UNIVERSITY OF EMBU

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MSc

2023

PREVALENCE AND HOST RESISTANCE TO COMMON BEAN RUST DISEASE IN CENTRAL AND WESTERN KENYA

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PLANT BREEDING AND BIOTECHNOLOGY OF THE UNIVERSITY OF EMBU

AUGUST, 2023

DECLARATION

This thesis is my original work and has not been presented elsewhere for a degree or any other award.

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ACKNOWLEDGEMENT

I would like to record my appreciation to those who were directly or indirectly involved in accomplishing this work. My special gratitude goes to my supervisors: Dr. Felix Rotich and Dr. Esther Arunga. They ensured that I got timely financial, administrative and academic support. I would also like to thank members of Kirkhouse Trust French bean Improvement Project, University of Embu; Sera N. Njau, Grace W. Watare, Nancy N. Munubi, Kevin L. Wanjala and Lilian Munene for their tremendous support during my study period. I share my sincere regards to Kirkhouse Trust for funding this study. I extend my best regards to the University of Embu for supporting my studies through the University scholarship program.

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LIST OF ABBREVIATIONS AND ACRONYMS

BSA Bulk Segregant Analysis

CIAT Centro Internacional de Agricultura Tropical (International Centre for Tropical Agriculture)

DH Doubled Haploids

DNA Deoxyribonucleic acid

dNTPs Deoxynucleotide triphosphates

FAOSTAT Food and Agriculture Organization of the United Nations Statistics

GPS Global Positioning System

HCD Horticultural Crops Directorate

KMD Kenya Meteorological Department

LG Linkage Group

MAS Marker Assisted Breeding

SDS Sodium Dodecyl Sulphate

SSR Simple Sequence Repeats

ABSTRACT

Rust (*Uromyces appendiculatus*) (Pers.:Pers.) Unger., is among the most destructive pathogens constraining the production of common beans (*Phaseolus vulgaris* L.) in Kenya. The study's objectives were to (i) determine the prevalence of bean rust in central and western Kenya; (ii) evaluate common bean genotypes for host resistance to rust disease under field and greenhouse conditions; (iii) characterize the *Ur* gene in a French bean breeding line *MU#13*; and (iv) identify SSR markers linked to the *Ur* gene in a French bean breeding line *MU#13*. A field survey was conducted in 150 farmers' fields in 5 counties in Kenya from September 2020 to January 2021. During the survey, germplasm and rust isolates were collected for screening in the field and under greenhouse conditions. A total of 77 common bean genotypes were subjected to natural infection under field conditions and inoculated with rust races 29-1, 29-3, 61-1, and 63-1 under greenhouse conditions at the University of Embu. The gene pool affiliation of the genotypes was determined through phaseolin protein marker analysis. Further, F_2 populations that were obtained from a cross between a French bean breeding line (*MU#13*) and 13 known *Ur* gene sources were screened using bean rust isolates to characterize the *Ur* gene in *MU#13*. Bulk segregant analysis (BSA) using 14 SSR primers and the DNA obtained from susceptible parent *Amy*, *MU#13* (resistant parent), and the F_2 plants, as well as RILs, was used in the identification of SSRs linked to the *Ur* gene in *MU#13*. The collected rust incidence and severity data were subjected to an analysis of variance using GenStat statistical software. The goodness-of-fit of hypothetical ratios in the F_2 mapping populations was assessed using the *Chi*-square test. The findings revealed a resurgence of bean rust, with Bungoma County displaying the highest disease incidence at 71% and a severity rating of 4. On the other hand, Embu County exhibited the lowest incidence at 38% and a severity rating of 2. The common bean cultivar grown, debri management, management of volunteer plants, use of fungicides, and crop spacing significantly ($p<0.01$) influenced bean rust incidence and severity. However, cropping system, source of seeds, and previous crop did not significantly influence rust prevalence. A significant level of variability was observed among the 77 bean genotypes in their reaction to bean rust. The genotypes *KMR 11* (*Angaza*), *Kat X56*, *UN6-Nakholo*, *UN2-Darkgreen*, *Enclave*, *Manakelly,* and *MU#13* were the most resistant, while *Mexico 222*, *Widusa*, *Mitchelite*, *Amy*, *Samantha*, *Julia*, *GBK-032805,* and *UN4-Yellow small* were the most susceptible to rust. Generally, common bean genotypes of Mesoamerican origin were more resistant to rust as compared to those of Andean origin. The study further revealed a potentially new gene in *MU#13*. The SSR *PV-ctt001*, the only informative marker identified through BSA, was loosely linked to the gene, limiting its utility and therefore the need for the development of robust markers tagging the gene. This study provides baseline information for common bean rust occurrence in the two regions in Kenya and resistance sources to aid in the control of the rust disease.

CHAPTER ONE
INTRODUCTION

1.1 Background of the study

The common bean (*Phaseolus vulgaris* L.) ranks among the most commonly consumed legumes globally, produced for direct human consumption and income generation for rural and urban populations (Myers and Kmiecik, 2017; Petry *et al.*, 2015; Singh *et al.*, 2020). Common bean is a major staple pulse, providing dietary proteins of about 20–25%, vitamins A, B_6, C, and K, folic acid, and other essential minerals (Blair *et al.*, 2013; Chavez-Mendoza and Sanchez, 2017; Petry *et al.*, 2015). These nutrients are useful in supplementing diets that are majorly composed of cereals, roots, and tuber crops (Messina, 2014). Common beans are mainly consumed as boiled dry grains, undried grains, green immature pods (French beans), or green leaves (Blair *et al.*, 2016). French beans are cultivated primarily for their tender pods for processing and fresh consumption (Beebe *et al.*, 2014; Hagerty *et al.,* 2016). In Kenya, French beans are produced mainly for the export market (Otieno *et al*., 2017). Common beans have medicinal value, for instance, in the treatment of diabetes, dysentery, eczema, hiccups, tenesmus, bladder burn, and some cardiovascular problems (Hutchins *et al.*, 2012; Singh *et al.*, 2017).

Despite the significance of common beans as a cash and food crop, its production and marketing potential have recently been declining, and this has been attributed to a combination of abiotic and biotic stresses (FAO, 2022). In Kenya, bean production declined by 7.92% from 887,603 tonnes in 2017 to 817,307 tonnes in 2020 (FAO, 2022). The major biotic factors limiting production include pests and diseases (Mwanauta *et al*., 2015; Rodriguez and Creamer, 2014). Key challenges to common bean production arise from significant diseases such as bean rust (*Uromyces appendiculatus* (Pers.:*Pers.)* Unger.), anthracnose (*Colletotrichum lindemuthianum*), angular leaf spot (*Pseudocercospora griseola*), common bacterial blight (*Xanthomonas axonopodis* pv. *Phaseoli*), bean common mosaic virus (BCMV), and bean common mosaic necrosis virus (BCMNV) (Chen *et al*., 2021; Liebenberg and Pretorius, 2010; Mohammed, 2013; Nay *et al*., 2019; Worrall *et al*., 2015).

Bean rust disease is distributed globally, limiting the cultivation of common beans in regions with humid sub-tropical and tropical climates. Additionally, intermittent but severe outbreaks of bean rust disease can occur in areas with moist temperate conditions (Souza *et al.,* 2013). The rust pathogen causes yield losses of about 65% to 100% in common beans due to premature leaf yellowing, senescence, and total leaf fall (Mersha and Hau, 2011). Crop rotation, pathogen eradication through incorporation of bean plant debri into the soil, use of resistant cultivars, timely planting, and timely spraying of fungicides have been utilized in controlling bean rust (Chhetry and Mangang, 2012). Crop rotation, however, is not effective in controlling bean rust due to the airborne fungal spores produced on infected volunteer, wild, or cultivated plants (Mmbaga *et al.*, 1996). The use of fungicides requires some form of technical knowledge. In addition, the associated high production cost as well as eco-toxicological concerns limit the utility of chemical control (Bon *et al.*, 2014; Okello, 2011). Cultivation of rust-resistant bean cultivars is therefore an efficient, inexpensive, safe, and sustainable alternative that can be accessed by common bean farmers. However, the extensive pathogenic variability of *U. appendiculatus* is a hindrance to the development of durable resistance to rust in common beans (Souza *et al.*, 2013). Consequently, *Ur* genes have consistently been identified and introgressed into commercially grown bean genotypes (Souza *et al.,* 2014).

The first step to successful gene deployment is the identification and selection of resistant genotypes as donors using predominant and virulent physiological pathogen races of bean rust (Souza *et al.*, 2013). Therefore, periodic race typing of bean rust is essential as it informs on virulence diversity, the dynamics of bean rust epidemics, and the breeding of resistant cultivars (Monclova-Santana, 2019; Nyang'au *et al.*, 2016). Furthermore, knowledge of the origin, evolution, and domestication of the *P. vulgaris* species and its relatives can improve the breeding process targeting disease-causing pathogens (Singh and Schwartz, 2010a). Bean breeders employ various sources of germplasm for common bean improvement, including landraces, introductions, registered cultivars (obsolete and current), breeding lines, and landraces (Singh and Schwartz, 2010a). These genetic materials can be screened for resistance by creating artificial infections or biochemical or molecular markers that can be directly or indirectly applied in the selection process

(Hadzhi, 2019; Steadman *et al*., 2002). Therefore, the objectives of this study were to assess the current status of bean rust disease and host plant resistance in western and central Kenya, screen a collection of locally grown common bean genotypes for resistance to rust under both field and greenhouse conditions, and characterize host resistance in a French bean line.

1.2 Statement of the problem

Bean rust disease is a significant constraint to common bean production in Western and Central Kenya. The disease leads to significant yield losses and has economic implications for smallholder farmers. Despite its impact on food security and livelihoods, there is a lack of comprehensive data on the prevalence and factors influencing the occurrence and severity of rust disease in the region. Farmers employ different management strategies to reduce disease pressure, either in combination or alone. The use of chemical control strategy is common for fungal diseases in French beans, whose produce fetches premium prices. However, fungicide use increases production costs, and the chemical residues on the harvested produce can affect the health of consumers, which is a major challenge for the French bean export market. Thus, host resistance remains one of the most sustainable and effective methods to manage this devastating disease. However, it is necessary to comprehensively assess the resistance levels of common bean germplasm to multiple races of rust disease. Unlike dry beans, the majority of French bean cultivars are introductions, mostly bred in Europe, and often have their resistance overcome due to the variability of the bean rust pathogen across the globe. French bean cultivars, *Monel* and *Teresa,* that were previously considered resistant to bean rust are susceptible to some rust pathogen races in Kenya. In this regard, efforts have been put in place to develop locally adapted French bean cultivars resistant to bean rust races in Kenya. However, information on bean rust resistance (*Ur*) genes in most French bean cultivars is scanty. Despite the fact that *Ur* genes have been characterized in dry bean cultivars, their utility in French bean crop improvement is limited due to the reduction of pod quality as a consequence of linkage drag. Furthermore, French bean breeders have been employing conventional methods involving different selection strategies, such as backcross breeding for disease resistance. Such procedures are coupled with a number of challenges, such as reduced

efficiency and lengthy breeding programs. Traits of low heritability are difficult to identify based on phenotypic attributes, which necessitates the need for molecular markers to aid in crop selection.

1.3 Justification

The common bean is one of Kenya's crucial legume crops for human nutrition and income generation (FAO, 2022; HCD, 2020; Ugen *et al*., 2017). In this regard, addressing the abiotic and biotic constraints that affect productivity and marketing prospects for common beans is of particular significance in filling the void of unmet demand in Kenya. One of the most widespread bean diseases in all production regions of the world is rust, which can be managed by host plant resistance (Liebenberg and Pretorius, 2010). An assessment of the prevalence and factors influencing bean rust disease would contribute to our knowledge of the epidemiology and drivers of the disease in Western and Central Kenya. The findings will provide critical insights for the development of targeted and sustainable control strategies to mitigate the impacts of bean rust disease, ultimately enhancing productivity of common bean.

Resistance to rust is generally conferred by *Ur* genes that are monogenically inherited and that may succumb to new strains of the pathogen; therefore, continuous field surveys will enable monitoring of the effectiveness of *Ur* genes in current cultivars. The availability and adoption of disease resistant bean cultivars will increase the area under production, the economic yields and meet the market demand for the produce (Jochua *et al.*, 2008; Odogwu *et al.*, 2017). One of the basic steps to achieving host plant resistance is the evaluation of a number of genotypes in order to select suitable parents for breeding. Evaluation and characterization of *Ur* genes have been achieved using field nurseries, known pathogen races, and molecular markers. Various *Ur* genes have been designated based on their reactions to the bean rust races and the presence of DNA markers tagging these resistance (Hadzhi, 2019; Meziadi *et al.*, 2016; Steadman *et al*., 2002). The development of randomly amplified polymorphic DNA (RAPD) and sequence-characterized amplified regions (SCAR), markers has enabled the tagging of several *Ur*-genes in common beans (Hurtado-Gonzales *et al.*, 2017; Souza *et al.*, 2011). Utilization

of these DNA markers can complement the selection process in the greenhouse during breeding and can help reduce the risk of 'escapes' during the inoculation procedure when virulent races are unavailable. However, the dominant nature of RAPDs and most SCAR markers calls for the utilization of easy-to-use codominant simple sequence repeats (SSR)/microsatellite markers. The SSR markers have not been fully exploited to tag important *Ur* genes, and therefore, it is necessary to identify high-throughput markers linked to the *Ur* genes. Molecular markers tightly associated with resistance to common bean diseases, including rust, can enhance efficiency and hasten breeding programs. Some attributes such as low costs, high efficiency, whole genome coverage, high polymorphism, high reproducibility, ease of detection, robustness, and minimum DNA requirements have enabled the utilization of SSR markers in common bean improvement for disease resistance (Tryphone *et al.*, 2013).

