWAS Statistical Model with Continuous and Binary Data.

Data	Rust Race	7PC	EMMA	7PC+EMMA
Binary	20-3	0.15	0.18	0.19
Binary	63-1	0.17	0.17	0.18
Binary	31-1	0.15	0.17	0.19
Continuous	20-3	0.14	0.16	0.16
Continuous	63-1	0.19	0.19	0.19
Continuous	31-1	0.15	0.18	0.19

Multiple genomic regions were associated with rust resistance in this climbing bean collection. SNPs falling in the 0.01 percentile were considered significant (Mamidi et al., 2014). Manhattan plot in figures 5, 6 and 7 show the genomic positions on the horizontal axis and the – log P-values on the vertical axis. The quantile-quantile plots show the adjustment of the data with the model in a regression line. Candidate gene models were searched from 50 Kb upstream and downstream of the significant SNP marker, based on the V.2 of the *P. vulgaris* genome, available at https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris (Schmutz et al., 2014).

Genomic Regions Associated with Resistance to Bean Rust Race 20-3

There are multiple factors significant GWAS peaks ($P<0.001$) related to resistance to race 20-3. (Fig. 5). Table 10 shows the 0.01 percentile of SNPs with the lowest P-value. One of the main factors is observe in Pv02 (38.13 Mb and 38.22Mb) which are in the same region as Phvul.002G212900 and Phvul.002G213600, which are predicted to encode for Haloacid dehalogenase-like hydrolase (HAD) superfamily protein and RNA-binding KH domain-containing protein. Also, the significant signal in Pv04 (379 kb) associated with resistance to bean rust (20-3) is located in the same region as between Phvul.004G005800 and Phvul.004G005900, which are predicted to encode for cytochrome P450, family 96, subfamily A, polypeptide 10.
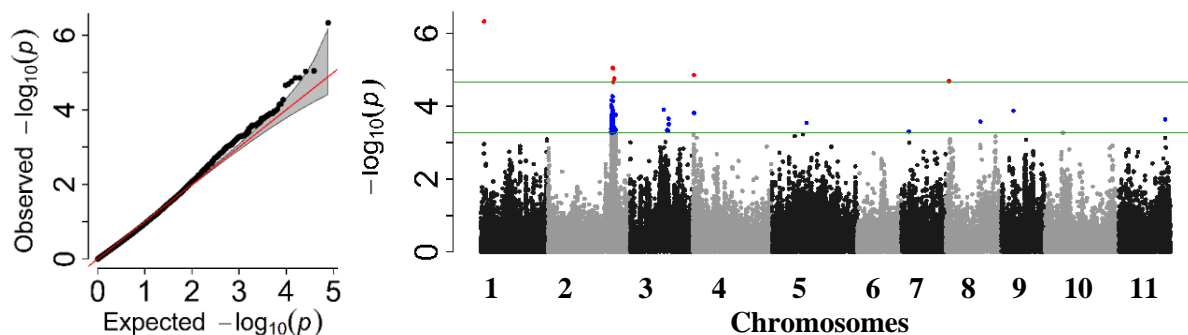
Figure 5. a) q-q plot by using mixed model and b) Manhattan plot for bean rust resistance to race 20-3 by using mixed model (EMMA+7PC). The green line is the cutoff value to identify a significant peak. SNPs that pass the 0.01 percentile are red colored, and those falling from 0.01 to 0.1 percentile are blue colored.

Table 10. SNPs markers (0.01%) significantly associated with rust resistance to race 20-3 on the Guatemalan climbing bean collection, sorted by the lowest *P*-value.

Chromosome	Position	P-value	SNP	MAF*
1	2563910	4.69E-07	S01_2563910	0.14571429
2	38134625	9.04E-06	S02_38134625	0.08285714
2	38223023	9.29E-06	S02_38223023	0.06571429
4	379943	1.39E-05	S04_379943	0.10000000
4	379989	1.39E-05	S04_379989	0.10000000
2	38701510	1.73E-05	S02_38701510	0.06285714
8	2303523	2.06E-05	S08_2303523	0.08285714

*MAF: Minor Allele Frequency.

Genomic Regions Associated with Resistance to Bean Rust Race 63-1

Significant associations controlling resistance to race 63-1 were identified at Pv10 and

Pv04 (Table 11). The GWAS signal in Pv10 (10.71-10.68 Mb) (Fig. 6) is located in the same

region as Phvul.010G061800 and Phvul.010G061900 predicted to encode for a SCAR homolog

2. And the GWAS signal Pv10 (11.09Mb) is located in between the region of Phvul.010G062300

and Phvul.010G062400 which are predicted to encode for an iron regulated and a 1 RNI-like

superfamily protein, respectively. The GWAS peak on Pv04 (1.42 Mb) (Fig. 6) is in the same

region as between Phvul.004G012700 and Phvul.004G012801, which are predicted to encode for

ATPase E1-E2 type family protein / haloacid dehalogenase-like hydrolase family protein and

NB-ARC domain-containing disease resistance protein. Also, these GWAS peak is surrounded

by genes that encode for NB-ARC domain protein for disease resistance.
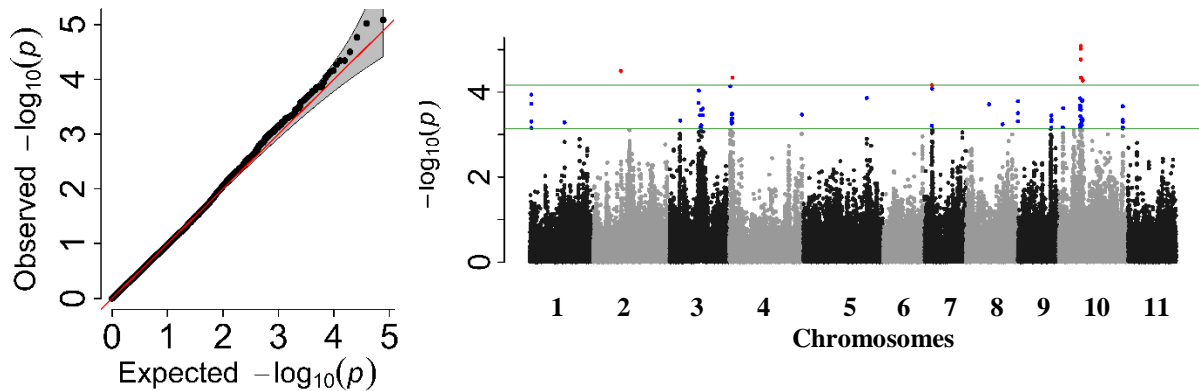
Figure 6. a) q-q plot of EMMA model and b) Manhattan plot of EMMA model for bean rust resistance to race 63-1. The green line is the cutoff value to identify a significant peak .and SNPs that pass the 0.01 percentile are red colored, and those falling from 0.01 to 0.1 percentile are blue colored.

Table 11. SNPs markers (0.01%) significantly associated with rust resistance to race 63-1 on the Guatemalan climbing bean collection, sorted by the lowest *P*-value.

Chromosome	Position	*P*-value	SNP	MAF[*]
10	10686825	8.26E-06	S10_10686825	0.16976744
10	10686721	9.55E-06	S10_10686721	0.16976744
10	10686722	1.71E-05	S10_10686722	0.17209302
2	21055579	3.17E-05	S02_21055579	0.11395349
10	10713869	4.55E-05	S10_10713869	0.12325581
4	1428498	4.58E-05	S04_1428498	0.31627907
10	11094935	5.37E-05	S10_11094935	0.12790698

*MAF: Minor Allele Frequency.

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Genome Regions Associated with Resistance to Bean Rust Race 31-1

The same association at Pv04 was found across all models. However, control for relatedness by EMMA model was selected because this model fit the most (Fig. 7). The significant GWAS peak in Pv04 (39.28 Mb) that is associated with resistance to rust race 31-1 is located in the same region as between Phvul.004G113400 and Phvul.004G113500 that are predicted to encode for C2H2-like zinc finger protein and ATP binding microtubule motor family protein, respectively (Table 12). This region is close to cluster of genes that are predicted to encode for different proteins for disease resistance. The significant association at Pv02 (35.92 Mb) is located as same region as between Phvul.002G195400 and Phvul.002G195500, which function have not been described yet.
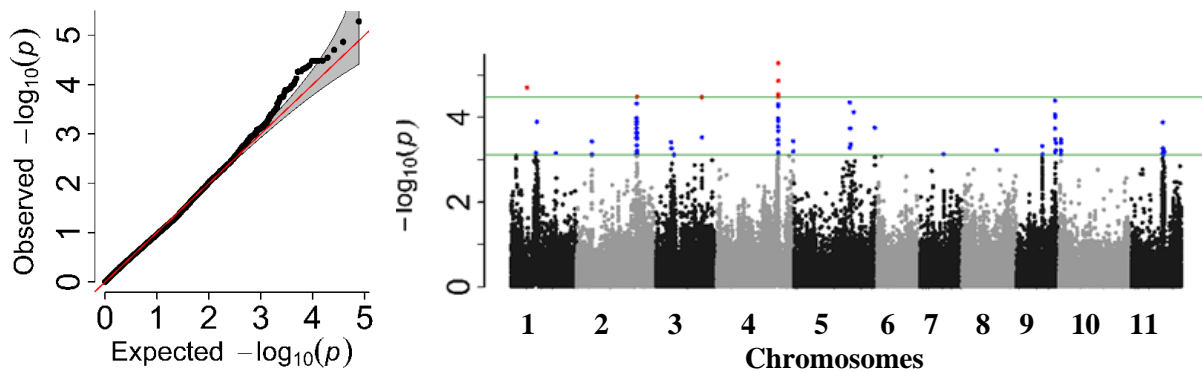
Figure 7. a) q-q plot by using EMMA model and b) Manhattan plot for bean rust resistance to race 31-1 by using EMMA model. The green line is the cutoff value to identify a significant peak. SNPs that pass the 0.01 percentile are red colored, and those falling from 0.01 to 0.1 percentile are blue colored.

Table 12. SNPs markers (0.01%) significantly associated with rust resistance to race 31-1 on the Guatemalan climbing bean collection, sorted by the lowest P-value.

Chromosome	Position	*P*-value	SNP	MAF*
4	39281554	5.27E-06	S04_39281554	0.38648649
4	39282041	1.37E-05	S04_39282041	0.37297297
1	12571938	1.99E-05	S01_12571938	0.07567568
4	39282007	2.85E-05	S04_39282007	0.41351351
4	39281577	3.29E-05	S04_39281577	0.42432432
2	35952312	3.31E-05	S02_35952312	0.06216216
2	35952366	3.31E-05	S02_35952366	0.06216216

*MAF: Minor Allele Frequency.

DISCUSSION

Pathogen Characterization

Based on the infection types, collected isolates showed a similar pattern, expect for PI 260418 (Table 5). By using the binary system described by Steadman et al. (2002), races 63-1 and 31-1 were identified. Both races were present across three Departments in the western highlands of Guatemala (Quetzaltenango, Chimaltenango, and San Marcos), which are important climbing bean producers. Those races were virulent to most of the genes of Andean gene pool. Race 31-1 has been previously reported and used for virulence test, as it was named as race 53 in the old system (Steadman et al., 2002; Hurtado et al., 2017). This race was used to successfully map the rust resistance gene *Ur-3* (Hurtado-Gonzales et al., 2016). However, the isolate used for Hurtado-Gonzales et al. (2016) is older than the isolate used for this study, it was collected in a different location, and has been maintained at Beltsville, MD (Pastor-Corrales et al., 2001). Contrastingly, isolate representing race 31-1 in this study was recently collected (2015) at Guatemala and may be genetically different compared with the isolate used in Beltsville, MD because pathogen isolates tend to change through the time. This is especially true for a highly variable pathogen such as *U. appendiculatus* (S. Markell, Pers. Comm., 2017).

Since race 63-1 and 31-1 were present across three Departments, it suggests that those isolates have adapted to climate conditions of Milpa cropping systems at mid-highlands, where altitude ranges from 1760 to 2440 meters above sea level (Table A.1). In addition, there is no clear geographical and/or altitudinal separation of races since both were present across all departments and altitudes. These results are in contrast with other rust diversity studies that have been done at Central America (Acevedo et al., 2013; Dardon et al., 2016), since previous studies have found higher diversity. However, the study by Dardon et al. (2016) in Guatemala cannot be

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totally compared with our study since it reported isolates and not races. First, Dardon et al. (2016) used bush type beans as susceptible cultivars in the field while mostly climbing beans were sampled in this study, which can have a different reaction. Second, this study evaluated different locations (towns) than the ones evaluated by Dardon et al. (2016). Lastly, mobile nurseries do not assess Single Ureidinium Isolates (SUI), then cultivars get infected by a mixture of spores and observed symptoms cannot be assigned to a single isolate and neither classify it as a race (Dardon et al., 2016). In contrast, this study report isolates that were increased from SUI which allowed to accurately score the IT caused by the infection of each isolate.

U. appendiculatus is one of the bean pathogens that has a strong affinity with host gene pool origin (Araya et al., 2004). Anthracnose and angular leaf spot have showed a similar host co-evolutionary interaction pattern as well (Sandin eta al., 1999; Araya 2003; Araya et al., 2004). Sandin et al., (1999) described that Mesoamerican rust isolates were virulent to genotypes of Mesoamerican and Andean origin. Moreover, Andean rust isolates were more frequent to cause a susceptible reaction to genotypes of Andean origin. However, isolates collected in Guatemala, 63-1 and 31-1, were virulent mainly to accessions of Andean origin and one of the six accessions of Mesoamerican gene pool (GN 1140 with *Ur-7*). It suggest that isolates used for race 63-1 and 31-1 might have Andean origin. However this needs to be further studied.

Germplasm Greenhouse Evaluation

Evaluation of the climbing bean germplasm to rust races 63-1, 31-1 and 20-3 was conducted under greenhouse conditions during summer 2016. However, many accessions were removed because they showed high phenotypic variability. In total, 157 of 372 were removed for race 63-1 evaluation, 187 accessions for race 31-1 and, 163 for race 20-3. This was expected due to the nature of the germplasm collection since the accessions in this climbing bean

collection are mostly landraces, and seed are kept from generation to generation. Also, climbing beans tend to invade other plant's space due to its indeterminate growth habit (type IV), and seed mixture and variation among accessions might happened during harvesting of seed increases. All those factors lead to variations within accessions. Phenotypic differences are also very common within production fields of climbing beans in Guatemala.

After removing inconsistent accessions, those with susceptible reaction and the susceptible check (pinto UI 114) showed strong symptoms, with maximum values of 6 on a scale of 1 to 6 (Fig.A.2). In addition, the majority of the accessions showed resistant reaction to rust races; 175 accessions were consistently resistant to race 63-1, 160 resistant to race 31-1, and 188 to race 20-3 (Table 8, A.2, A.3, and A.4). Accessions with IT of 1 to 2 (highly resistant) might be considered in bean breeding programs to improve climbing bean accessions and/or perhaps bush type beans. The accessions: c696cm4cm3cm3cm, c8315cm6cmb5cm4cm and guate1165 had IT of 1 to 2 to the three evaluated races. Those accessions can be cross with ICTA Utatlán or ICTA Labor Ovalle, which are climbing bean released varieties in Guatemala.

After collecting data from the race characterization and the greenhouse evaluation, it is suggested that resistant accessions to race 63-1 might have *Ur-3, Ur-5, Ur-3+, Ur-Unknown* (CNC) or *Ur-11* resistant genes of Andean origin, or novel genes that have not been reported yet. Resistant accessions to race 31-1 might have *Ur-Unknown* (PI 260418), *Ur-3, Ur-5, Ur-3+, Ur-Unknown* (CNC) or *Ur-11* resistant genes. Also, is possible that resistant accessions may involve more than one gene in epistatic interaction (Liebenberg et al., 2006). For example, the *Ur-5* gene is epistatic to *Ur-4*, which are located at Pv04. The *Ur-3* and *Ur-6* genes are epistatic to *Ur-11*. In addition, resistant climbing bean accessions to race 20-3 might have *Ur-3, Ur-5, Ur-3+, Ur-*

Unknown (CNC) or *Ur-11* resistant genes (Markell et al., 2008). However all these phenotypic results were refined with following GWAS results.

<div align="center">

Genome–Wide Association Studies

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Resistance to Race 20-3

Based on GWAS results, is suggested that resistance to race 20-3 is controlled by a factor on Pv02 (38.13 Mb and 38.22Mb) and Pv04 (379 kb). The significant peak in Pv02 (Fig. 5) is nearby the same region as Phvul.002G212900 and Phvul.002G213600, which are predicted to encode for Haloacid dehalogenase-like hydrolase (HAD) superfamily protein and RNA-binding KH domain-containing protein, respectively (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris). The Haloacid dehalogenase-like hydrolase (HAD) superfamily protein together with protein kinases control the state of phosphorylation of cell proteins and thereby provide an important mechanism for regulating cellular activity (www.ebi.ac.uk). These might play a role triggering signal mechanisms. The RNA-binding KH domain-containing protein had been reported to play a key role for heat stress-responsive gene regulation and thermotolerance in Arabidiopsis (Guan et al., 2013). However, there are not rust specific resistance genes (*Ur*) previously reported in this chromosome (Meziadi et al., 2016), which suggests this could be a new resistance region not previously reported.