1.4 Hypotheses

i. Common bean rust is not prevalent in Central and Western Kenya.
ii. Common bean genotypes do not vary significantly in their resistance to the bean rust pathogen.
iii. The *Ur* gene in *MU#13* is similar to other characterized genes.
iv. There are no SSR markers linked to the *Ur- MU#13* gene in a French bean line.

1.5 Objectives

1.5.1 General objective

To establish the current status of common bean rust disease in Central and Western Kenya and characterize bean rust resistance in a French bean breeding line.

1.5.2 Specific objectives

i. To determine the prevalence and severity of bean rust disease in Central and Western Kenya.
ii. To evaluate common bean genotypes for host resistance to bean rust disease under field and greenhouse conditions.

iii. To characterize the *Ur* gene in a French bean breeding line, *MU#13*.

iv. To determine the SSR markers linked to *Ur-MU#13* gene in a French bean breeding line.

CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and domestication of common bean

The common bean is an autogamous diploid (2n=2*x*=22 linkage groups) and has approximately 514 Mb total genomic size (Blair *et al*., 2018). It belongs to the domesticated species *Phaseolus vulgaris* in the *Fabaceae* family, classified into dry and snap beans (Myers and Kmiecik, 2017; Wallace *et al*., 2018). The crop originated in America (Ron *et al*., 2015). The gene pools of the Andes and Mesoamerica make up the main common bean domestication centres (Pathania *et al.*, 2014). The gene pools have been suggested considering the geographical distribution of *P. vulgaris* as well as other characteristics based on morphological features and molecular and biochemical analyses (Bitocchi *et al.*, 2012). Analysis of genetic variation within the Andean and Mesoamerican gene pools through various molecular investigations unveiled the population arrangement of *P. vulgaris* (Nkhata *et al*., 2020; Pathania *et al.*, 2014). The identification of domestication origins for wild beans involved the utilization of seed protein phaseolin analysis (Gepts and Bliss, 1988), multiple allozyme markers (Bellucci *et al*., 2014; Koenig and Gepts, 1989), and diverse types of molecular markers (Arunga and Odikara, 2020; Bitocchi *et al*., 2013; Nanni *et al*., 2011). These markers are still helpful in elucidating the genetic diversity of common bean germplasm. For example, the Phaseolin protein molecular marker was utilized by Arunga and Odikara (2020) to designate Kenyan French beans into the two common bean gene pools.

2.2 Production and importance of common beans

Common bean cultivation and utilization take place in numerous countries globally, notably across Africa, South America, Central America, North America, India, Europe, Asia, and Australia (Akibode and Maredia, 2012). India, Myanmar, Brazil, the USA, and China are the leading dry bean producers in the world, with production quantities of 5.46, 3.053, 3.035, 1.495, and 1.282 million tonnes, respectively (FAO, 2022). China, Indonesia, India, Turkey, and France, with 17.964, 0.889, 0.644, 0.547, and 0.394 million tonnes production quantities, respectively, are the leading French bean producers globally. The

world's total common bean production has declined over time against ever-increasing demand due to the increasing world population. In Africa, the leading dry bean and French bean producers include Tanzania, Kenya, Uganda, Ethiopia, Burundi, Rwanda, Egypt, Cameroon, Mozambique, and the Democratic Republic of Congo. Kenya is the leading French bean producer in East Africa (FAO, 2022). There has been a marked decline in the total production of dry and French beans in Kenya, and this may be attributed to biotic and abiotic factors (FAO, 2022; HCD, 2020). *Per capita* common bean consumption in Kenya is relatively low, ranging from 14 kg to as high as 66 kg per year (Katungi *et al.* 2010). By 2050, there will be a requirement for around a 30% increase in common bean production to adequately address the food and dietary requirements of the growing global population (Palomino, 2012).

The common bean is an important crop for dietary nourishment and income generation for most urban and rural households in Kenya (Myers and Kmiecik, 2017; Ugen *et al*., 2017). They are largely grown for the domestic market, with French beans grown mainly for the export market as fresh or canned produce. Beans provide high protein and mineral contents, which are essential in protecting the body against micronutrient deficiencies and malnutrition (Celmeli *et al*., 2018; Hayat *et al.*, 2014; Petry *et al.*, 2015). They have medicinal benefits as they contribute to the treatment of human ailments such as diabetes, cancer, and heart diseases (Hutchins *et al.*, 2012; Singh *et al.*, 2017). Interaction between bean plants and *Rhizobium* bacteria can fix more than 160 kg/ha of atmospheric nitrogen, contributing to soil nitrogen replenishment (Beshir *et al*., 2015).

2.3 Agroecological requirements for common bean production

The common bean is produced as an annual crop in elevated areas between 600 and 2700 m above sea level (Esilaba *et al*., 2021). The crop is mainly cultivated in warm mid-elevation and cool highland areas, although its production is being extended to lowlands (Katungi *et al*., 2009; Ramirez-Cabral *et al*., 2016). Temperatures of 18 to 24 °C are considered optimal for bean production, with a maximum temperature of 30 °C experienced during flowering (Esilaba *et al*., 2021). Elevated temperatures can affect common bean production; for instance, flower abortion, reduced pod formation and

malformation of pods all of which contribute to a decrease in overall yield (Vargas *et al*., 2021). Additionally, the crop is not well-suited to prolonged exposure to near freezing temperatures due to its sensitivity to frost, making it unsuitable in areas at elevations of more than 2700 m above sea level that characteristically receive extremely low temperatures (Esilaba *et al*., 2021; Katungi *et al*., 2009). When cultivated without irrigation, the crop requires well-distributed rainfall of at least 400 to 500 mm throughout its growth period. Although an annual total of 500 to 1500 mm of well-distributed rainfall is considered optimal in common bean production, high amounts of precipitation during flowering cause flower drop and increased disease incidences (Katungi *et al*., 2009).

2.4 Production challenges in common beans

Common bean production in Kenya is adversely affected by both abiotic and biotic factors. Aluminium (Al) and magnesium (Mg) toxicity, nutrient deficiency, and drought are common abiotic stresses constraining common bean production (Rodriguez and Creamer, 2014). Biotic stresses often constraining common bean farmers include fungal, bacterial, and viral pathogens, as well as pathogenic nematodes and pests (Singh, 2013). Diseases often cause severe common bean yield losses and a reduction in the quality of dry and snap bean produce worldwide (Schwartz and Singh, 2013; Singh and Schwartz, 2010a). Fungal diseases include bean rust, anthracnose, and angular leaf spot (Nay *et al*., 2019; Padder *et al.*, 2017; Souza *et al*., 2013). Bean common mosaic necrosis virus (BCMNV), bean golden mosaic virus (BGMV), bean common mosaic virus (BCMV), and cucumber mosaic virus (CMV) viral diseases have all been linked to severe yield losses (Jacquemond, 2012; Worrall *et al.*, 2015). The common bean bacterial diseases include bacterial brown spot (*Pseudomonas syringae* pv. *Syringae),* halo blight (*Pseudomonas syringae* pv. *Phaseolicola*), and common bacterial blight (Belete and Bastas, 2017; Felix-Gastelum *et al*., 2016). Some pests inflicting damage on common beans include bean fly (*Ophiomyia phaseoli*), aphids (Aphis fabae), leaf hoppers (*Empoasca kraemeri*), thrips (*Callothrips fasciatus*), pod borers (*Maruta vitrata*), cutworms (*Striacosta albicosta),* and red spider mites (*Tetranuchus spp.*) (Mwanauta *et al.*, 2015; Singh and Schwartz, 2010b). Root-knot nematodes (*Meloidogyne incognita* and *Meloidogyne javanica*) constrain common bean production, especially in sandy soils (Adomako *et al*., 2022).

2.5 Bean rust disease

2.5.1 Aetiology, symptoms and signs of bean rust infection

The rust pathogen, a member of the phylum Basidiomycota, cannot survive without its common bean host and other alternative hosts (Gautam *et al.*, 2022; Souza *et al.*, 2008). This pathogen is also macrocyclic, producing diverse types of spores such as urediniospores, teliospores, basidiospores, pycnyospores, and aeciospores (Souza *et al.*, 2013). The basidiospores germinate and penetrate the leaf surface through natural plant openings such as stomata, necessitated by a layer of free water (Boddy, 2016). Eight to ten days after infection, a uredium with uredospores is produced (McMillan *et al.*, 2003). Uredospores are thin-walled, spiny, unicellular, globoid to ellipsoid in shape, and light brown in colour (Gautam *et al.*, 2022; Liebenberg and Pretorius, 2010). The uredospores can germinate and develop an infectious hypha that infects susceptible common bean plants, forming a new uredium in which new uredospores are produced (Liebenberg and Pretorius, 2010). Re-infection of beans occurs due to the production of several generations of uredospores, which are a source of inoculum (Souza *et al*., 2013). The latent period takes 7 to 15 days for symptom and sign development after infection, depending on temperature and humidity factors (Liebenberg and Pretorius, 2010).Bean rust epidemics can lead to a reduction in the overall leaf area, diminished leaf size due to leaf shrinking, and an accelerated process of leaf defoliation. Symptoms first appear on upper and lower leaf surfaces as circular chlorotic or white spots forming reddish-brown pustules (Liebenberg and Pretorius, 2010; Seebold, 2014; Souza *et al*., 2008). Pustules may also occur on petioles or on pods. The tissue surrounding large or small single groups of uredia usually turns yellow. Premature plant defoliation, stunted growth, and low yields may occur due to severe infection (Souza *et al*., 2013).

2.5.2 Pathogen variability and race typing

Plant pathogens may exhibit pathogenic variability, and this is prevalent in *U. appendiculatus* (Acevedo *et al.*, 2013). New bean rust races emerge due to sexual mechanisms like genetic recombination during sexual reproduction, exchange of DNA in somatic cells, heterokaryosis, mutagenesis, gene flow, and extrachromosomal variation

(Araya *et al.*, 2004). The pathogenic virulence diversity rust is extensive, with more than 300 races reported globally (Araya *et al*., 2004). This substantial diversity in pathogenicity underscores the adaptability of the fungus to various host cultivars, thereby posing considerable challenges for disease management (Souza *et al*., 2013). This phenomenon highlights the ongoing necessity for vigilant monitoring, research, and breeding efforts to develop bean varieties with durable resistance against the constantly evolving and diverse rust fungus races (Souza *et al*., 2013). Nine bean rust races were identified in Kenya by Arunga *et al.* (2012), 4 of which were verified by Nyang'au *et al.* (2016).

Race typing of *U. appendiculatus* isolates and understanding of its virulence diversity is basic to subsequent knowledge of bean rust dynamics and guides the breeding of resistant cultivars. This necessitates the identification of *U. appendiculatus* races that can be used in monitoring *Ur* genes and their introgression in breeding programs (Arunga *et al.*, 2012; Acevedo *et al.*, 2013; Souza *et al.*, 2007a). A proper classification scheme for the bean rust pathogen (*U. appendiculatus*) into physiological races was effectively established, including a collection of differential cultivars and a binary naming system, as outlined by Steadman *et al*. (2002). The differential series consists of 6 Andean and 6 Mesoamerican bean cultivars with known *Ur* genes (Table 2.1). Evaluation of the reaction to bean rust pure isolates in race characterization considers a 1-6 disease rating scale divided into 2 reaction degrees: resistant and susceptible (Souza *et al.*, 2013).

Table 2.1. Twelve common bean differential cultivars for characterization of bean rust into physiological races.

Binary value	**Genotype**	**Resistance loci**	**Gene pool**
1	*Early Gallatin*	*Ur-4*	Andean
2	*Redlands Pioneer*	*Ur-13*	Andean
4	*Montcalm*	Unnamed	Andean
8	[1]*PC-50*	*Ur-9, Ur-12*	Andean
16	*Golden Gate Wax*	*Ur-6*	Andean
32	*PI 260418*	Unnamed	Andean

Binary value	Genotype	Resistance loci	Gene pool
1	*Great Northern 1140*	*Ur-7*	Mesoamerican
2	*Aurora*	*Ur-3*	Mesoamerican
4	*Mexico 309*	*Ur-5*	Mesoamerican
8	*Mexico 235*	*Ur-3*$^{+}$	Mesoamerican
16	[2]*CNC*	Unnamed	Mesoamerican
32	*PI 181996*	*Ur-11*	Mesoamerican

[1]*Pompadour Checa-50*, [2]*Compuesto Negro Chimaltenango*. Source: (Steadman *et al.*, 2002).

2.5.3 Common bean rust host-pathogen coevolution

Fungal pathogens such as *Uromyces appendiculatus*, *Colletotrichum lindemuthianum,* and *Phaeisoriopsis griseola* have co-evolved with their bean hosts, driven by the ongoing process of adapting to alterations in bean morphology, biochemistry, and ecological conditions (Acevedo, 2008; Araya *et al.*, 2004; Pastor-Corrales, 2004). The rust pathogen can be grouped into the Andean and Mesoamerican races which reflect the diversity of its bean host (Pastor-Corrales, 2004). Araya *et al*. (2004) observed a distinct separation of 90 pathogen populations following a similar pattern to that of the bean host. This indicates a parallel evolutionary process within the bean rust pathosystem. This antagonistic coevolution enables the coexistence of individual components in dynamic equilibrium with each other. Paralleled bean host reactions relative to the bean rust pathogen suggest host-pathogen coevolution, which explains the occurrence of *U. appendiculatus* as a biotroph comprising different pathotypes (Araya *et al*., 2004).

2.5.4 Factors influencing rust prevalence in common bean

The spread of bean rust spores is primarily through wind, birds, insects, water, contaminated farm tools, and infected crop debri, leading to severe epidemics (Liebenberg and Pretorius, 2010). Bean rust disease prevalence can be attributed to a convergence of multiple and variable factors such as the altitude of the growing region, agronomic practices, and the prevailing environmental conditions. (Helfer, 2014; Lin, 2011).