The significant signal at the beginning of Pv04 was search in the bean genome annotation and it falls within the same region as Phvul.004G005800 and Phvul.004G005900 (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris). Those genes are predicted to encode for cytochrome P450, family 96, subfamily A, and polypeptide 10, which are involved on catalysis of an oxidation-reduction (redox) reactions (www.ebi.ac.uk). The

resistance gene *Ur-5* (Stavely, 1984) and *Ur-OuroNegro* (Correa et al., 2000; Faleiro et al., 2001; Miklas 2002; Alzate-Marin et al., 2002; de Souza et al., 2007) or currently known as *Ur-14* (de Souza et al., 2011) have been mapped at the end of Pv04 (Miklas eta al., 2006; Meziadi et al., 2016). The accession carrying *Ur-5* showed a resistance reaction to race 20-3 (Table 6). The accession with *Ur-OuroNegro* is not part of the set of differential cultivars and was not evaluated in this study. However, the GWAS signal in this study is located at the beginning of Pv04 (379 kb) while *Ur-5* and *Ur-OuroNegro* are mapped at the end of the chromosome.

Resistance to Race 63-1

Resistance to race 63-1 is controlled for two major regions in Pv10 and Pv04 (Table 12). The GWAS signal in Pv10 (10.71-10.68 Mb) is located in the same region as Phvul.010G061800 and Phvul.010G061900 predicted to encode for a SCAR homolog 2, which molecular function is unknown yet (www.ebi.ac.uk). Other GWAS association was found in Pv10 (11.09Mb) that fall in the region between Phvul.010G062300 and Phvul.010G062400, which are predicted to encode for an iron regulated and a 1 RNI-like superfamily protein, respectively (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris). The 1 RNI-like superfamily protein is involved in different biological process (http://supfam.org). There are not rust resistance genes yet reported on Pv10. Therefore, these genomic region on Pv10 associated with resistance to 63-1 might be unique for climbing beans.

The GWAS signal on Pv04 (1.42 MB) is in the same region as between Phvul.004G012700 and Phvul.004G012801, which are predicted to encode for ATPase E1-E2 type family protein / haloacid dehalogenase-like hydrolase family protein and NB-ARC domain-containing disease resistance protein. Also, these GWAS peak is surrounded by genes that encode for NB-ARC domain protein for disease resistance. This supports the possible association

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between the GWAS peak Pv04 and the resistance mechanism to race 63-1. In addition, this region in Pv04 is nearby a cluster of resistance genes to different diseases including *Ur-5, Ur-Dorado* and *Ur*-OuroNegro (Correa et al., 2000; Faleiro et al., 2001; Miklas 2002; Alzate-Marin et al., 2002; de Souza et al., 2007) or currently known as *Ur-14* (de Souza et al., 2011) (Miklas eta al., 2006; Meziadi et al., 2016). In addition, the differential accession Mexico 309 carrying *Ur-5* showed resistance reaction to race 63-1 (Table 6). Further fine mapping might be needed to target the GWAS region and compare its location with *Ur-5*.

Resistance to Race 31-1

Resistance to race 31-1 was found to be controlled by two major factors, one of them in PV04 (39.28 Mb Mb) where numerous SNPs were in Linkage Disequilibrium (LD) (Fig. 7). Candidate genes in this genomic region (Phvul.004G113400 and Phvul.004G113500) are predicted to encode for C2H2-like zinc finger protein and ATP binding microtubule motor family protein. The C2H2-like zinc finger protein family is involved in the response to abiotic and biotic stresses (Huang et al., 2004; Liu et al., 2015). Then, region might be related with defense mechanism to race 31-1, in addition, this GWAS peak is nearby a block of disease resistance family protein (https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris). This genomic region is favorable for disease resistance NL gene cluster (NB-LRR) due to the presence of heterochromatic blocks (higher recombination) (Meziadi et al., 2016), which are the dominant class of disease resistance genes in the common bean genome. Compared with the location of previous reported genes (*Ur-5*, *Ur-Dorado* and *Ur-OuroNegro*), the GWAS peak found in this study fall at the opposite arm of the chromosome (Meziadi et al. 2016). The second significant association was found in Pv02 (35.92 Mb), however is located in a region where genes function

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have not been described yet

(https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris). Although, this association in Pv02 falls in presence of heterochromatic region where there is high recombination, which is favorable for development of disease resistance genes. There had been some diseases resistance genes nearby this genomic region of Pv02, however, not rust resistance gene has been reported here before. This highlight the unique genetic composition of climbing beans.

Results of the genomic region in Pv04 conferring resistance to race 31-1 differ from the ones reported by the previous study by Hurtado-Gonzales et al. (2017) which used race 31-1 to map *Ur-3* located in Pv11. It can be due to changes on isolates and accessions evaluated. First, the isolate used by Hurtado et al. (2017) is not the same used for this study (G1522) which was recently collected (2015) in Guatemala. Both isolates have been under different weather and host conditions. Therefore, as it happens with other pathogens, both isolates might have differentiated and so its mechanisms to reproduce on the host. This is especially true in the case of a highly variable pathogen such as *U. appendiculatus*. In addition, climbing beans type were used for this study while Hurtado et al. (2017) used bush type accessions. Therefore, the genomic regions conferring resistance could differ as well.

Some of those reported genomic regions associated with rust resistance might have not been reported before: Pv02 (38.13 Mb and 38.22Mb), Pv10 (10.71-10.68 Mb), and Pv02 (35.92 Mb). Since SNPs for this study were generated from Mesoamerican climbing beans, its genome sequences may have not been included on previous annotations of common bean genome. A recent Genetic Diversity Study was reported on the same climbing bean germplasm evaluated in this project by Tobar et al. (2017), using 78,754 SNPs (MAF <0.05). Preliminary results of

principal components analysis (PCA), population structure (PC) and phylogenic tree, showed a genetic differentiation of climbing beans to other Middle America bean races (Durango-Jalisco and Mesoamerica). Suggesting that climbing beans from Guatemala are a different group of beans, and are a new source of genetic diversity. Moreover, some of the GWAS signals in this study are located in genomic regions not described previously; and do not coincide with currently *Ur* reported genes. Hence, it suggests that climbing beans might have novel regions conferring rust resistance to races 63-1, 31-1, and 20-3, perhaps to other rust races not evaluated yet.

Results of bean rust diversity on this study give an important sight of the current status of the pathogen at the highlands in Guatemala, as is reported by this study, race 63-1 has not been analyzed previously. However, isolates from more locations still need to be characterized. In addition, bean rust tends to change trough the time and therefore, population should be periodically tracked across bean producing regions. Moreover, GWAS results showed the potential genetic information for disease resistance in climbing beans. Some regions might have been reported previously by using other accessions and rust races, and others are being reported for the first time in this study. The climbing group of beans from Guatemala will be helpful for bean improving and host-pathogen interaction studies. Accessions with resistance reaction (IT of 1 to 2) should be selected and crossed with those climbing landraces that are widely used by farmers and commonly consumed. Based on pathogen characterization results, genes of Mesoamerican origin (except for *Ur-7*) should be combined for durable resistance, especially in Quetzaltenango, San Marcos and Chimaltenango, where races 63-1 and 63-1 where found. In addition, it has been proved that *Ur-11*, *Ur-5* and *Ur-3* confer broad resistance and must be

combined. Reduction of seed yield losses of those regions with malnutrition problems can be achieved by improving climbing bean, which in turn can enhance food security.

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APPENDIX

Table A.1. Geographical coordinates of bean rust samples collected at the western Guatemalan Highlands.

Isolate ID	Town	Altitude (masl)	N	O
G1501	CEIPRA La Esperanza	2440	14° 51´ 56.00"	91° 33' 14.00"
G1502	ICTA, Labor Ovalle	2380	14° 52' 12.00"	91° 30' 50.00"
G1503	ICTA, Labor Ovalle	2380	14° 52' 12.00"	91° 30' 50.00"
G1504	ICTA, Labor Ovalle	2380	14° 52' 12.00"	91° 30' 50.00"
G1505	ICTA, Labor Ovalle	2380	14° 52' 12.00"	91° 30' 50.00"
G1506	ICTA, Labor Ovalle	2380	14° 52' 12.00"	91° 30' 50.00"
G1507	ICTA, Labor Ovalle	2380	14° 52' 12.00"	91° 30' 50.00"
G1508	ICTA, Labor Ovalle	2380	14° 52' 12.00"	91° 30' 50.00"
G1509	Parramos, Chimaltenango	1760	14° 37' 30.00"	90° 48' 31.00"
G1510	Parramos, Chimaltenango	1760	14° 37' 30.00"	90° 48' 31.00"
G1511	Parramos, Chimaltenango	1760	14° 37' 30.00"	90° 48' 31.00"
G1512	Patzicía, Chimaltenango	2090	14°39' 13.00"	90°56' 28.00"
G1513	Patzicía, Chimaltenango	2090	14°39' 13.00"	90°56' 28.00"
G1514	Santa Cruz, Balanyá, Chimaltenango	2190	14° 42'02.00"	90° 57' 00.00"
G1515	Patzicía, Chimaltenango	2090	14°39' 13.00"	90° 56' 28.00"
G1516	Patzicía, Chimaltenango	2090	14°39' 13.00"	90° 56' 28.00"
G1517	Loma linda, San Pedro Sacatepequez, SM.	2167	14° 56' 33.04"	91° 45' 45.19"
G1518	El centro, las Barranquitas, San Antonio, SM.	1956	14° 55' 37.5"	91° 44' 34.23"
G1519	El centro, las Barranquitas, San Antonio, SM.	1956	14° 55' 38.58"	91° 44' 39.26"
G1520	Caserío Nueva Reforma, San Pedro Sac, SM	1938	14° 55' 47.70"	91° 45´ 13.7´´
G1521	Aldea Champollap, San Pedro Sac, SM	1938	14° 56' 30.3"	91° 45´ 42.9´´
G1522	Loma linda, San Pedro Sacatepequez, SM.	2167	14° 56' 33.53"	91° 45' 44.63"
G1523	La Esperanza, Quetzaltenango	2345	14° 25' 21.70"	89° 44´ 11.2´´

Figure A.1. Germplasm greenhouse evaluation a) Climbing bean accessions and susceptible control 6 days after planting, b) Inoculation of 8 days plants with bean rust, c) Inoculated accessions in the humidity chamber, d) and e) Symptoms development under greenhouse conditions, 8 days after inoculation, and f) virulence phenotype evaluation, 14 days after inoculation.
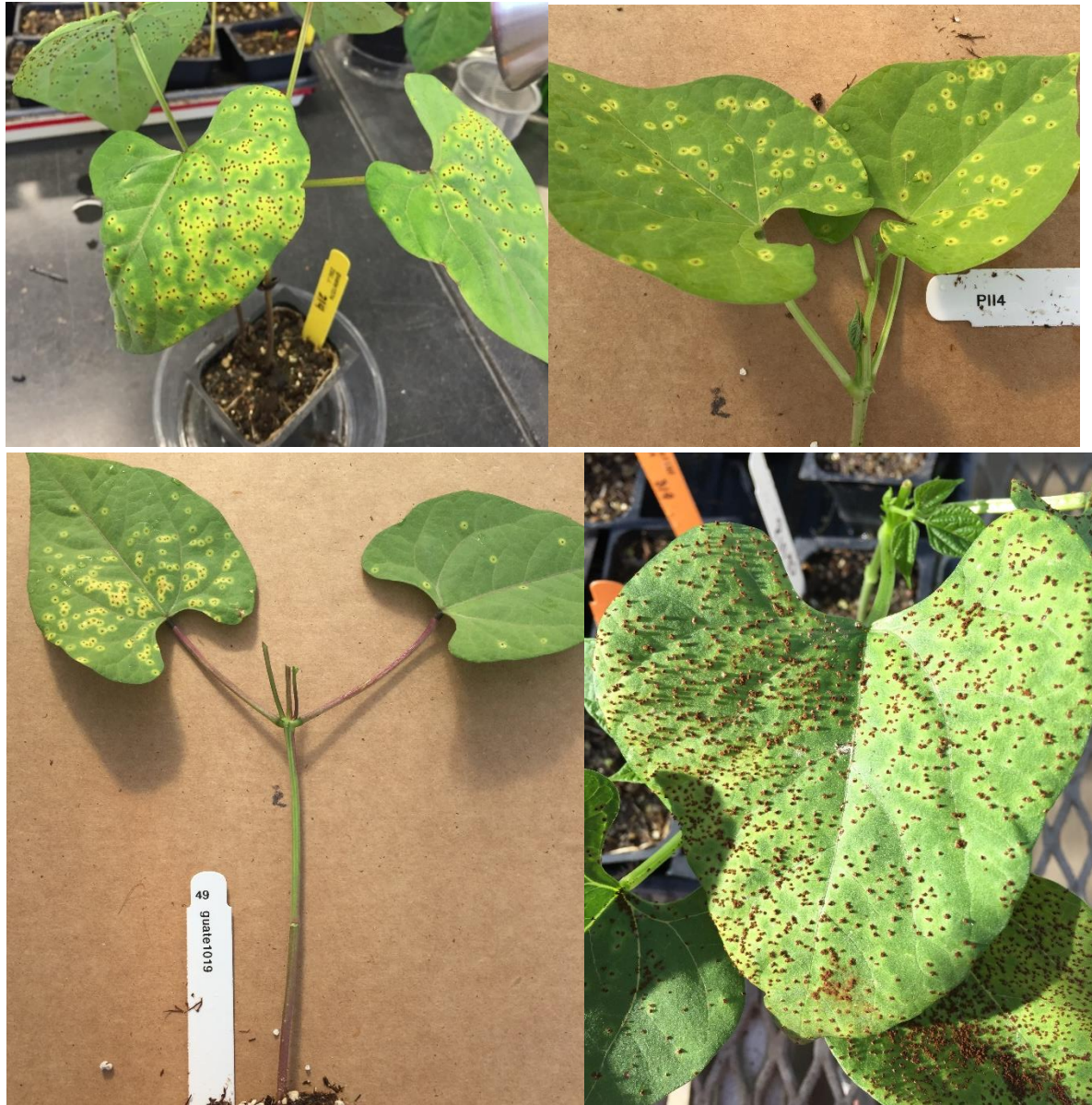
Figure A.2. Bean rust symptoms on susceptible bean accessions, 14 days after inoculation. a), c) and d) climbing bean susceptible plants, b) Susceptible check pinto UI114.

Table A.2. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 63-1 collected in Guatemala, at the seedling stage[e]

Accession ID	Infection type					
AT188422cmcm	1	1	1	2+	2+	2+
c12311cmcm	1	3	1	2+	2+,3	2+,3
c1231cmcmcm	4	4	4	4,3	4,3	4,3
c12332cmcm	1	3,2+	1	2+	2+	2+
c1831cmcmcm	3	1	3	3	2+	2+
c274cm6cm9cmb5cm	1	1	1	2+	2+	2+
c695cm6cm9cm5cm	1	4	1	2+	2,3	1
c696cm4cm3cm3cm	1	1	1	1	1	1
c697cm4cm9cm3cm	1	1	1	1	1	1
c8110cm4cm2cm2cm	2+	2+	2+	2+	2+	2+
c8116cm6cm7cmb2cm	1	2+	1	2+	2+	1
c813cma4cm6cm6cm	1	2+	1	3	1	1
c819cm6cm9cm5cm	1	2+	1	2+	1	2+
chajmaquiaj40	4	4	4	1	4	4
frijolboloj	3	1	1	1	1	1
frijolbonilla	1	2+	1	2+,3	2+	2+
G6076	1	1	1	3	1	1
guate1000	4	5,4	4	4	3,4	4,3
guate1001	1	2+	1	2+	2+	2+
guate1002	3	3	3	3	2,3	3
guate1005	1	1	2+	2+	1	1
guate1007	2+	2+	1	1	1	1
guate10071	1	1	1	1	1	1
guate1009	1	5	4,5	5	4	4,5
guate1016	1	2+	1	1	2+	1
guate1019	1	6	6	6	6	6
guate1024	1	3	1	3	2+	2+
guate1029	1	3	1	3	1	1
guate1032	1	2+	1	2+	2+	1
guate1036	1	1	1	1	2+	1
guate1038	2+	2+	2+	2+	2+	2+
guate1041	1	5	6	6	6	6
guate1045	1	1	1	2+	2+	1
guate1051	1	1	1	2+	1	1
guate1053	1	1	1	2+	2+	2+
guate1059	1	1	1	2+	2+	1
guate1063	2+	2+	2+	2+	1	1

[e]ITs are based on the 0 to 6 scale of Stavely, 1984; Mmbaga et al., 1996; Steadman et al., 2002.