Temperature, relative humidity (RH), leaf surface moisture, and host factors contribute to the prevalence of *U. appendiculatus* (Helfer, 2014; Singh, 2018). A temperature range of 17 to 21 °C favours *U. appendiculatus* germination and infection (Liebenberg and Pretorius, 2010). Singh and Gupta (2019) observed a decline in bean rust pustules on infected bean leaves at a temperature range of 21–30 ºC with no disease observed at 35 ºC. In addition, a high level of bean rust severity was observed as relative humidity increased from 70% to 100%, as long as the leaf had free surface moisture for about 6–12 hours. However, lower humidity levels and high temperatures resulted in reduced infection levels. Cropping systems have also been reported to influence the distribution of bean rust disease (Lin, 2011). According to Liebenberg and Pretorius (2010) and Sanyang *et al*. (2019), various cultural practices influence bean rust infection and dissemination. Farming practices such as monocropping, use of cultivar mixtures, sowing time, intercropping, and the selection of common bean cultivars for planting all have the potential to influence the degree of bean rust dissemination and infection. High rust disease severity in monocropping systems may be due to re-infection with inoculum from crop debri or volunteer plants. Dispersal and retention of rust spores, as well as their infection efficiency, may be influenced by the crop diversity level in an intercropping system (Odogwu *et al*., 2016). High rust prevalence during the mid- and late-season associated with high relative humidity and moderate temperatures that influence the dynamics of bean rust spread and infection necessitates the recommendation for early planting to ‘escape’ the disease (Ronner and Giller, 2013). Production of susceptible cultivars may result in high incidences and severity of rust under ideal environmental conditions and in the presence of a virulent rust pathogen.

2.5.5 Control of bean rust disease

The rust disease control relies on integrated disease management that utilizes some cultural practices, fungicides, and host plant resistance (Lin, 2011; Odogwu *et al*., 2014; Paparu *et al*., 2014; Singh *et al*., 2022). Host plant resistance is essential in attaining the principles of integrated bean rust management (Hadzhi, 2019). Crop rotation, soil incorporation of common bean plant debri bearing overwintering bean rust spores, timely planting, use of resistant common bean cultivars, and timely application of chemical fungicides have been

utilized to minimize bean rust infection (Buruchara *et al.*, 2010; Souza *et al.*, 2008). The prevalence of bean rust disease may be reduced due to low plant density. Adjustments in planting dates in particular cultivation areas may substantially reduce bean rust infection. Such adjustments minimize exposure to optimal temperatures and relative humidity for bean rust infection during the vegetative and flowering growth stages (Moore *et al*., 2020). However, the infection rate of rust and the spread of spores may increase due to favourable environmental conditions. Additionally, the dynamics of the cropping and disease control systems may influence adjustments in agronomic practices with the goal of reducing inoculum (Mmbaga *et al.*, 1996).

Biological control suppresses or inhibits bean rust disease development. When introduced before inoculation with *U. appendiculatus* uredospores in the greenhouse, some fungal and bacterial bioagents are antagonists against bean rust disease (Ismail *et al*., 2019). Assante *et al.* (2004) evaluated the interactions between *U. appendiculatus* and the mycoparasite *Cladosporium tenuissimum* and observed that uredospore germination decreased, possibly due to antibiosis mechanisms. Abo-Elyousr *et al*. (2021) attributed rust reduction to the mycoparasitic activity of *Pseudomonas putida* and direct suppression of *U. appendiculatus* spore germination under greenhouse conditions.

Chemical control involves the use of fungicides, for instance, protectants such as chlorothalonil ($C_8Cl_4N_2$) and dithiocarbamates (CH_2NS_2-), as well as systemic fungicides such as triazoles ($C_2H_3N_3$) and carboxins ($C_{12}H_{13}NO_2S$) (Liebenberg and Pretorius, 2010). Commercial fungicides reduce bean rust severity with over 90% efficacy levels (Devi *et al.,* 2020; Sharma *et al.,* 2018). However, since fungicides are expensive, they are often used in subsistence and small-scale bean production systems in Africa (Paparu et al., 2014). Bean rust infection that may occur before the flowering stage results in severe common bean yield losses and, thus, the need for timely fungicide application during early plant development stages for effective control of the disease (Sharma *et al.,* 2018). Continuous fungicide application may result in the development of fungicide-resistant rust pathotypes, rendering chemical control ineffective (Oliver, 2014).

The use of genetic resistance is certainly a major strategy in the integrated control of bean rust disease. Common bean rust disease can be effectively controlled using genetic resistance (Liebenberg and Pretorius, 2010; Souza *et al*., 2013). However, the breeding of rust-resistant bean cultivars is constrained by the great variability of *U. appendiculatus* pathogenicity, thus leading to resistance being overcome in released cultivars. Therefore, combining different race-specific genes into a common bean background is an ideal strategy for wide and sustainable resistance (Hadzhi, 2019; Souza *et al.*, 2008; Ragagnin *et al.*, 2009).

2.5.6 Genetics and breeding for resistance to bean rust

The resistance of common beans to the rust pathogen is determined by distinct interactions between the host and the pathogen. These interactions primarily adhere to the gene-for-gene model, as elucidated in studies by Christ and Groth (1982), Sayler *et al*. (1995), and Souza *et al*. (2008). Characterization of novel *Ur* genes from both gene pools is essential in breeding common beans with the aim of attaining bean rust resistance, as this broadens the scope for gene pyramiding (Pastor-Corrales *et al.*, 2008; Souza *et al*., 2011).

Host plant resistance against bean rust disease is generally conditioned by a single major dominant gene (Souza *et al.*, 2007b; Souza *et al*., 2011). Additionally, a single recessive gene (Zaiter *et al.*, 1989), two epistatic genes (Finke *et al.*, 1986), two complementary dominant genes (Grafton *et al.*, 1985), or polygenes with minor effects (Edington *et al.*, 1994) may also control resistance to bean rust disease. Ten major dominant *Ur* genes, including *Ur-3*, *Ur-5*, *Ur-7*, *Ur-11,* and *Ur-14* from the Mesoamerican gene pool and *Ur-4*, *Ur-6*, *Ur-9*, *Ur-12*, and *Ur-13* from the Andean gene pool, have been identified and documented (Souza *et al.*, 2013). Furthermore, some cultivars, including *Montcalm* (McClean and Myers, 1990), *CNC* (Rasmussen *et al.*, 2002), *BAC6* (Jung *et al.*, 1996), *PI 260418* (Pastor-Corrales *et al.*, 2008), and *Dorado* (Miklas *et al.*, 2000), have unnamed *Ur* genes that have been identified. In Kenya, the Mesoamerican genes *Ur-5*, *Ur-11*, and *Ur-CNC* are the most effective against *U. appendiculatus* races (Arunga *et al.* 2012).

2.5.7 Molecular markers linked to bean rust resistance

Genomic regions controlling inheritance and expression of genetic traits can be located using molecular genetic maps and quantitative trait loci (QTL) mapping techniques (Gonzalez *et al*., 2017). The localization of genomic regions allows for the architecture of significant genetic traits like resistance to diseases (Ebrahim and Zeleke, 2020). It is necessary that genetic maps be saturated with molecular markers tagging specific gene loci for use in common bean improvement (Perez-de-Castro *et al*., 2012). The availability of molecular markers would be helpful in comprehending the mode of action and the effects of the loci controlling important traits in common bean improvement programs using marker-assisted selection (MAS) (Assefa *et al*., 2019).

The identification of genetic loci controlling disease resistance in common beans can be achieved through genetic linkage analysis, which is based on the genetic recombination principle (Ott *et al*., 2015). Most molecular markers utilized in breeding programs were identified and developed through linkage mapping. A bi-parental population is generated for identification of the genomic region that segregates with the trait (Osorno and McClean, 2014). The segregating F_2, F_3, and backcross populations are commonly used in the development of molecular markers. Additionally, double haploid (DH) and recombinant inbred lines (RILs) populations can be utilized in developing molecular markers. RILs and DH populations, being homozygous, can be maintained for continuous use over a long period of time (Collard and Mackill, 2008).

Bean rust resistance traits can be introgressed into common bean cultivars using high-throughput markers that are associated with the underlying genes (Tryphone *et al.*, 2013). Classical breeding of common bean is presently combined with MAS to hasten and increase selection efficiency (Mondo *et al*., 2019; Njuguna, 2014). Basic to the utilization of MAS, DNA markers are frequently developed through linkage analysis to identify markers co-segregating with traits of interest (Assefa *et al*., 2019). MAS has been used in early generation selection, thus accelerating varietal development, gene pyramiding, parental selection, germplasm fingerprinting, and phylogenetic analysis (Kumar *et al.*, 2011). A good molecular marker should be high-throughput in tagging the loci of interest,

reliable and stable in the selected breeding lines, easy to use, and cost-effective for successful MAS (Tryphone *et al.*, 2013). Therefore, the efficacy of the molecular marker in identifying progeny phenotypes must be validated.

In MAS, RAPD, SCAR, SSR, and single nucleotide polymorphism (SNP) markers have extensively been used (Kumar *et al.*, 2011). Bean rust race-specific *Ur* genes have been mapped, co-segregating with specific molecular markers tagging the resistance loci (Souza *et al*., 2013). RAPD markers (*OPF10* tagging the *Ur-5* in cultivar *B-190* and *OPX11* tagging the *Ur-14* in *Ouro Negro*) and SCAR markers (*S119* tagging the Mexico 309 gene *Ur-5*, *SAE19* tagging the *Ur-11* in *BelmiDak,* and *SBA08* tagging the *Ur-14* in *Ouro Negro*) have been utilized in pyramiding these genes into the background of cultivar *Ruda* (Souza *et al.*, 2014).

Certain RAPD and SCAR molecular markers associated with major *Ur* genes in common beans can produce inaccurate positive and negative results (Nemchinova and Stavely, 1998; Valentini *et al*., 2017a). This phenomenon is attributed to the loose linkage between certain molecular markers and the *Ur* gene, as in the case of marker $OA14_{1100}$ linked to the *Ur-4* gene in all Andean bean genotypes (Valentini *et al*., 2017a). In addition, the close proximity of genes such as *Ur-3* and *Ur-11*, as well as the lack of a reference genome for common beans, hindered the development of precise and high-throughput molecular markers, contributing to the inefficiencies of SCAR and RAPD markers (Nemchinova and Stavely, 1998). The advancement of high-throughput genotyping technologies and the publication of the reference genome for common beans have hastened the identification of markers for MAS in bean improvement (Hao *et al*., 2020; Kelly and Bornowski, 2018).

SSR markers are advantageous as codominant markers in detecting homozygote and heterozygote genotypes more efficiently with a high percentage of polymorphism. Odogwu *et al*. (2017) identified three markers (*BARC_PV_SSR04725, bean_ssr_2892,* and *bean_ssr_0778*) associated with bean rust resistance. In addition, Shin *et al*. (2014) observed that genotype *PI 310762* harbours the *Ur-15* gene, which co-segregates with the *bean_ssr_0778* SSR marker. Valentini *et al*. (2017a) developed SSR markers that are

linked to the *Ur-4*, enabling the detection of common bean genotypes belonging to the two gene pools. Furthermore, the SSR markers *BARC_PV_SSR14078* and *BARC_PV_SSR04582* linked to *Ur-5* at 0.0 cM were identified (Valentini *et al*., 2017a).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Prevalence, severity and factors influencing occurrence of bean rust

3.1.1 Area of study

The study was conducted during the second cropping season (September 2020–January 2021) in five counties located in six major agro-ecological zones in Kenya. The zones are located in the warm lower humid midlands (LM1, LM2, LM3, and LM4), cool upper midlands (UM1), and lower highlands (LH1) of western and central Kenya. The counties represent major bean production areas in Kenya. The sampled fields were at an altitudinal range of between 1027 and 2429 m above sea level. Overall, a total of 150 fields were targeted in central Kenya (Embu and Kirinyaga Counties) and western Kenya (Uasin-Gishu, Bungoma, and Kakamega Counties).

3.1.2 Sample units

Purposive and simple random sampling methods based on intensity of bean production, crop stage, and geographical and ecological location were used, targeting 30 fields in each county. At a range of 5 to 10 km on the main roadways, randomly selected fields with bean plants at the flowering (R6) and pod formation (R7) growth stages were identified (Odogwu *et al*., 2016). The approximate size of each selected field was determined, and the developmental stage of the crops was confirmed. Equidistant steps following an inverted "V" outline were made at the edge of the field, from which 20 sample plants were selected. The sample unit was selected as the plant closest to the right foot at each predetermined pace. Evaluations were done on a cultivar found in a sample field. Whenever necessary, the number of single plants randomly selected for a field was adjusted to match crop distribution and the size of the field. Small-holder farmers with an average farm size of below 1.2 hectares were targeted.

3.1.3 Data collection and analysis

Bean rust disease assessment was done on 20 plants of the same cultivar randomly sampled within each field. Rust incidence was established as the proportion of symptomatic plants among the twenty plants sampled in the sample field. Bean rust disease severity was assessed using a modified scale of 1–9 (Van-Schoonhoven and Pastor-Corrales, 1991) (Table 3.1). Disease scores of 1–3 was categorized as resistant, 4–6 as intermediate, and 7–9 as susceptible. In addition, GPS measurements of latitude, longitude, and altitude were gathered from each field using the GPS Map Camera Lite application (version 1.0.7). The evaluation of factors affecting disease prevalence was based on farmers' responses. These factors included the cropping system (intercrop or sole crop), common bean cultivar under production, seed source (farmer-saved seeds, local market or certified seed from merchants), previous crop planted, and other cultural practices (fungicide use, crop debri management, crop spacing, and management of volunteer plants).

Table 3.1. Modified bean rust severity rating scale (1–9).

Reaction rating	Description	Category
1	No visible pustule	Resistant
2	Pustules covering 1% of leaf area	Resistant
3	Few pustules covering 2% of leaf area	Resistant
4	Intermediate pustules covering 5% of leaf area	Intermediate
5	Small pustules covering 8% of leaf area	Intermediate
6	Pustules covering 10% of leaf area, often surrounded with chlorotic halos	Intermediate
7	Large pustules covering 15% of leaf area, surrounded with chlorotic halos	Susceptible
8	Large pustules covering 20% of leaf area surrounded with chlorotic halos	Susceptible
9	Very large pustules covering more than 25% of leaf area, often with defoliation	Susceptible

Source: (Van-Schoonhoven and Pastor-Corrales, 1991).

A rust disease severity map was generated using the ArcGIS 10.4 software package by the Environmental Systems Research Institute (ESRI) using field coordinates for each farm. Using the GenStat Discovery Edition 14.0 statistical software, incidence and severity data were subjected to a one-way analysis of variance (ANOVA) (Payne *et al*., 2011). In this analysis, location (counties), cropping system, cultivar, source of seeds, debri management, previous crop, fungicide use, and management of volunteer plants were considered fixed factors. Tukey's studentized range test was used to compare multiple mean values for incidence and severity of rust at a significance level of $\alpha = 0.05$. At harvest maturity, seeds were collected from the visited farms for the purpose of screening for resistance to rust. Infected common bean leaves were collected from each sampled field for subsequent single-spore isolation and multiplication for use in further screening for rust resistance.

3.2 Germplasm screening for resistance to bean rust

3.2.1 Experimental site

The genotypes were phenotyped for host resistance to bean rust under field and greenhouse conditions at the University of Embu research station (0° 30′ S and 37° 27′ E). The area experiences a temperature ranging between 10 °C and a 25 °C with the mean temperature of 19 °C and an average annual rainfall of 1120 mm (Kisaka *et al.* 2015). The well-aerated and deep *Humic nitisol* soils formed from basic volcanic rocks are inherent in this field (FAO, 2011).

3.2.2 Germplasm

The germplasm panel was made up of 77 common bean genotypes obtained from farmers in the surveyed counties, the Kenya Agricultural and Livestock Research Organization (KALRO) seed unit, and the French bean improvement program at the University of Embu. They consisted of 14 landraces, 20 French bean cultivars, 28 dry bean cultivars, 3 breeding lines, and 12 bean rust differential cultivars. Codes UN1 to UN8 were used as identities for the 8 landraces that were unnamed. Based on information on their known resistance genes and gene pools, the susceptible cultivar *GLP X92* and the 12 differential genotypes were utilized as checks (Souza *et al.* 2013).

3.2.3 Field experimental procedure

The field experiment was conducted from May to July 2021 during the long-rain cropping season. A randomized complete block design with three replicates was used in this experiment. The experimental field had previously been used for screening common bean breeding materials for resistance to multiple diseases. To attain a moderately fine tilth for sowing, the experimental plot was pulverized using a hoe. Each entry had 21 seeds sown in a 2 m-long row with 30 cm and 10 cm inter- and intra-row spacing, respectively. To amplify disease pressure, a susceptible cultivar, *GLP X92*, was planted as a spreader row after every five entries at a comparatively high plant density. During sowing, 200 Kg/ha of di-ammonium phosphate (DAP) fertilizer was applied. After sowing, standard agronomic practices, including irrigation, weeding, pest control, and topdressing, were carried out by two split applications of 50 kg/ha of calcium ammonium nitrate (CAN) fertilizer, the first at the V4 growth stage and the second at the flowering (R6) stage. Disease inoculation was based on natural infection. Bean rust disease severity was recorded as described in Section 3.1.3.

3.2.4 Screening for resistance under greenhouse conditions

3.2.4.1 Isolation and characterization of common bean rust isolates

Ten viable bean rust isolates obtained during the survey were purified through single-spore isolation (Souza *et al*., 2013). An individual unopened pustule including a 25 mm^2 of surrounding leaf tissue for each isolate was separately cut, the spores collected, and transferred to susceptible seedlings of cultivar *GLP X92*. The single-pustules were collected and multiplied on the susceptible cultivar for 3 consecutive cycles and then characterized into physiological races using a set of 12 differentials, as described by Steadman *et al*. (2002). The bean germplasm was tested for resistance to rust disease using four pure races and one set of mixed races.

3.2.4.2 Rust inoculation and disease evaluation

Ten seeds from each entry were sown on seedling trays filled with sterile soil and set out in a three-replicate randomized complete block design (RCBD). The disease inoculum was

sprayed on 8–10-day-old plants that had reached the V2 growth stage with approximately about ⅔ enlarged primary leaves. Inoculation was done by manually spraying viable *U. appendiculatus* urediospores in distilled water at a concentration of 2.0×10^4 urediospores per ml, which was adjusted using a haemacytometer. Inoculated plants were subsequently moved to a screen house maintained at 20±1 °C and a relative humidity of >95% under a 12-hour light/dark schedule for 48 hours before being shifted to a greenhouse at 20±5 °C for 14 days. The severity of bean rust was assessed using a 1–6 disease severity scale (Steadman *et al*., 2002) (Table 3.2). Cultivars with reaction values of 1–3 were categorized as resistant and 4–6 as susceptible. The most prevalent infection grade was chosen in case of several infection grades.

Table 3.2. Virulence reaction scale of 1–6 used for scoring common bean rust under greenhouse conditions.

Reaction rating	**Description**	**Category**
1	No apparent pustule	Resistant
2	Necrotic spots with no spores	Resistant
3	Non-spore-producing tiny pustules having a diameter of less than 300 µm	Resistant
4	Spore-producing pustules having a diameter of between 300 µm and 500 µm commonly surrounded with chlorotic halos	Susceptible
5	Spore-producing pustules having a diameter of between 500 µm and 800 µm commonly surrounded with chlorotic halos	Susceptible
6	Spore-producing pustules having a diameter >800 µm in diameter commonly surrounded with chlorotic halos	Susceptible

Source: (Steadman *et al*., 2002).

3.2.5 DNA analysis for common bean gene pool affiliations

3.2.5.1 DNA extraction

Young leaf tissue samples were collected from each of the 77 common bean genotypes, and DNA was extracted using a modified Mahuku (2004) protocol. 150 mg of leaf sample was placed in a mortar containing acid-washed sand and macerated for 2 minutes using a pestle. The powder was transferred into a 1.5-mL Eppendorf (microcentrifuge) tube. 500µL of TES extraction buffer (0.2 M Tris-HCl [pH 8], 10mM EDTA [pH 8], 0.5 M NaCl, 1% SDS) was added into the microcentrifuge tube. The samples were then vortexed for 30 seconds to thoroughly mix, and the tubes were placed in a water bath at 65 °C for 30 minutes. One-half the volume (250 L) of 7.5M Ammonium acetate was added into the tubes, mixed, and incubated at -5 °C in a refrigerator for 10 minutes. The samples were then centrifuged at 15,000 rpm for 15 minutes. The supernatant was then transferred to a new microcentrifuge tube, and an equal volume (500µL) of ice-cold isopropanol was added. The samples were then incubated for 2 hours at -20°C. Thereafter, the samples were centrifuged at 15,000 rpm for 10 minutes to pellet the DNA. The supernatant was decanted, and the DNA pellet was washed with 800µL of cold 70% ethanol. The alcohol was drained off, and the pellets were air-dried on a clean, sterile paper towel for 15 minutes. The DNA was eluted from the pellet by adding 250µL 1xTE buffer (10mM Tris-HCl [pH 8], 1mM EDTA) and centrifuging each time for 5 minutes at 15,000 rpm to avoid collecting pelleted polysaccharides. The RNA was removed by adding 2µL of RNAse (10mg/mL) to the DNA solution and incubating the sample at 37 °C for 60 minutes. The DNA was recovered and air-dried as described above. The DNA was eluted in 50µL and stored at 4°C.

3.2.5.2 DNA amplification

The Phaseolin seed protein SCAR marker (forward- 5′-ACGATATTCTAGAGGCCTCC-3′; reverse- 5′-GCTCAGTTCCTCAATCTGTTC-3′) was used in PCR amplification (Kami *et al.* 1995). A reaction volume of 10 µl was prepared in FrameStar® Break-A-Way PCR tubes. This volume contained 1X Dream *Taq* buffer with 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of both forward and reverse primers, 0.1 U of *Taq* Polymerase (from Thermo Fisher Scientific), and 5 ηg/µl of genomic DNA. The process began with an initial denaturation

phase at 94 °C lasting for 3 minutes. This was ensued by 35 cycles of the subsequent three stages: denaturation at 94 °C for 10 seconds, annealing at 55 °C for 40 seconds, and extension at 72 °C for 2 minutes. Finally, a last extension phase at 72 °C was held for 5 minutes. The analysis of amplification products was done through gel electrophoresis, according to Green and Sambrook (2019). To each PCR product, 2 µl of 6x DNA loading dye (NEB) was added. A 50-bp DNA ladder (https://www.thermofisher.com/order/catalog/product/10416014) was loaded in the first well to the extreme left and right of the gel, and then PCR product contents were loaded in subsequent wells on a 1.5% agarose gel pre-stained with 5 µM Ethidium bromide ($C_{21}H_{20}BrN_3$) in 1x Sodium borate buffer ($Na_2[B_4O_5(OH)_4]\cdot 8H_2O$). The amplicons were separated at 100 volts for 3 hours. The gel was then viewed using the UVP® Benchtop Variable Transilluminator (https://www.uvp.com/products/lab-equipment/uvp-uv-white-blue-light-transilluminators/) and photographed using a Canon® camera. The gel was scored for the presence of either two or three DNA fragments of different sizes.

3.3 Characterization of bean rust resistance gene in *MU#13*

3.3.1 Genotypes

The common bean seeds for genotypes *MU#13* (a French bean breeding line resistant to rust) and 13 *Ur* source cultivars (Table 3.3) were provided by the French bean Improvement Program, University of Embu (provided by Dr. Edith Esther Arunga). The *MU#13* is a breeding line that was selected in 2008 with the aim of improving French bean cultivars for rust resistance (Arunga, 2012).

3.3.2 Development of mapping populations

In the greenhouse, seeds were planted in planting pots with a 3:1:1 mix of top soil, sand, and manure. Crosses were performed in the greenhouse using a modified method proposed by Bliss (1980). This method involved hand emasculation of the flower buds of the female parent using tweezers and the application of pollen collected from the male parent by gently rubbing and then hooking the stigmas of both parents. *MU#13* was used as the male parent in all the crosses except in the cross with *PI 181996* due to difficulties in crossing based on its morphology. Cultivar *PI 260418* could not be successfully crossed owing to

difficulties in flowering, podding, and seed setting. All F_1 plants were grown in the greenhouse and analyzed phenotypically, based on hypocotyl pigmentation, flower colour, seed colour, seed size, and seed shape, to identify true hybrids. The F_1 plants were selfed to generate corresponding F_2 populations, as outlined in Figure 3.1.

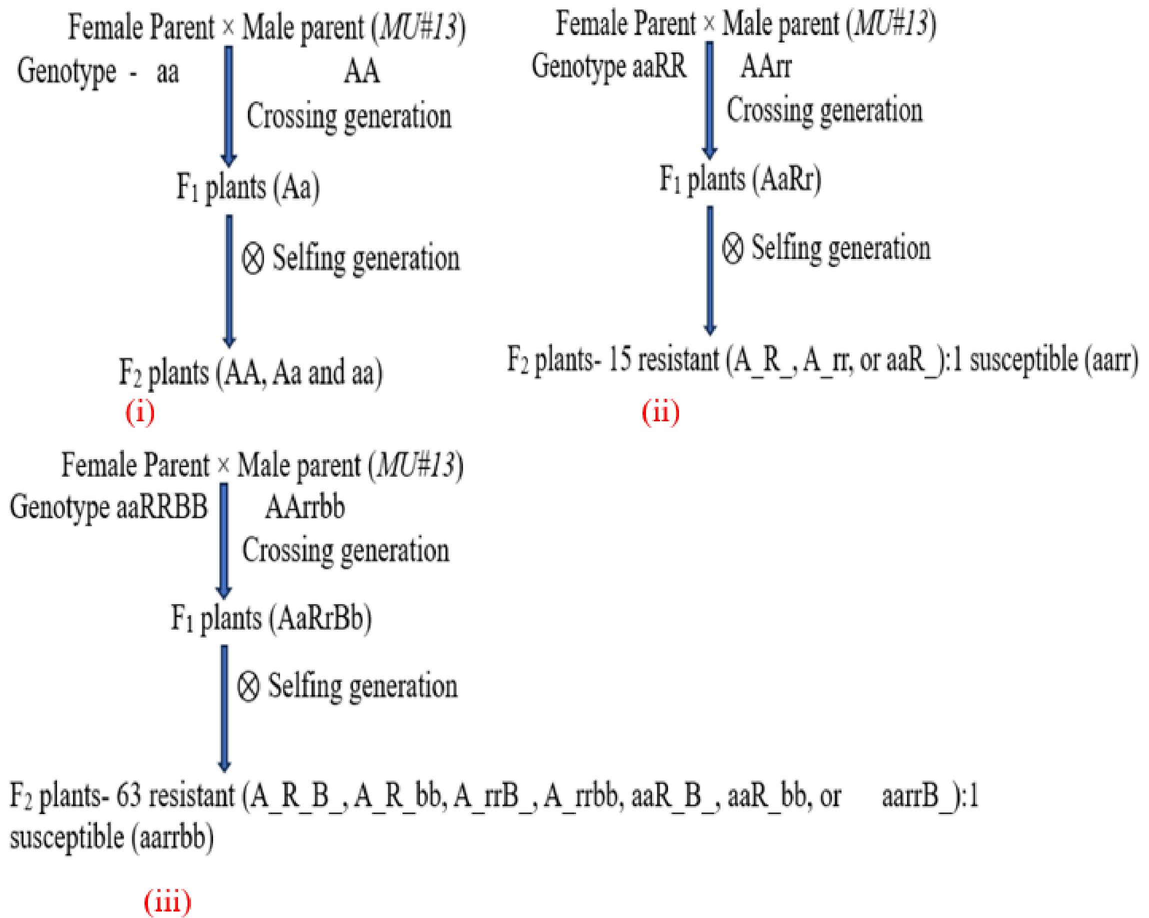

Figure 3.1 Crossing schemes for development of F_2 mapping populations considering scheme (i) for a single major dominant gene, (ii) for two major dominant independent genes and (iii) for three major dominant independent genes.