Table A.2. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 63-1 collected in Guatemala, at the seedling stage[e] (continued)

Accession ID	Infection type					
guate1064	2+	2+	2+	3,2+	2+	4,5,6
guate1066	1	1	1	2+,3	3	3
guate1069	2+	2+	2+	2+	2+	1
guate1073	1	2+	1	1	1	3
guate1074	4	4	4	5,6	4	1
guate10764PM	3	2+	3	3	2+	2+
guate1077	3	3	1	1	2+	2+
guate1079	5	5	5	4	6	4,5
guate1080	1	1	1	4,5	4	1
guate1081	2+	2+	2+	2+	1	2+
guate1088	2+	2+	2+	2+	1	1
guate1091	1	6	5	6	4,3	5,6
guate1098	5	6	5	5	6	5
guate1104	3	2+	3	3	0	1
guate1105	1	3	1	1	2+	2+
guate1109	4,3	4,3	4,3	4	4,3	4
guate1112	1	3	1	2+	3	2+
guate1117	3	4	4	4	4,5	3,4
guate1121	6	6	6	1	6	6
guate1123	1	3	3	3	3	2+
guate1132	4	5,6	4	5	3,4	4,5
guate1135	1	1	1	3	1	1
guate1137	1	1	1	1	1	1
guate1142	1	1	1	3	2,3	2,3
guate1143	1	3	1	2+	2+	2+
guate1149	1	3	1	3	2+	2+
guate1151	3	1	3	3	2+	1
guate1161	4	4,5	4	4,5	4	4
guate1161.3PMA	1	2+	1	2+	1	1
guate1163	1	6	5,6	6	5,6	5,6
guate1164	3	3	3	2+	2+	2+
guate1165	1	2+	1	1	2+	1
guate1166	1	3	1	2+	2,3	2,3
guate1168	1	1	1	2+	2+	2+
guate1170	1	3	1	3	1	1
guate1172	1	2+	1	1	1	1
guate1175	1	2+	1	1	2+	1

Table A.2. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 63-1 collected in Guatemala, at the seedling stage[e] (continued)

Accession ID	Infection type					
guate1177	1	2+	1	1	2+	1
guate1182	1	1	1	2+	1	1
guate1191	1	2+	1	1	1	1
guate1192	1	3	1	2+	2+	2+
guate1198	1	1	1	2+	1	1
guate1200	1	1	1	2+	3	1
guate1201	1	2+	1	1	2+	2+
guate1211	4	4	4	6,4	5,6	3,2
guate1213	1	2+	1	2+*	1	1
guate1214	1	1	1	2+	1	1
guate1216	1	0	2+	2+	2+	2+
guate1217	1	2+	1	2+	2+	2+
guate1218	1	3	1	1	1	1
guate1222	3	3	2+	2+	2+	1
guate1223	1	1	1	1	1	2+
guate1226	1	2+	1	2+	2+	2+
guate1234	1	1	1	4	1	1
guate1236	2+	2+	2+	2+	2+	2+
guate1238	1	4	1	2+	2+	2+
guate1241	4	3,4	3,4	6	4,5	4
guate1242	1	1	1	1,3	1	1
guate1245	1	5	1	1	1	5
guate1253	3	3	3	3	3	3
guate1257	5	5	5	5	5	4
guate12803PM	1	1	1	1	2+	2+
guate135	2+	2+	2+	2+	2+	2+
guate1378	1	2+	1	2+	2,3	1
guate138	4	4,5	4	4,5	4,3	3
guate1387	1	3	1	1	1	1
guate1390	1	2+	1	2+	1	2+
guate1394	1	1	1	1	1	1
guate1396	3	3	3	3	3	3
guate1407	1	4	1	2+	2+	1
guate1418	1	3	1	1	3	2+
guate1420	3	3	3	2+	2+	2+
guate1422	2+	2+	2+	2+	2+	2+
guate1424	1	1	1	1	1	1

Table A.2. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 63-1 collected in Guatemala, at the seedling stage[e] (continued)

Accession ID	Infection type					
guate1428	1	1	1	2+	1	1
guate1429	1	3	1	2+	1	1
guate1430	2+	2+	2+	2+	2+	1
guate1434	1	1	1	2+	2+	5
guate1511	6	6	6	4,5	6	6
guate1514	1	3	1	2+	2+	1
guate1515	1	2+,3	1	2+	2+	2+
guate182	5	5,6	5	5	3,4	3,4
guate183	1	1	1	1	1	1
guate186	4,3	4,3	1	4,3	4,3	4,3
guate188	1	2+	1	2+	1	1
guate190	5	5,4	5	5,4	5,6	4,3
guate192	1	1	1	3	1	1
guate200	2+	2+	2+	2+	1	1
guate205	1	2+	1	2+	2,3	2+
guate218	1	4	1	1	1	1
guate219	1	3	1	2+	2+	2+
guate221	3	3	2+	2+	1	1
guate223	1	1	1	2+	2+	1
guate230	5	5,4	5,6	5,6	5,6	4
guate233	3	3	3	3	1	1
guate237	1	4,3	4	4,3	4,3	4
guate242	1	1	1	1	5,6	1
guate245	3	3	2+	2+	2+	2+
guate247	1	2+	1	3	2+ 3	2+
guate257	1	2+	1	2+	2+	2+
guate258	1	3	1	3	2+ 3	1
guate264	1	1	1	1	2+	2+
guate266	1	2+	1	2+	2,3	3
guate385	1	1	1	1	1	2+
guate418	1	1	1	2+	3	1
guate458	2+	2+,3	2+	2+	2+	1
guate633	1	2+	1	3	2,3	2+
guate652	1	3	1	2+	2+	2+
guate660	2+	1	2+	2+	2+	2,2+
guate674	2+	3	2+	3	2+	2+
guate675	3	3	3	2+	2+	2+

Table A.2. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 63-1 collected in Guatemala, at the seedling stage[e] (continued)

Accession ID	Infection type					
guate678	1	2+	1	2+	2+	2+
guate683	1	3	1	2+	2+	2,3
guate684	1	2+	1	1	1	1
guate83	1	3	1	3,4	3	1
guate884	1	1	1	2+	1	1
guate887	1	3	1	2+,3	3	2+,3
guate894	1	1	1	2+	2+	2+
guate896	3	3	3	2+	1	2+
guate898	1	2+	1	1	1	1
guate899	1	2+,3	1	2+,3	2+	1
guate900	6	6	6	6	5,6	1
guate904	1	3	1	2+	2+	1
guate905	1	3	1	3	3	3
guate906	1	3	1	2+	2+	2+
guate912	4	4,5	4	4	4,5	4
guate913	1	3	1	2+	3	2+
guate918	1	3	1	3	2+	1
guate919	3	3	2+	2+	2+	2+
guate92	1	1	1	2+,3	3	3
guate920	2+	2+	2+	2+	1	1
guate926	1	5	5,6	6	5,6	5
guate9282PM	1	1	1	1	2+	2+
guate930	3,2+	3,2+	3,2+	2+	1	1
guate933	1	3	1	1	2+	2+
guate935	1	4,5	4,5	5	4,5,6	4
guate936	1	2+	1	2+	2+	2+
guate937	1	4,5	5,6	4,5	5,6	4
guate940	2+	3	2+	2+	2+	2+
guate941.3	1	2+	1	3	1	1
guate944	1	1	1	1	1	1
guate945	3	3	3	3	2+	1
guate947	3	2+	3	3	2,3	5,6
guate950	5,6	5	5	5	5,6	4
guate952	2+	2+	2+	2+	2+	2+
guate955	1	1	1	1	2+	2+
guate956	1	2+	1	1	1	2+
guate959	1	5	1	5	2+	1

Table A.2. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 63-1 collected in Guatemala, at the seedling stage[e] (continued)

Accession ID	Infection type					
guate962	1	1	1	1	1	2+
guate963	1	1	1	2+	1	1
guate966	4	4	4,5	5	5	5,6
guate967	1	1	1	3	2+	2+,3
guate968	1	1	3	3,2+	2+	2+
guate970	4	4,5	4,3	4,5	4,5	3
guate985	1	2+	1	1	1	1
guate988	1	1	1	3,2+	2++	2+
guate993	5,6	6	5	5,6	3,4	3
guate995	6	6	4	6	6	1
jardinero	1	1	1	1	2+	2+
LaborOvalle	3	3	3	3	2+	2+
LCH86V13	3	3	3	2+	3	3
LCH86V17	1	2+	1	5,6	6	6
LCH86V19	1	2+	1	1	1	1
LCH86V23	1	2+	1	1	2+	2+
LCH86V31	1	5,6	5	5	4,3	3,4
LCH86V41	1	1	1	4	1	1
LCH86V71	1	3	1	3	2+	2+
LCH86V77	1	1	1	2+	2+	2+
LCH86V83	1	6	4	4	4	4
mediamilpa1	1	3	1	3	2+	3
mediamilpa2	1	2+	1	1	2+	2+
Purplepod1	3	3	3,4	3	3	3
V4614315	4	4	4	4	4	4
V4616320	3	3	1	3	3	3
V4616324	1	3	1	1	1	3
V5746313	1	2+	1	4	2+	1
V7966	1	3	1	3	2+	1
xenimajuyu	5	4,5	5	6	4	6

Table A.3. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 31-1 collected in Guatemala, at the seedling stage[f]