Table 3.3. Gene constitution, disease reaction and morphology of 13 bean genotypes used in allelism study.

Genotype[a]	Resistance gene	Disease reaction[c]		Flower colour	Seed type	Hypocotyl colour	Growth habit[d]
		1	2				
MU#13	Unknown	+	+	purple	small black	black	D
Early Gallatin	*Ur-4*	−	−	white	medium white	green	D
Redlands Pioneer	*Ur-13*	−	+	white	large brown yellow	green	D
Montcalm	*Ur-?*[b]	−	−	white	dark red kidney	green	D
PC-50	*Ur-9, Ur-12*	−	−	white	large red mottled	green	D
Golden Gate Wax	*Ur-6*	−	−	white	large brown	green	ID
PI 260418	*Ur-?*	−	−	white	large black/brown/white speckled	green	ID
Great Northern 1140	*Ur-7*	−	−	white	medium white	green	ID
Aurora	*Ur-3*	+	+	purple	small black	black	ID
Mexico 309	*Ur-5*	+	+	purple	small black	black	ID
Mexico 235	*Ur-3*$^{+}$	+	+	purple	medium pink	green	ID
CNC	*Ur-?*	+	+	purple	small black	black	ID
PI 181996	*Ur-11*	+	+	purple	medium black	black	ID
Ouro Negro	*Ur-14*	+	+	purple	small black	black	ID

[a]*PC-50= Pompadour Checa-50*, *CNC= Compuesto Negro Chimaltenango*; [b]*Ur-?=* unnamed gene; [c]Disease reaction to 1= race 63-1 and 2= race 61-1 of bean rust, −= Susceptible, += Resistant; [d]Growth habit, D= Determinate, ID= Indeterminate.

3.3.3 Rust inoculation for F_2 population phenotyping

In the greenhouse, 200 F_2 plants from each cross, as well as 10 seeds from each parental genotype and F_1 plant, were sown in 7.5-cm-diameter plastic pots filled with sterile soil. Rust races 63-1 and 61-1 were used in allelism studies as they were virulent to most differential genotypes and avirulent to genotype *MU#13*. The 8–10-day-old plants at V2 growth stage with about ⅔ expanded primary leaves were inoculated by hand spraying viable *U. appendiculatus* urediospores at a concentration of 2.0×10^4 urediospores per ml of dH_2O containing 0.05% Tween 20. In order to prevent cross-contamination, plant materials that had been inoculated with distinct races were moved to distinct partitions of the screenhouse (Plate 3.1). The facility was maintained at a temperature of 20±1 °C and relative humidity >95% under a 12-hour light/dark regime for 48 hours, after which the plants were moved to a greenhouse at 20±5 °C for 14 days.

Plate 3.1. Inoculated F_2 population bean plants in the screening chamber.

3.3.4 Data collection and analysis

Bean rust severity was rated using the 1–6 scale as described in section 3.2.4.2. The most prevalent infection grade was chosen in case of several infection grades. The *Chi*-square test was used to test goodness-of-fit to expected ratios of 3:1, 15:1 or 63:1 in the F_2 populations for inheritance and allelism tests as follows:

$$\chi 2 = \sum \frac{(O-E)^2}{E}$$ ……………………………………………….*Formula 1* (Pandis, 2016).

where *O* is the observed value and *E* is the expected value.

3.4 Determination of SSR markers linked rust resistance gene in *MU#13*

3.4.1 Genotypes

The common bean seeds for genotypes *Amy* (susceptible) and *MU#13* (a French bean breeding line resistant to rust) were grown in the greenhouse. Genetic crosses using *MU#13* as the male parent were performed in the greenhouse to obtain F_1 seeds using a modified method proposed by Bliss (1980), as described in Section 3.3.2. The F_1 plants were grown in the greenhouse and analyzed phenotypically based on hypocotyl pigmentation, flower colour, seed colour, seed size, and seed shape to identify true hybrids. The F_1 plants were selfed to generate corresponding F_2 populations. Furthermore, 80 recombinant inbred lines (RILS) previously developed using single seed descent from a cross between the *MU#13* and *Amy* parents were included in the study.

3.4.2 Bean rust inoculation and disease evaluation

Bean rust race 63-1 was selected for inoculations as it is avirulent to genotype *MU#13* and virulent to genotype *Amy*. In the greenhouse, 200 F_2 plants from the crosses and 10 seeds from each of the RILs, parental genotypes, and F_1 plants were sown in 7.5-cm-diameter plastic pots filled with sterile soil. Rust inoculum was introduced on 8–10-day-old plants at V2 growth stage with about ⅔ expanded primary leaves by hand spraying of urediospores of rust race 63-1 at a concentration of 2.0×10^4 urediospores per ml of dH_2O containing 0.05% Tween 20. Inoculated plants were transferred to the screenhouse maintained at 20±1 °C and a relative humidity >95% under a 12-hour light/dark regime for

approximately 48 hours, after which they were transferred to a greenhouse at 20±5 °C for about 14 days. Bean rust severity was rated using the 1–6 scale (Table 3.2), considering cultivars with reaction values of 1–3 as resistant and 4–6 as susceptible. The most prevalent infection grade was chosen in case of several infection grades.

3.4.3 Extraction, quantification and preparation DNA samples for BSA

Genomic DNA was extracted from young leaf tissues of the mapping population and parental genotypes (*Amy* and *MU#13*) following a modified protocol by Mahuku (2004). The DNA quality and quantity were assessed on a 1.5% agarose gel pre-stained with 5 µM ethidium bromide ($C_{21}H_{20}BrN_3$). Samples of DNA (5 µl), lambda DNA standards of 50, 100, and 200 ηg/µl and a 50bp ladder were loaded on the gel in 1x sodium borate buffer ($Na_2[B_4O_5(OH)_4]\cdot 8H_2O$) and separated at 100 V for 30 minutes. The gel was then visualised under ultraviolet light using the UVP® Benchtop Variable Transilluminator and photographed using a Canon® camera. The quantity of the DNA was estimated using the band sizes and intensity of the standards. Equal amounts of standardized DNA (10 ng/l) were produced from 10 homozygous resistant and 10 homozygous susceptible F_2 plants and RILs chosen based on phenotypic data for response to race 63-1. Sample genomic DNA from these genotypes was evaluated for polymorphism using 14 primers.

3.4.4 SSR Primers

Fourteen SSR markers were used in this study. Located in linkage group 4 in the common bean genome, 6 of the markers are alternatives to the loosely linked marker (PVctt001) that was identified by Arunga (2012). In summary, *PV-cct001*, an SSR marker located on linkage group LG 4, is located at 19.3 cM from the resistance gene in *MU#13*. The 6 SSR markers were selected from the Phaseolus Genes marker Database (http://phaseolusgenes.bioinformatics.ucdavis.edu/) (three on either side of *PV-ctt001*). A total of 14 primers, including the query sequence *PV-ctt001,* were selected (seven already in the database and the other seven designed using the sequence of each of the seven markers in the Primer 3 program) (Table 3.4).

Table 3.4. 7 SSR primers from Phaseolus Genes Database together with 7 primers designed from primer 3 software.

SSR marker	Loc[a]	Size	TM (°C)	Forward primer sequence	Reverse primer sequence
Primers in the Phaseolus genes database					
PV-ctt001 (query)		152	47	5′-GAGGGTGTTTCACTATTGTCACTGC-3′	5′-TTCATGGATGGTGGAGGAACAG-3′
SSR-IAC215	Right	191	53	5′-AAAAATCTGATCAAAACACAA-3′	5′-AAGCCTGCACCCACATT-3′
BMb583	Right	160	45	5′-ATTTCAAATTTCCTTCACCC-3′	5′-AAATCTTCTAATCCCTGTTACT-3′
BMb431	Right	159	52	5′-TCACGTTATTGGGAGTCAA-3′	5′-ACAAGTCAATTATTACGTTTGAA-3′
PvM156	Left	219	53	5′-CACACTTCAACTCCAAAGG-3′	5′-CCAACCCTCGCAAAT-3′
BM161	Left	185	52	5′-TGCAAAGGGTTTGAAAGTTCGAGAG-3′	5′-TGCAAAGGGTTTGAAAGTTCGAGAG-3′
BMb388	Left	275	58	5′-TCCAAATAGAACAATTGGAAA-3′	5′-TTTGTTACCCTTTCTAGAATAAA-3′
Primers designed in Primer 3 software					
SSR marker	**Loc[a]**	**Size**	**TM (°C)**	**Forward primer sequence**	**Reverse primer sequence**
PV-ctt001-P3		152	58	5′-GACCCTCTTCCTTCTGGGAC-3′	5′-CACACCATTTGGCTCACAAC-3′
SSR-IAC215-P3	Right	191	53	5′-TTTGCGCACTCTCAATCAAC-3′	5′-TAATCAACTCCCACATGCCA-3′
BMb583-P3	Right	160	53/47	5′-TGCTCATGGTGAAGATGGAG-3′	5′-AAAAAGCGTTTGGGTTTACAGTCA-3′
BMb431-P3	Right	159	50	5′-TGGTTGTGATCAATGTGTTAGC-3′	5′-CCTGTTCCCAATAAAAACAACC-3′
PvM156 (P3)	Left	219	53	5′-GGAGACTTTGTGCAGGCTTC-3′	5′-CCAGCGAATGGTAAGGATGT-3′
BM161-P3	Left	185	52	5′-CTGGATCTGTGCAAAGGGTT-3′	5′-TTTGCCCACAAAAGTTCC-3′
BMb388-P3	Left	275	58	5′-CGACAGTGATTGAAAGTTAACAAA-3′	5′-ATTACCGCCAAATGCAAAAA-3′

[a]Location, either on left or right of *Pvctt001*, TM- annealing temperature.

3.4.5 Molecular analysis

Amplification was carried out in FrameStar® Break-A-Way PCR tubes with a reaction volume of 10 µl for each individual sample. 1X Dream *Taq* buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of both reverse and forward primers, 0.1 U of Taq Polymerase (from Thermo Fisher Scientific), and 2.5 ηg/µl genomic DNA composed the mixture. The PCR process followed a specific pattern, beginning with a one-minute initial denaturation stage at 95 °C, ensued by 30 cycles of the following three phases: denaturation at 95 °C for 15 seconds, a 15-second annealing step, and an extension at 72 °C for 10 seconds. A 5-minute final extension phase followed at 72 °C. The specific temperatures used for annealing in this study are outlined in Table 3.4. Following the amplification, 5 µl of the PCR products along with a 50-bp ladder were loaded onto a 1.5% agarose gel pre-stained with 5 µM ethidium bromide ($C_{21}H_{20}BrN_3$). The amplicons were separated at a voltage of 100 volts for a duration of 60 minutes in a 1x Sodium borate buffer ($Na_2[B_4O_5(OH)_4]\cdot 8H_2O$). The UVP® Benchtop Variable Transilluminator was used to visualize the gel under ultraviolet light, and subsequently, a Canon® camera was used to photograph it. The DNA fragment sizes were documented.

CHAPTER FOUR

RESULTS

4.1 Prevalence of bean rust

4.1.1 Bean rust incidence and severity

Bean rust was observed across the 5 surveyed counties with varying degrees of incidence and severity. The severity of bean rust ranged from 1 to 9, with an incidence of between 0 and 100%. Notably, the extent of incidence and severity of *U. appendiculatus* showed substantial variations among the counties within the regions, with statistical significance ($p<0.001$), as illustrated in Table 4.1. The bean rust severity map developed revealed the distribution of rust across the surveyed counties, as depicted in Figure 4.1

Table 4.1. ANOVA table showing the effect of various factors on the incidence and severity of bean rust within counties in Western and Central Kenya.

Source of variation	df[1]	Incidence MS[2]	Severity MS
County	4	46.214***	11.398***
Cropping system	1	74.8	0.958
Altitude	2	8887.8***	15.835***
Cultivar	23	1039.3**	3.722**
Seed source	2	225.5	0.191
Previous crop	10	794.2	2.923
Debri management	3	2017.8**	5.026*
Fungicide use	1	6300.6***	19.364**
Management of volunteer plants	2	1251.0***	3.623***
Crop spacing	19	1193.7***	2.030**

[1]df= degree of freedom; [2]MS= Mean square values with *, ** and *** indicating significance at p=0.05, p<0.01 and p<0.001, respectively.

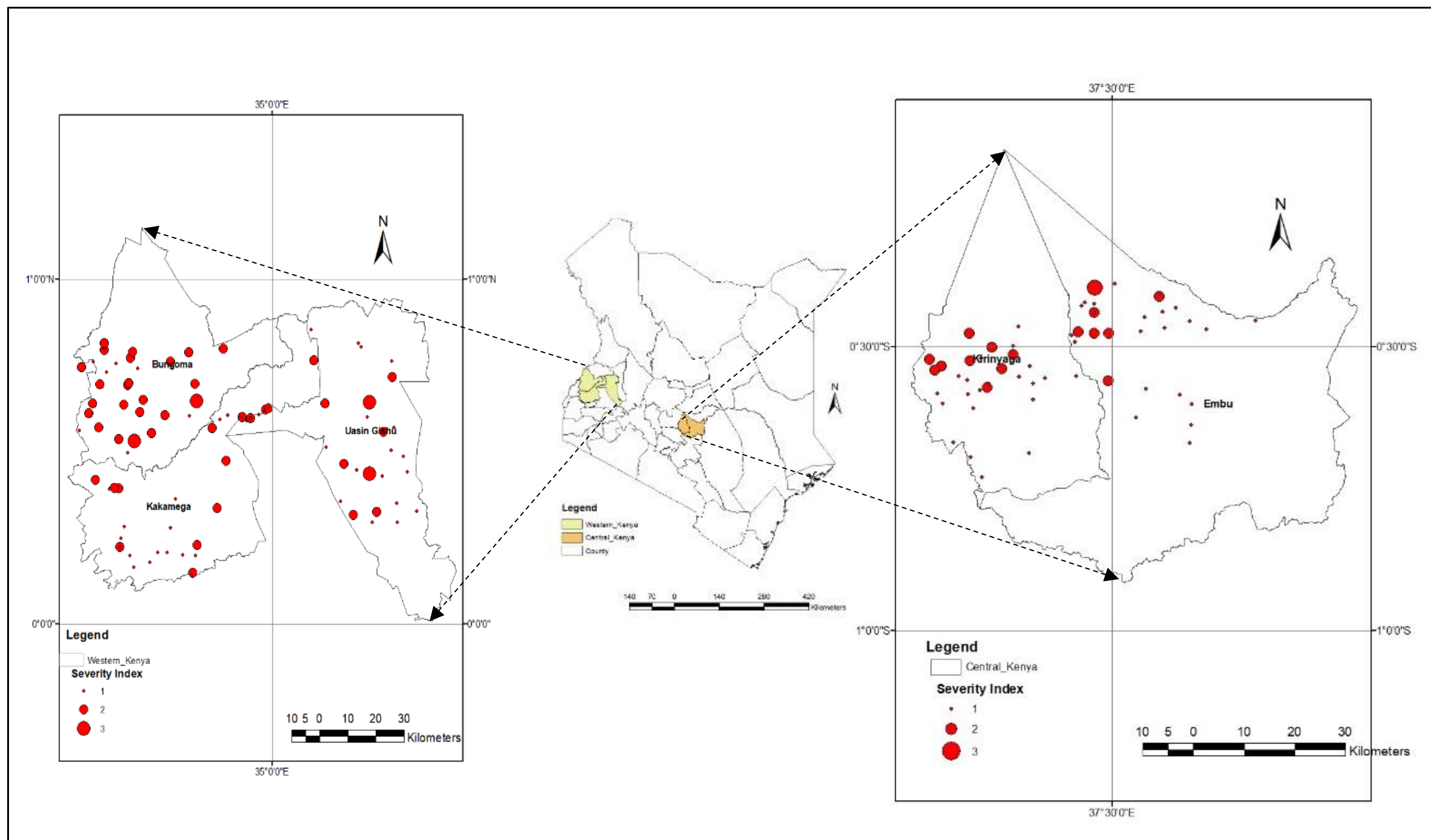

Figure 4.1. Bean rust severity mapping in five counties in Western and Central Kenya. Score of 1 represents resistant, 2 represents intermediate reaction and 3 highly susceptible to rust.

The overall mean rust incidence for the counties surveyed was 55.20%, with the highest mean rust incidence of 70.80% observed in Bungoma County, followed by Uasin Gishu with a value of 61.20%, Kakamega with a value of 57.30%, Kirinyaga with a value of 48.30%, and lastly Embu with a value of 38.30%. Bungoma, Uasin Gishu, Kakamega, Kirinyaga, and Embu counties had mean rust severity values of 3.99, 3.12, 3.00, 2.69, and 2.34, respectively, with an overall mean severity of 3.03 (Table 4.2). Therefore, beans grown in Kirinyaga and Embu counties had a mean severity below average by 11.22% and 22.77%, respectively.

Table 4.2. Incidence and severity of bean rust in western and central Kenya.

County	Number of fields surveyed	Bean rust[1]	
		Incidence (%)	Severity
Bungoma	30	70.83[a]	3.99[a]
Uasin Gishu	30	61.17[ab]	3.12[ab]
Kakamega	30	57.33[b]	3.00[bc]
Kirinyaga	30	48.33[bc]	2.69[bcd]
Embu	30	38.33[c]	2.34[cd]
Mean		55.20	3.03

[1]Values in the same column with identical letters are not significantly different from each other ($p<0.05$).

4.1.2 Effects of cultural practices on bean rust prevalence

Common bean production practices significantly influenced the incidence and severity of bean rust disease in the surveyed regions. Production of common beans as a sole crop or intercrop did not influence disease incidence and severity (Plate 4.1 and Table 4.3). The cropping system, source of seeds used for planting, and previous crop grown had no significant influence on the incidence and severity of bean rust in the surveyed counties (Tables 4.1 and 4.3). Generally, 11 different crops had been used as previous crops on some farms, with common beans as the only alternative host for rust.

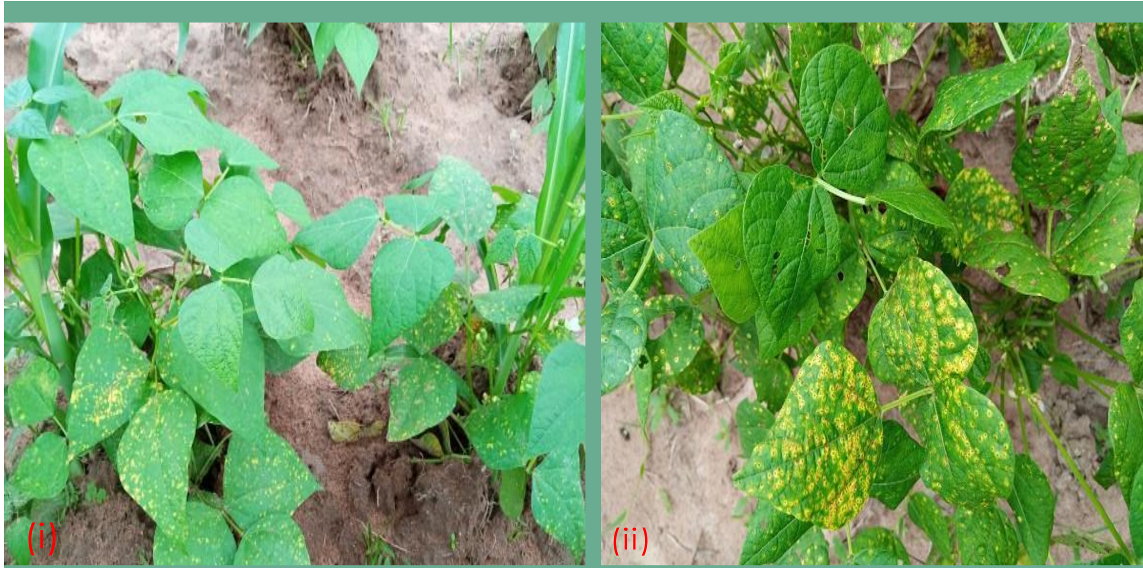

Plate 4.1. Bean rust in scored with severity of 9 in (i) maize-bean intercrop and (ii) common bean sole-crop in Bungoma county.

Differential rust incidence and severity were observed across different elevations. At lower elevations, significantly lower rust incidence and severity were observed compared to areas of altitude exceeding 1,200 meters above sea level (Table 4.3). Fungicide use significantly ($p<0.01$) affected the incidence and severity of bean rust, with reduced disease in fields sprayed with fungicides such as Dithane M45® (Mancozeb) and Funguran® (Copper hydroxide, 770g/kg). Similarly, strategies used in management of common bean debri (making trash lines, soil incorporation, compost manure preparation, burning, leaving on the soil surface, and use as livestock feed), management of volunteer plants (incorporating in the soil, spraying herbicides, and no management), and crop spacing (different crop spacing measurements) had significant ($p<0.05$) effects on mean rust incidence and severity (Tables 4.1 and 4.3). Incidences and severity of rust were cultivar-dependent, with the most susceptible cultivars being *Kisii*, *Sungura*, *GLP-24* (*Canadian Wonder),* and *Kablanketi,* while the most resistant cultivars were *Kamusele*, *Vanilla*, *Embean 14,* and *KAT B11* (Figures 4.2 and 4.3).

Table 4.3. Factors affecting incidence and severity of bean rust in western and central Kenya.

Factor	Factor classification as variables	Bean rust[1]	
		Incidence (%)	Severity
Altitude	1200-1800 masl	58.33[a]	3.19[a]
	>1800 masl	57.24[a]	2.98[a]
	<1200 masl	14.5[b]	1.34[b]
Cropping system	Sole crop	55.70[a]	3.09[a]
	Intercrop	54.20[a]	2.92[a]
Fungicide use	No fungicide spray	57.30[a]	3.14[a]
	Fungicide spray	35.00[b]	1.91[b]
Seed source	Certified Seed Agents	49.50[a]	3.05[a]
	Local Market	56.20[a]	3.06[a]
	Saved seed	54.70[a]	2.96[a]
Management of volunteer plants	No management	57.10[a]	3.12[a]
	Soil incorporation	52.90[b]	2.91[a]
	Herbicide spray	30.00[c]	1.65[b]
Debri management	Trash-lines	77.50[a]	4.05[a]
	Compost manure	65.33[b]	3.48[a]
	Burn	62.77[b]	3.41[a]
	Leave on soil surface	59.50[b]	3.33[ab]
	Livestock feed	47.47[c]	2.64[b]
	Soil incorporation	45.00[c]	2.38[c]

[1]Values within a column that are followed by identical letters are not significantly different from one another ($p<0.05$).

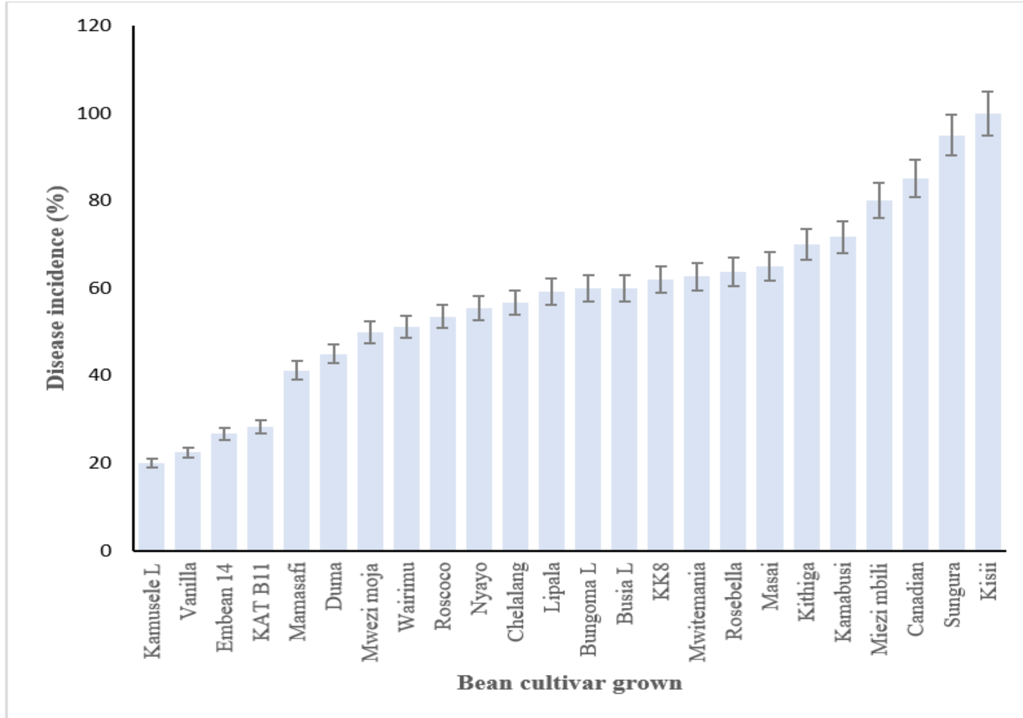

Figure 4.2. Incidence of bean rust on different common bean genotypes from western and central Kenya. The error bars represent standard errors.

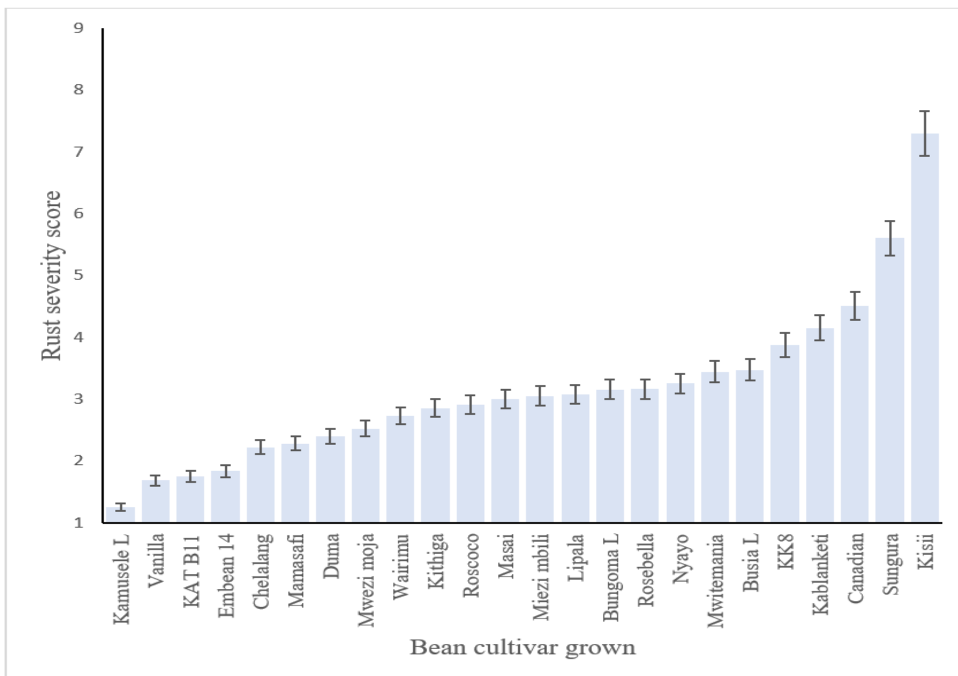

Figure 4.3. Severity of bean rust on different common bean genotypes from western and central Kenya.