Accession ID	Infection types					
AT18435cmcma	2+	2+	2+	2+	2+	2+
AT188422cmcm	1	1	1	1	1	1
c12332cmcm	2+	1	1	1	1	1
c273cm2cm12cm6cm	1	4	4	4	1	4,3
c274cm6cm9cma5cm	2+	1	1	2+	3, 2+	3
c274cm6cm9cmb5cm	1	1	1	1	2+	2+
c495cm6cma12cm6cm	3	3	3	3	3	3
c495cm6cmb3cm2cm	3	2+	2+	3	2+	2+
C61cm8cm9cmcm5	2+	2+	2+	2+	2+	2+
c695cm6cm9cm5cm	2+	2+	2+	2+	1	2+
c696cm4cm3cm3cm	1	2+	2+	2+	1	1
c697cm4cm9cm3cm	1	1	1	1	1	1
c698cm4cm12cm6cm	4	4	5	5	4	5
c8116cm6cm7cmb2cm	2+	2+	2+	2+	1	1
c8116cm6cm7cmb2cm.2	2+	2+	2+	2+	2+	2+
c813cma4cm6cm6cm	2+	1	1	1	1	1
c815cm6cm9cm6cm	2+	2+	2+	2+	4	2+
c819cm6cm9cm5cm	2+	1	1	1	1	1
c8315cm6cmb5cm4cm	2+	2+	2+	2+	2+	2+
c934cmb8cm3cm6cm	2+	3	2+	3	2+	1
frijolboloj	4	4	4	4	4	4
frijolbonilla	3	3	3	3	3	3,4
G6076	2+	1	1	1	1	1
guate1004	3	2+	3	2+	2+	2+
guate1007	1	2+	1	1	1	1
guate10071	1	1	1	1	2+	2+
guate1019	6	6	6	6	6	6
guate1025	2+	3	3	2+	1	1
guate10261	4	4	4	4	4	4
guate1029	1	2+	1	1	2+	1
guate1032	4	1	1	1	1	1
guate1036	1	1	1	1	2+	2+
guate1040	2+	2+	2+	2+	2+	2+
guate1044	1	3	1	3	3	3
guate1045	1	1	1	1	1	1
guate1051	2+	2+	2+	2+	2+	2+
guate1053	2+	2+	2+	2+	1	1

[f]ITs are based on the 0 to 6 scale of Stavely, 1984; Mmbaga et al., 1996; Steadman et al., 2002.

Table A.3. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 31-1 collected in Guatemala, at the seedling stage[f] (continued)

Accession ID	Infection types					
guate1055	3	2+	3,4	2+	3	3
guate1059	1	1	1	1	1	1
guate1063	2+	1	1	3	3	3
guate1069	1	1	1	1	1	1
guate1071	2+	1	1	1	3	3
guate1073	1	1	1	1	2+	1
guate1079	6	6	5	5	6	4
guate1081	2+	1	1	1	2+	1
guate1082	2+	2+	2+	2+	2+	2+
guate1084	1	3	1	1	1	3
guate1088	2+	1	1	1	1	1
guate1089	6	6	5	6	4	5
guate1098	6	6	6	6	6	6
guate1100	4	5	4	5	5	5
guate1105	2+	2+	2+	1	1	1
guate1118	2+	2+	2+	2+	2+	2+
guate1121	4	5	5	5	4	5
guate11242	5	4	4	4	5	4
guate1127	6	6	6	6	5	6
guate1132PMB	2+	2+	2+	2+	2+	2+
guate1134	2+	3	2+	3	2+	2+
guate11352	2+	1	2+	1	1	1
guate1136	2+	3	3	3	3	3
guate1137	2+	1	1	1	1	1
guate1142	3	3	3	3	3	3
guate1143	1	2+	1	1	1	1
guate1148	5	5	4	5	4	4
guate1150	4	2+	2+	2+	2+	2+
guate1154	5	4	4	5	5	4
guate1159	2+	2+	2+	2+	2+	2+
guate1161.3PMA	2+	2+	2+	2+	1	1
guate1163	6	6	6	6	6	6
guate1165	1	1	1	1	1	1
guate1168	2+	2+	2+	2+	1	1
guate1170	2+	2+	2+	2+	1	1
guate1172	2+	2+	2+	2+	1	1
guate1175	1	1	1	1	1	1

Table A.3. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 31-1 collected in Guatemala, at the seedling stage[f] (continued)

Accession ID	Infection types					
guate1177	1	2+	1	1	2+	1
guate1182	2+	1	1	1	3	2+
guate1191	1	1	1	1	2+	2+
guate1198	1	1	1	1	1	1
guate1200	1	1	1	1	1	1
guate1201	1	1	1	1	1	1
guate1213	1	1	1	1	1	1
guate1214	2+	1	1	1	1	1
guate1216	1	1	1	1	1	1
guate1217	2+	1	1	1	1	1
guate1218	1	2+	1	1	1	1
guate1223	1	1	1	1	1	1
guate1231	2+	2+	2+	2+	2+	2+
guate1232	2+	2+	2+	2+	2+	2+
guate1234	2+	1	1	1	1	1
guate1242	2+	1	1	1	2+	1
guate1244	2+	2+	2+	2+	2+	2+
guate1246	2+	2+	2+	2+	2+	2+
guate1248	3	2+	2+	3	3	3
guate12564PM	2+	3	2+	2+	3	3
guate1257	4	4	4	5	5	4
guate12803PM	2+	1	2+	2+	2+	2+
guate1375	3	2+	2+	3	2+	2+
guate1376	2+	2+	2+	2+	2+	2+
guate1378	1	2+	1	1	1	1
guate13853PMB	2+	2+	2+	2+	2+	2+
guate1386	2+	3	2+	3	2+	2+
guate1387	1	2+	1	1	2+	1
guate1390	1	1	1	1	1	1
guate1394	1	1	1	1	1	1
guate1424	1	1	1	1	1	1
guate1428	1	1	1	1	1	1
guate1429	2+	2+	2+	2+	1	1
guate143	1	3	3	2+	2+	2+
guate1430	1	2+	1	2+	2+	2+
guate147	3	1	1	1	3	3
guate148	2+	1	1	1	1	1

Table A.3. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 31-1 collected in Guatemala, at the seedling stage[f] (continued)

Accession ID	Infection types					
guate1511	5	5	5	5	4	4
guate1514	2+	1	1	1	2+	1
guate1515	2+	2+	2+	2+	1	2+
guate182	5	5	5	5	4	3
guate183	1	1	1	1	2+	1
guate188	2+	1	1	1	2+	1
guate192	1	1	1	1	2+	1
guate200	2+	1	1	1	2+	2+
guate204	3	3	3	3	3	1
guate205	2+	2+	2+	2+	2+	2+
guate218	1	1	1	1	2+	1
guate219	1	2+	2+	1	2+	1
guate223	2+	1	1	1	1	1
guate230	6	5	5	5	6	5
guate233	1	1	1	1	2+	1
guate240	3	2+	3	1	1	1
guate242	2+	1	1	1	1	1
guate251	3	3	3	3	3	3
guate254	2+	2+	2+	2+	2+	2+
guate257	1	1	1	1	2+	2+
guate264	1	1	1	1	1	1
guate266	2+	1	1	1	1	1
guate381	2+	2+	2+	2+	2+	2+
guate385	1	1	1	1	1	1
guate418	2+	2+	1	1	1	2+
guate458	1	1	1	1	2+	1
guate660	1	1	1	1	1	1
guate678	2+	1	1	1	1	1
guate683	1	2+	1	1	3	2+
guate684	2+	1	1	1	1	1
guate884	1	1	1	1	1	1
guate891	2+	2+	2+	2+	2+	2+
guate894	2+	1	2+	1	2+	1
guate898	1	1	1	1	2+	2+
guate899	1	1	1	1	1	1
guate900	6	5	5	5	6	5
guate904	2+	1	2+	1	2+	1

Table A.3. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 31-1 collected in Guatemala, at the seedling stage[f] (continued)

Accession ID	Infection types					
guate908	3	1	3	3	2+	1
guate915	2+	2+	2+	2+	2+	2+
guate920	2+	1	1	1	2+	2+
guate923	2+	3	3	3	2+	2+
guate9282PM	2+	3	3	2+	1	1
guate931	2+	2+	2+	2+	2+	2+
guate932	2+	3	3	2+	3	1
guate933	1	1	1	1	2+	1
guate936	2+	1	1	1	2+	2+
guate941.3	3	1	1	3	1	1
guate944	2+	1	2+	1	2+	1
guate951	2+	2+	2+	2+	2+	2+
guate955	2+	1	1	1	2+	1
guate956	2+	2+	2+	1	1	1
guate962	2+	1	1	1	1	1
guate963	1	1	1	1	1	1
guate966	2+	1	1	1	1	1
guate967	2+	1	1	1	2+	1
guate968	2+	1	1	1	1	1
guate969	5	4	4	4	5	4
guate979	4	4	4	5	5	4
guate984	3	2+	2+	2+	3	2+
guate985	2+	2+	2+	2+	2+	1
guate988	1	1	1	1	3	3
guate997	3	1	5	1	3	3
jardinero	2+	1	1	1	1	2+
LCH86V19	2+	1	1	1	1	1
LCH86V23	1	1	1	1	3	1
LCH86V41	1	2+	1	1	1	1
LCH86V77	1	1	1	1	2+	2+
lineach86B39	2+	2+	2+	2+	3	3
mediamilpa2	1	2+	1	1	2+	2+
Pascueno2	2+	2+	2+	2+	2+	2+
V461634	4	1	1	1	1	1
V5746313	2+	1	1	1	2+	1
V7966	2+	1	1	1	4	4
xenimajuyu	6	4	5	6	6	5