4.2 Response of common bean germplasm to bean rust

4.2.1 Profiles of common bean germplasm to rust under field conditions

Relatively low bean rust disease pressure was observed under field conditions compared to greenhouse conditions. Thirty-three genotypes were classified as resistant, while 44 were susceptible to rust (Table 4.5). The common bean cultivars harbouring the known genes *Ur-3, Ur-3*$^{+}$, *Ur-5*, *Ur-11*, *Ur-14,* and the unnamed gene *Ur-CNC*, which are of Mesoamerican origin, were the most resistant (Table 4.5).

4.2.2 Characterization of bean rust isolates using differential cultivars

Four bean rust races (29-1, 29-3, 61-1, and 63-1) were obtained from single spores of the ten bean rust isolates. Race 61-1 was the most common race characterized using isolates obtained from Kirinyaga, Kakamega, Uasin Gishu, and Bungoma counties (Table 4.4).

Table 4.4. Characterization of bean rust isolates based on their reaction on the 12 differential cultivars.

Isolate	Andean gene pool[a]						Mesoamerican gene pool							
ID	1	2	3	4	5	6	7	8	9	10	11	12	Race	Gene pool
Uas1	−	+	−	−	−	+	−	+	+	+	+	+	29-1	Andean
Kak17	−	+	−	−	−	+	−	+	+	+	+	+	29-1	Andean
Emb27	−	+	−	−	−	+	−	+	+	+	+	+	29-1	Andean
Kir14	−	+	−	−	−	+	−	−	+	+	+	+	29-3	Andean
Emb4	−	+	−	−	−	+	−	−	+	+	+	+	29-3	Andean
Kir24	−	+	−	−	−	−	−	+	+	+	+	+	61-1	Andean
Kak11	−	+	−	−	−	−	−	+	+	+	+	+	61-1	Andean
Uas16	−	+	−	−	−	−	−	+	+	+	+	+	61-1	Andean
Bun13	−	+	−	−	−	−	−	+	+	+	+	+	61-1	Andean
Bun25	−	−	−	−	−	−	−	+	+	+	+	+	63-1	Andean

[a]−= Susceptible, += Resistant, 1= *Early Gallatin*, 2= *Redlands Pioneer*, 3= *Montcalm*, 4= *Pompadour Checa-50*, 5= *Golden Gate Wax*, 6= *PI260418*, 7= *Great Northern 1140*, 8= *Aurora*, 9= *Mexico 309*, 10= *Mexico 235*, 11= *Compuesto Negro Chimaltenango (CNC)* and 12= *PI181996*.

4.2.3 Profiles of common bean resistance to rust under greenhouse conditions

The common bean germplasm evaluated exhibited different reactions to *U. appendiculatus*. Typical bean rust symptoms were observed (Plate 4.2). The rust genes *Ur-3, Ur-3*$^{+}$, *Ur-5*, *Ur-11*, *Ur-14,* and the unknown gene *Ur-CNC*, which are of Mesoamerican origin, were resistant to races 29-1, 29-3, 61-1, 63-1, and mixed isolates used for evaluation under the greenhouse. Common bean genotypes of Andean origin such as *Enclave*, *Kat X56*, *Kablanketi,* and *KMR 11* (*Angaza*) were resistant, whereas cultivars *Hawaii*, *Julia*, *Amy*, *Samantha,* and *UN3-Yelow Small* were susceptible to all the Andean races. However, some genotypes of Mesoamerican origin (*MU#13*, *Manakelly,* and *UN6-Nakholo)* were also resistant to the races (Table 4.5).

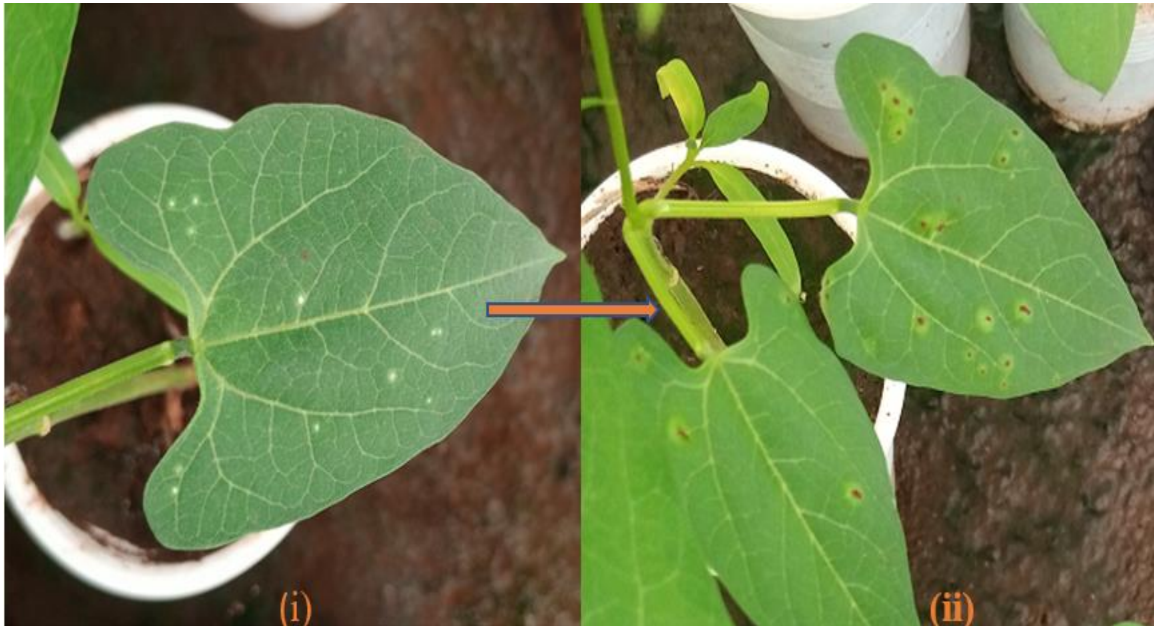

Plate 4.2. Progression of rust uredospore on leaf and petiole of bean plant. (i) White to cream-coloured circular specks on the leaf; initial bean rust symptoms. (ii) Rust-coloured pustules often surrounded by chlorotic halo.

Table 4.5. The characteristics of common bean germplasm and their response to rust under field and greenhouse conditions.

S/No	Genotype	Growth habit[a]	Seed type[b]	Gene pool[c]	Field disease reaction[d]	Greenhouse disease reaction				
						Mixed isolate	29-1	29-3	61-1	63-1
1	*MU#03*	I	S	MA	+	−	+	−	−	−
2	*MU#13*	I	S	MA	+	+	+	+	+	+
3	*Rosebella*	I	L	A	+	−	+	−	+	−
4	*KMR 11 (Angaza)*	II	L	A	+	+	+	+	+	+
5	*Embean14 (Mwende)*	I	L	MA	−	−	+	+	+	+
6	*Rosecoco (GLP 2)*	I	L	A	+	−	−	+	−	−
7	*GLP-585 Red haricot*	I	S	MA	−	−	−	+	−	−
8	*GLP X92*	II	L	MA	−	−	−	−	−	−
9	*Kablanketi*	II	S	A	+	+	+	+	+	−
10	*Kat/B1 (Katheka)*	I	L	M	−	−	+	−	+	+
11	*Kat X56*	I	L	A	+	+	+	+	+	+
12	*KK Rosecoco-194*	I	L	A	+	−	+	−	+	−
13	*KK8*	I	L	A	−	−	+	−	−	−
14	*New Rose Coco*	I	L	MA	+	−	−	−	+	+
15	*Rio Rojo*	I	L	A	−	−	+	+	+	−
16	*Tasha*	I	L	MA	+	+	+	+	−	−
17	*Wairimu Dwarf*	I	M	MA	−	−	+	−	+	−
18	*AB 136*	II	M	MA	+	+	+	+	+	−
19	*Cornell 49-242*	II	M	MA	+	−	+	−	−	−
20	*G 2333*	II	M	MA	−	−	+	+	+	−
21	*Kaboon*	I	L	A	−	−	+	−	−	−
22	*MDRK*	I	L	A	−	−	−	−	−	−
23	*Mexico 222*	I	M	MA	−	−	−	−	−	−
24	*Mexico 54*	II	M	MA	+	−	−	−	+	−
25	*Mitchelite*	II	S	MA	−	−	−	−	−	−
26	*Ouro Negro*	II	M	MA	+	+	+	+	+	+

S/No	Genotype	Growth habit[a]	Seed type[b]	Gene pool[c]	Field disease reaction[d]	Greenhouse disease reaction				
						Mixed isolate	29-1	29-3	61-1	63-1
27	*Perry marrow*	I	L	A	+	−	−	−	−	−
28	*PI 207262*	II	S	MA	−	−	−	+	−	−
29	*TO*	II	M	MA	−	−	−	+	−	−
30	*TU*	II	M	MA	−	−	−	−	+	−
31	*Widusa*	I	M	A	−	−	−	−	−	−
32	*Aurora*	II	M	MA	+	+	+	−	+	+
33	*CNC*	II	M	MA	+	+	+	+	+	+
34	*Early Gallatin*	I	M	A	−	−	−	−	−	−
35	*Golden Gate Wax*	II	L	A	−	−	−	−	−	−
36	*Great Northern 1140*	II	M	MA	−	−	−	−	−	−
37	*Mexico 235*	II	M	MA	+	+	+	+	+	+
38	*Mexico 309*	II	M	MA	+	+	+	+	+	+
39	*Montcalm*	I	L	A	−	−	−	−	−	−
40	*PC-50*	I	L	A	−	−	−	−	−	−
41	*PI 181996*	II	L	MA	+	+	+	+	+	+
42	*PI 260418*	II	L	A	−	−	+	+	−	−
43	*Redlands pioneer*	I	L	A	+	−	+	+	+	−
44	*Amy*	I	S	A	−	−	−	−	−	−
45	*Blazer*	I	M	A	−	−	−	−	−	−
46	*Boston*	I	S	A	+	−	+	+	+	−
47	*Edge*	I	S	A	+	+	+	−	+	−
48	*Enclave*	I	S	A	+	+	+	+	+	+
49	*Fanaka*	I	S	A	−	−	−	+	−	−
50	*Hawaii*	I	S	A	−	−	−	−	−	−
51	*Julia*	I	S	A	−	−	−	−	−	−
52	*Konza*	I	S	A	+	−	+	+	+	−
53	*Lomami*	I	S	A	−	−	−	+	−	−
54	*Manakelly*	I	S	MA	+	+	+	+	+	+

S/No	Genotype	Growth habit[a]	Seed type[b]	Gene pool[c]	Field disease reaction[d]	Greenhouse disease reaction				
						Mixed isolate	29-1	29-3	61-1	63-1
55	*Mara*	I	S	A	+	+	+	+	+	−
56	*Moonstone*	I	S	A	−	−	+	−	−	−
57	*Samantha*	I	S	A	−	−	−	−	−	−
58	*Seagull*	I	S	A	−	−	+	−	−	−
59	*Serengeti*	I	S	A	−	−	+	+	−	−
60	*T19*	I	M	MA	+	−	+	+	−	+
61	*Teebus*	I	M	MA	−	−	−	+	−	+
62	*Teresa*	I	S	A	−	−	+	−	−	+
63	*Vanilla*	I	S	A	−	−	+	+	−	+
64	*GLP-24*	I	L	A	+	+	+	+	+	−
65	*GBK 032805*	I	M	MA	−	−	−	−	−	−
66	*GBK 032928*	I	M	MA	−	−	−	−	+	+
67	*Kamusele*	II	S	A	−	−	−	+	+	−
68	*MCM 1015*	II	S	MA	+	+	+	+	−	+
69	*MCM 2001*	II	M	MA	+	+	+	+	+	−
70	*MCM 5001*	II	S	MA	−	+	+	+	+	−
71	*UN 1- Khaki small*	II	S	MA	−	−	−	−	−	+
72	*UN 2- Dark-green round*	II	M	A	+	+	+	+	+	+
73	*UN 3- Yellow medium*	II	M	A	−	−	−	−	−	−
74	*UN 4- Yellow small*	II	S	MA	−	−	−	−	−	−
75	*UN 5- Libya*	I	S	MA	−	−	−	+	−	−
76	*UN 6- Nakholo*	I	L	MA	+	+	+	+	+	+
77	*UN 8- Tanzania*	I	L	MA	−	−	+	+	−	+

S/No. 1-3= breeding lines, 4-31= dry bean cultivars, 32-43= differential cultivars, 44-64= French bean cultivars, 64-77= landraces. [a]Growth habit; I= Determinate, II= Indeterminate. [b]Seed Size; S= Small, M= Medium, L= Large. [c]Gene Pool; A= Andean, MA= Mesoamerican. [d]Field disease reaction; += Resistant, −= Susceptible.

4.2.4 Gene pool affiliations of common bean genotypes using the phaseolin marker

Molecular analysis based on the phaseolin protein marker; 37 genotypes were affiliated to the Mesoamerican gene pool while 40 belong to the Andean gene pool (Table 4.5). A profile of 2 bands of 249 bp and 270 bp implies the genotype is of the Mesoamerican gene pool, while a profile of 3 fragments of 249 bp, 264 bp, and 285 bp is of the Andean gene pool, as demonstrated in Plate 4.3.