Table A.4. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 20-3 collected in Guatemala, at the seedling stage[g]

Accession ID	Infection types											
AT18419cmcm	1	1	1	1	3	3	3	3	3	1	1	1
AT18430cmcm	3	1	1	1	1	1	1	2+	1	1	MD	1
AT18432cmcm	1	1	1	1	1	1	1	1	1	1	1	1
c1231cmcmcm	1	1	1	1	3	3	3	3	1	3	4	1
c1235cmcmcm	1	1	1	1	1	1	1	1	1	1	1	1
c1831cmcmcm	1	1	1	1	3	1	1	3	1	1	1	1
c274cm6cm9cma5cm	1	1	1	1	1	1	3	3	1	1	1	1
c274cm6cm9cmb5cm	1	1	1	1	1	1	1	1	1	1	1	1
c495cm6cmb3cm2cm	1	3	1	1	3	3	1	3	1	1	1	1
C61cm8cm9cmcm5	1	1	1	1	1	1	1	1	1	3	1	1
c6910cm6cm9cm7cm	2+	1	1	1	1	1	1	1	1	1	1	1
c695cm6cm9cm5cm	1	2+	3	1	2+	2+	2+	2+	1	1	1	1
c696cm4cm3cm3cm	1	1	1	1	1	1	2+	1	1	1	1	1
c697cm4cm9cm3cm	1	1	1	1	2+	2+	2+	2+	1	3	1	1
c698cm4cm12cm6cm	1	1	1	1	1	1	3	1	1	1	3	3
c8110cm4cm2cm2cm	1	1	1	1	1	MD	3	1	1	1	1	1
c813cma4cm6cm6cm	1	3	1	1	1	1	1	2+	3	1	1	1
c815cm6cm9cm6cm	1	1	1	1	1	1	1	1	1	1	1	1
c819cm6cm9cm5cm	1	1	1	1	1	1	3	1	1	1	1	1
c8315cm6cmb5cm4cm	1	1	1	1	1	3	3	2+	1	1	1	1
c934cmb8cm3cm6cm	1	1	1	1	1	1	1	1	3	1	1	3
chajmaquiaj40	2+	2+	1	1	1	1	MD	1	1	1	1	1
frijolboloj	1	1	1	1	MD[h]	1	4	1	1	1	1	1
G6076	1	1	1	1	MD	MD	1	1	3	3	MD	3
guate1000	1	1	1	1	3	3	1	1	1	3	3	3
guate1002	1	1	1	1	3	3	3,4	3	3,4	1	1	1
guate1004	3	3	2+	2+	1	1	1	1	1	1	1	1
guate1005	1	1	1	1	1	1	3	3	1	1	1	1
guate1007	1	1	1	1	1	2+	2+	1	1	3	3	3
guate10071	2+	2+	1	1	1	1	1	1	1	1	1	1
guate1012	1	6	1	1	1	1	1	1	1	1	1	3,4
guate1016	1	1	1	1	MD	3	3	3	1	1	3	1
guate1019	6	6	6	6	4	4	5	4	5	5	5	5
guate1024	1	1	3,4	1	1	1	1	1	1	1	1	1
guate1029	1	1	1	1	1	1	1	1	1	1	1	1
guate1032	2+	2+	2+	2+	1	MD	1	1	1	1	1	1
guate1036	1	1	1	1	1	1	1	1	1	1	3	3

[g]ITs are based on the 0 to 6 scale of Stavely, 1984; Mmbaga et al., 1996; Steadman et al., 2002.
[h]MD: Missing data because seed did not germinate

Table A.4. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 20-3 collected in Guatemala, at the seedling stage[g] (continued)

Accession ID	Infection types											
guate1038	1	1	1	1	1	3	1	1	1	3	1	3
guate1040	1	1	1	1	1	1	1	1	3	1	1	1
guate1042	4	4,5	4	4,5	5	5	5	5	5	5	5	5
guate1045	1	1	1	1	1	1	1	1	1	3	1	1
guate1051	2+	3	1	2+	1	1	1	1	1	1	1	1
guate1053	1	1	1	1	1	3	3	1	3	1	1	1
guate1059	3	1	1	1	1	MD	2+		1	1	1	MD
guate1064	3	3	1	1	1	1	1	3	1	1	1	1
guate1067	4,5	4,5	4,5	4,5	4	5	5	4,3	4	4	4	4
guate1069	1	3	1	1	3	1	1	1	1	1	3	1
guate1071	1	1	1	1	1	1	1	1	3	3	1	1
guate1073	3	1	3	1	1	1	2+	1	1	1	1	1
guate1074	6	6	6	5	5	5	5	4	4	MD	5	5
guate1077	1	1	1	1	1	1	1	1	3	3	1	3
guate1080	1	1	1	1	1	1	1	1	1	1	1	1
guate1081	1	3	1	1	3	3	3	3	1	1	3	1
guate1082	2+	1	1	1	3	1	3	3	3	1	3,4	3
guate1091	1	5	6	4	4	4	4	4	6	6	5	5
guate1100	6	6	6	6	5	4	1	4	6	6	6	6
guate1104	1	1	1	1	1	1	3	3	1	3	1	1
guate1105	1	1	1	1	1	MD	1	1	3	3	1	1
guate1107	4	4	4	5	5	1	5	5	5	5	5	5
guate1117	1	1	1	1	1	1	1	1	1	3	1	1
guate11242	5	5	5	5	4	4	5	4.5	5	5	4	5
guate1127	4	5	1	5	5	5	5	4	6	5	6	6
guate1135	1	1	1	1	1	1	1	1	3	3	3	3
guate1136	4	5	4	4	3	4	4	4	6	6	5	5
guate1137	1	1	1	1	1	1	1	1	1	1	3	1
guate1142	5	5	5	5	5	MD	5	5	5	5	5	5
guate1143	2+	2+	1	1	1	1	1	1	1	1	1	3
guate1148	1	4	6	6	4	5	4	5	5	5	5	5
guate1149	1	1	1	1	3	3	1	1	1	1	1	1
guate1150	1	1	1	1	1	2+	1	1	3	1	1	1
guate1151	1	1	1	1	MD	MD	3	3	3	1	1	3
guate1152	1	1	1	1	1	1	5	1	1	1	3	1
guate1154	1	1	1	1	3	3	1	1	1	1	1	3
guate1164	1	1	1	1	2+	1	1	2+	1	1	1	1

Table A.4. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 20-3 collected in Guatemala, at the seedling stage[g] (continued)

Accession ID	Infection types											
guate1165	1	1	1	1	1	1	1	1	1	1	1	1
guate1168	2+	1	1	2+	1	3	3	1	1	1	1	1
guate1172	1	1	1	1	1	1	1	1	1	1	3	3
guate1173	1	2+	1	2+	MD	1	1	1	1	1	1	1
guate1175	1	1	1	1	1	1	3	1	1	3	3	3
guate1177	1	1	1	1	1	3,4	3	3	3	3	1	1
guate1182	1	1	1	1	1	1	2+	2+	1	4	1	3
guate1191	1	1	1	1	1	1	3,4	1	1	1	1	1
guate1198	1	1	1	1	1	1	2+	2+	1	1	1	3
guate1200	1	1	1	1	3	3	MD	3,4	3	3	3	1
guate1201	1	1	1	1	1	1	2+	1	1	1	1	1
guate1213	2+	1	1	1	1	1	1	1	3	1	1	3
guate1214	3,4	1	1	1	1	1	1	1	1	3	1	1
guate1216	1	1	1	1	1	1	2+	MD	1	1	3	3
guate1217	1	1	3	1	1	1	1	1	1	1	1	1
guate1218	1	1	1	1	1	1	1	1	1	1	1	1
guate1221	1	1	2+	1	2+	2+	1	2+	MD	3	1	1
guate1222	1	1	1	1	3	3	1	1	1	1	1	MD
guate1223	1	1	1	1	1	1	1	1	1	1	1	1
guate1226	1	1	1	3	MD	3	3,4	3,4	1	1	1	1
guate1231	1	1	1	1	1	1	1	1	1	3	3	3
guate1232	1	1	3	3	2+	2+	2+	2+	1	1	1	1
guate1233	1	1	1	1	1	1	3,4	3	3,4	1	3	1
guate1234	1	1	1	1	1	1	1	1	1	1	1	1
guate1236	1	1	1	1	1	1	1	1	1	1	1	1
guate1237	1	1	1	1	1	1	1	1	1	1	1	1
guate1238	1	1	1	2+	1	1	1	1	1	1	1	1
guate1242	1	1	1	1	1	1	1	3	1	1	1	1
guate1244	1	1	1	1	1	MD	1	1	1	1	1	1
guate1245	1	1	1	1	1	1	1	1	1	1	1	1
guate1253	1	3	1	1	1	1	1	1	1	1	1	1
guate12803PM	1	1	1	1	1	1	1	1	1	1	1	1
guate1375	1	1	1	1	1	1	MD	1	1	3	1	1
guate1376	1	1	2+	1	5	1	1	1	3	3	3	4
guate1378	1	1	1	1	1	1	1	1	1	1	1	1
guate138	5	6	5	5	4	4	1	4	6	6	6	6
guate1387	3	3,4	1	1	1	MD	1	1	1	1	1	1

Table A.4. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 20-3 collected in Guatemala, at the seedling stage[g] (continued)

Accession ID	Infection types											
guate1390	1	2+	2+	1	1	3	3		1	1	1	
guate1394	1	1	1	1	1	1	1	1	1	1	1	1
guate1396	1	1	1	1	1	1	1	3	3	1	1	1
guate1422	1	1	1	1	3	MD	3,4	3	1	1	1	1
guate1424	1	1	1	1	1	1	1	5	1	1	1	1
guate1428	1	1	1	1	MD	MD	2+	2+	1	3	3	3
guate1429	1	1	1	1	1	3	1	1	1	1	2+	3
guate1430	1	1	1	1	1	1	1	1	1	1	1	1
guate1434	3,4	1	1	2+	1	1	3	3	1	1	1	1
guate147	1	1	3	1	MD	1	1	3,4	1	3	1	1
guate148	1	1	1	1	MD	3	1	1	1	3	1	3
guate1514	2+	2+	2+	2+	1	1	1	1	3	1	3	1
guate1515	1	1	1	1	1	1	1	3,4	1	1	1	1
guate183	2+	1	1	1	1	1	3	1	1	1	3	1
guate186	2+	1	1	2+	1	1	2+	1	1	1	1	1
guate188	1	1	1	1	1	1	1	1	1	1	1	1
guate192	1	2+	1	1	2+	2+	1	1	1	1	1	1
guate200	1	1	1	1	MD	MD	1	1	1	1	1	1
guate205	1	1	1	1	1	1	1	1	1	1	1	3
guate219	1	1	1	1	MD	MD	2+	2+	1	3	3	1
guate221	1	1	1	1	1	1	1	1	1	1	1	1
guate223	1	1	1	1	1	1	1	1	1	1	1	1
guate230	5,6	5,6	5,6	5,6	5	5	4	4	5	5	5	5
guate233	1	1	1	1	1	1	1	1	1	1	1	1
guate234	5	5	5	5	1	4	4	MD	6	6	6	6
guate237	1	1	1	1	2+	1	1	2+	1	1	1	3
guate240	1	2+	2+	1	1	1	1	1	1	1	1	1
guate242	1	1	1	1	1	1	1	1	1	1	1	1
guate245	1	1	1	1	2+	MD	2+	2+	1	1	1	1
guate251	4,5	4,5	4	4,5	4,3	4	4	4	4	4	4	4
guate254	1	1	1	1	1	1	3	2+	1	3	3	1
guate257	2+	1	2+	1	1	1	1	1	3	1	1	1
guate264	1	1	1	3	1	1	1	2+	1	1	3	3
guate266	1	1	1	1	1	1	1	1	1	1	1	1
guate381	1	1	1	1	3	3	3	3	1	3	1	3
guate385	1	1	1	1	1	1	1	1	1	1	1	1
guate418	1	1	1	1	1	1	1	1	1	1	1	1

Table A.4. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 20-3 collected in Guatemala, at the seedling stage[g] (continued)

Accession ID	Infection types											
guate458	1	1	1	1	1	3	3	3	1	1	1	1
guate578	1	1	1	1	3	1	1	1	1	1	3	1
guate639	3	3,4	3,4	1	1	1	3	1	1	3	3	3
guate660	1	1	1	1	1	1	1	3	1	1	1	3
guate674	1	1	1	1	3	3	3	3	1	1	1	3
guate675	3	1	1	1	MD	MD	1	1	1	1	1	1
guate683	1	1	1	1	1	1	MD	MD	1	1	3	1
guate83	1	1	1	3,4	1	1	1	3	1	1	3	1
guate884	1	1	2+	1	1	1	3	1	1	1	1	1
guate894	3	1	1	3	1	3	1	2+	1	1	1	1
guate896	1	1	1	1	1	1	1	1	3	1	1	1
guate898	1	1	1	1	1	1	MD	MD	3	3	1	1
guate902	1	1	1	1	2+	2+	MD	1	3	1	1	4
guate904	1	1	1	1	1	1	1	1	1	4	1	1
guate905	1	1	1	1	2+	2+	2+	3	1	1	3	1
guate908	4	4	4	5	5	5	5	1	5	4	5	5
guate910	2+	3	1	3	1	1	1	1	1	3	1	1
guate913	1	2+	1	1	1	3	1	1	3	3	3	1
guate915	1	2+	2+	2+	1	2+	1	1	1	1	3	3,4
guate919	1	1	1	1	1	3	1	1	1	1	1	1
guate92	3,4	3	2+	1	1	1	2+	2+	1	1	1	1
guate920	1	1	1	1	1	1	1	1	1	3	1	1
guate927	1	1	1	1	1	1	1	2+	3	3	MD	3
guate9282PM	3	1	1	1	1	3	1	1	1	3	3	1
guate930	1	1	1	1	1	1	3	1	1	3	3,4	1
guate931	1	1	1	1	3	1	1	1	1	1	1	1
guate933	1	1	1	1	1	1	2+	1	1	1	1	1
guate935	1	6	6	6	5	5	5	5	5	5	5	5
guate937	5	5	5	5,6	4	4	5	5	4	4	4	4
guate940	1	1	1	1	MD	1	2+	1	3	3	3	3
guate943	1	3	1	2+	1	1	1	1	1	1	1	1
guate944	1	1	1	1	1	1	1	1	3	1	1	1
guate945	1	1	1	1	1	3	3	1	3	1	1	3,4
guate947	1	1	1	1	1	1	1	1	1	1	1	1
guate955	2+	1	1	1	1	1	1	1	1	1	1	1
guate956	1	1	2+	2+	1	1	3	1	3	1	3	1
guate962	1	1	1	1	1	3	1	1	1	1	1	3

Table A.4. Infection types for climbing bean accessions from Guatemala from *Uromyces appendiculatus* race 20-3 collected in Guatemala, at the seedling stage[g] (continued)

Accession ID	Infection types											
guate966	1	1	1	3	3	3	2+	1	3	3	1	3,4
guate968	1	1	1	1	1	1	1	1	1	1	1	1
guate969	1	1	1	1	1	1	1	1	1	1	1	1
guate971	3	2+	2+	2+	1	1	1	1	1	3	1	1
guate977	1	1	2+	1	1	1	1	1	3	MD	1	1
guate979	1	1	1	1	1	1	1	1	3	1	1	1
guate984	1	4	1	1	1	1	3,4	1	1	1	3	3
guate995	1	1	3	1	1	1	2+	1	3	1	3,4	3
guate997	3	1	3	3	MD	MD	MD	1	1	1	3	1
jardinero	2+	2+	1	1	1	1	1	1	1	1	1	3
LaborOvalle	1	1	1	1	1	3	3	3	1	1	1	1
LCH86V23	1	2+	1	1	2+	2+	2+	2+	1	1	1	1
LCH86V31	1	6	5	6	6	6	6	6	6	5	5	5
LCH86V41	1	1	1	1	3,4	3	3	1	1	3	1	1
LCH86V71	1	3,4	1	1	1	3	3	1	1	1	1	1
LCH86V77	1	3	3	2+	1	1	1	1	1	1	3	1
LCH86V87	1	1	1	1	3	3,4	3	3	1	1	1	1
lineach86B39	1	1	1	1	1	1	1	1	1	1	1	3
mediamilpa1	1	1	1	1	1	1	3	3	1	1	1	1
mediamilpa2	1	1	1	1	1	1	1	1	1	3	1	1
Pascueno2	1	1	1	1	2+	1	1	1	3	3	3	3
V4616324	1	1	1	1	1	1	3	1	1	3	1	1
V461634	1	1	1	1	1	3	3,4	3	3	3	3,4	1
xenimajuyu	1	6	6	6	5	5	3	5	5	5	5	5