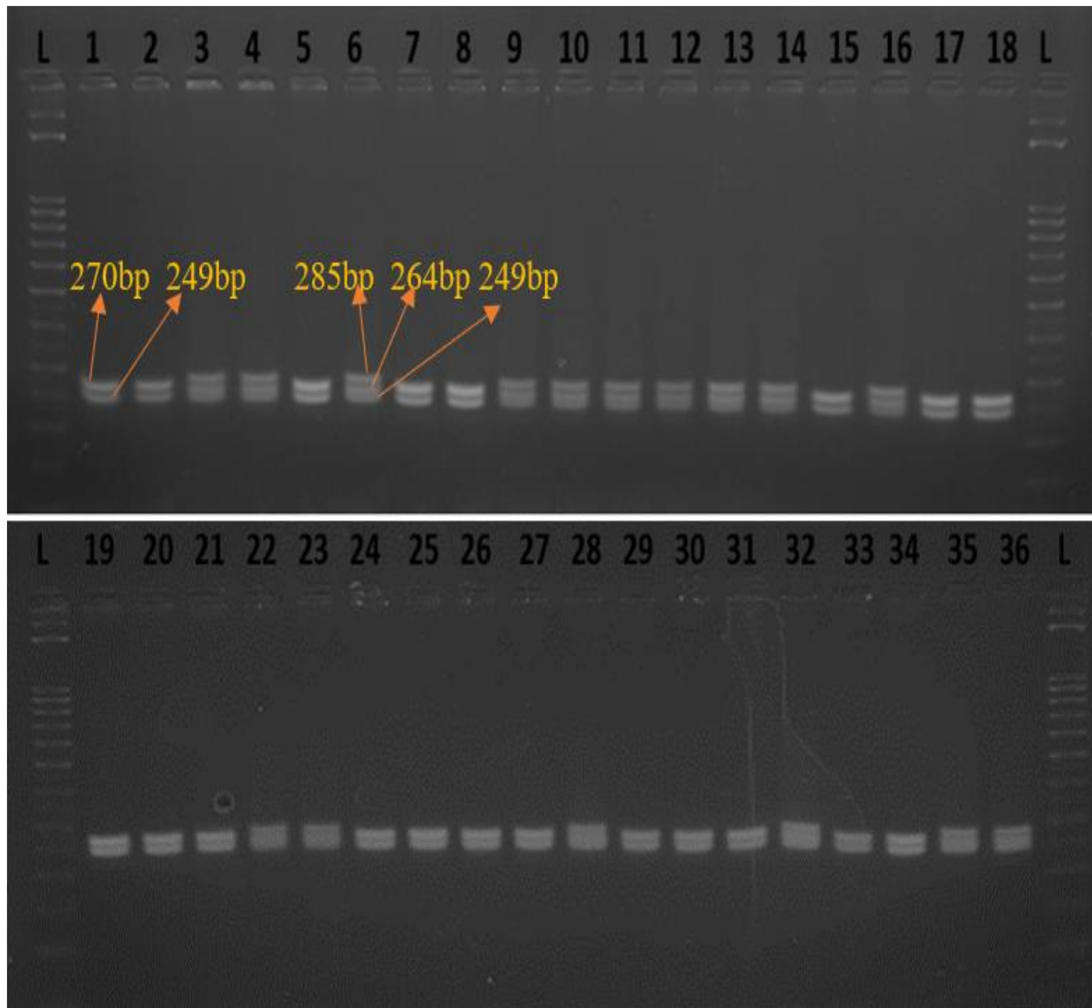

Plate 4.3. Gel photos showing PCR amplification products of genomic DNA of common beans using the Phaseolin seed protein marker electrophoresed on 1.5% agarose gel at 100V for 3 hours. L= 50 bp ladder, 1-36= genotype serial numbers as outlined in Table 4.5.

4.3 Characterization of the resistance gene(s) in *MU#13*

Seven of the 13 differential cultivars were susceptible to race 63-1 of *U. appendiculatus* used in this study (Tables 4.4 and 4.5). The segregation ratio of 3R_:1rr was observed in the F_2 mapping populations derived from the crosses between cultivars *Early Gallatin* (harbouring the *Ur-4* gene), *Montcalm* (with the unnamed resistance gene), *Golden Gate Wax* (with the *Ur-6* gene), *PC-50* (carrying the *Ur-9* and *Ur-12* genes), *Great Northern 1140* (harbouring the *Ur-7* gene), and the French bean line *MU#13* (Table 4.6). In addition, the segregation ratios observed in the F_2 populations resulting from crosses between resistant Mesoamerican genotypes *Ouro Negro* (*Ur-14*), *Redlands Pioneer* (*Ur-13*), *Mexico 235* (*Ur-3*$^+$), *Mexico 309* (*Ur-5*), *Aurora* (*Ur-3*), *PI181996* (Ur-11), and *MU#13* French bean line did not deviate significantly from the expected ratio of 15 resistant to 1 susceptible (15R:1S) (Table 4.6). The F_2 population for testing the *CNC* (*Ur-?*) resistance loci showed a segregation ratio of 63R (resistant):1S (susceptible).

Table 4.6. Phenotypic distribution and Chi-square test based on the expected ratios in the mapping populations

Locus tested	Rust race	Reaction[a]	No. of plants	Expected ratio	Observed ratio	χ^2	P(%)[b]
Ur-4	63-1	R × S	243	3R:1S	183R:60S	0.0123	91.152
Ur-13	61-1	R × R	179	15R:1S	167R:12S	0.0629	80.191
Ur-? (*Montcalm*)	63-1	R × S	185	3R:1S	140R:45S	0.0450	83.192
Ur-9, Ur-12	63-1	R × S	120	3R:1S	89R:31S	0.0444	83.302
Ur-6	63-1	R × S	200	3R:1S	128R:40S	0.1270	72.158
Ur-7	63-1	R × S	121	3R:1S	90R:31S	0.0248	87.488
Ur-3	63-1	R × R	182	15R:1S	171R:11S	0.0132	90.857
Ur-5	63-1	R × R	158	15R:1S	147R:11S	0.1367	71.157
Ur-3$^+$	63-1	R × R	200	15R:1S	188R:12S	0.0213	88.387
Ur-? (*CNC*)	63-1	R × R	164	63R:1S	161R:3S	0.0759	78.296
Ur-11	63-1	R × R	150	15R:1S	141R:9S	0.0160	89.934
Ur-14	63-1	R × R	105	15R:1S	99R:6S	0.0514	82.059

[a]Reaction; Resistant (R), Susceptible (S). [b]The percentage probability (P) derived from the *chi*-square (χ^2) test; α= 5% and 1 degree of freedom (d.f=1).

4.4 Molecular analysis

Differential disease reactions to race 61-3 of *U. appendiculatus* on *Amy*, *MU#13* (Plate 4.4), F_1, the F_2 population, and RILs were observed (Table 4.7). All F_1 plants showed a similar resistance pattern to *MU#13*. The observed segregation ratios did not differ significantly from the expected ratios of 3R:1S and 1R:1S in the F_2 population and RILS, respectively (Table 4.7).

Plate 4.4. (i) Resistant and susceptible reaction of genotypes *MU#13* and *Amy*, respectively. (ii) Resistant (R) and susceptible (S) reaction in a segregating F_2 population.

Table 4.7. Segregation of the resistance gene in *MU#13*.

Locus tested	Pop[a]	Reaction[b]	No. of plants	Expected ratio	Observed ratio	χ^2	P(%)[c]
MU#13	Parent	R	10	1R:0S	10R:0S	-	-
Amy	Parent	S	10	0R:1S	0R:10S	-	-
Ur-MU#13	F_1	R	40	1R:0S	40R:0S	-	-
Ur-MU#13	F_2	R × S	325	3R:1S	242R:83S	0.0503	82.262
Ur-MU#13	RILs	R × S	400	1R:1S	205R:195S	0.2500	61.708

[a]Population inoculated with race 63-1, F_1= First filial generation, F_2= Second filial generation, RILs= Recombinant Inbred Lines. [b]Reaction, R= Resistant, S= Susceptible. [c]Percentage probability (P) of the *chi*-square (χ^2) test; α= 5% and d.f=1.

All 14 primers amplified the DNA of the parental genotypes, with non-polymorphic single bands observed between *Amy* and *MU#13* for markers *BMb431*, *BMb431-P3*, *BMb538-P3*, *SSR-IAC215*, *SSR-IAC215-P3*, *BMb388*, *BMb388-P3*, *BM161*, *BM161-P3*, *PvM156,* and *PV-ctt001-P3*. Three markers, *Bmb583*, *PVM156-P3,* and *PV-ctt001,* showed polymorphism between the resistant (*MU#13*) and susceptible (*Amy*) parents.

The SSR marker *Bmb583* showed polymorphic bands of 160 bp in *Amy* and 200 bp in *MU#13*. Two DNA fragments of 160 bp and 190 bp were observed in each of the bulks (Plate 4.5). However, 2 bands were observed for each of the resistant and susceptible bulks obtained from the mapping populations.

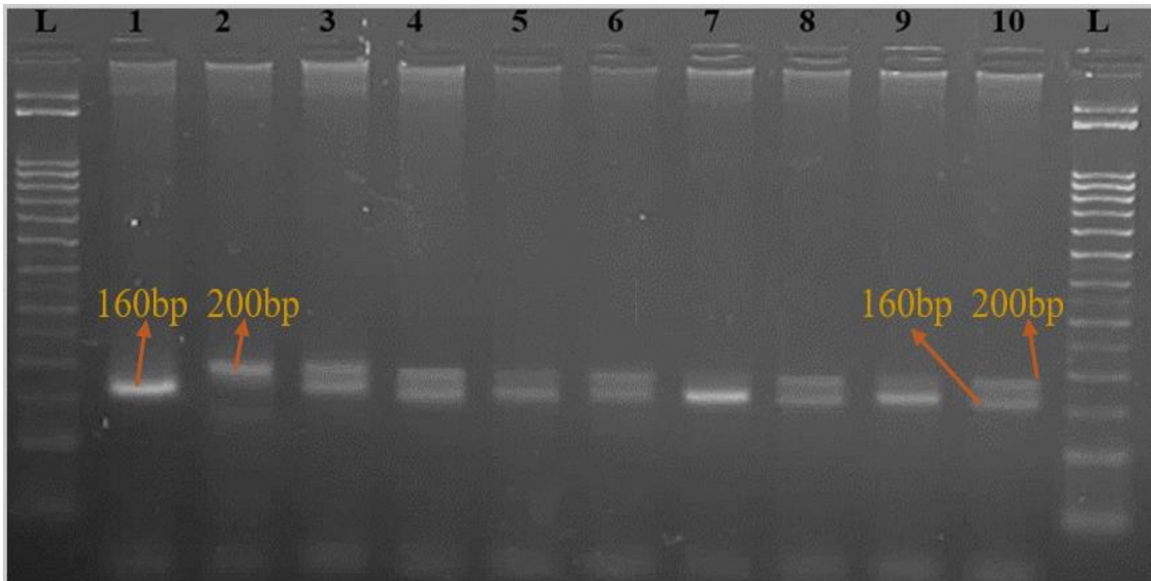

Plate 4.5. *BMb583* marker amplification. L= 50 bp ladder, 1=*Amy*, 2=*MU#13*, 3= Susceptible bulk 1 RILs, 4= Susceptible bulk 2 RILs, 5= Susceptible bulk 1 F_2s, 6= Susceptible bulk 2 F_2s, 7= Resistant bulk 1 RILs, 8= Resistant bulk 2 RILs, 9= Resistant bulk 1 F_2s and 10= Resistant bulk 2 F_2s.

The SSR marker *PVM156-P3* showed polymorphic bands of 200 bp in *Amy* and 140 bp in *MU#13*. However, a non-polymorphic band of 140 bp was observed in the two bulks from the F_2 and RILs (Plate 4.6).

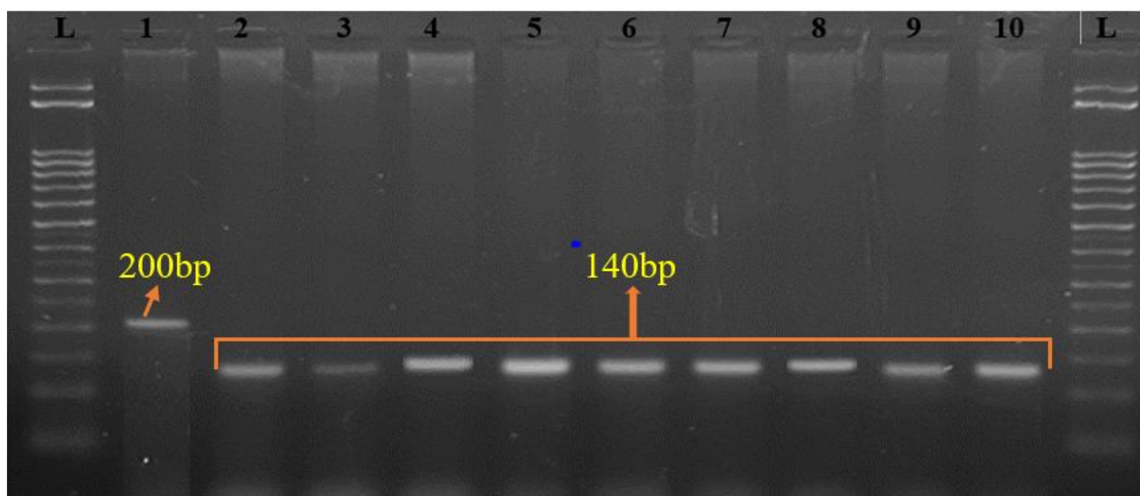

Plate 4.6. *PVM156-P3* marker amplification. L= 50 bp ladder, 1=*Amy*, 2=*MU#13*, 3= Susceptible bulk 1 RILs, 4= Susceptible bulk 2 RILs, 5= Susceptible bulk 1 F_2s, 6= Susceptible bulk 2 F_2s, 7= Resistant bulk 1 RILs, 8= Resistant bulk 2 RILs, 9= Resistant bulk 1 F_2s and 10= Resistant bulk 2 F_2s.

The SSR marker *PV-ctt001* showed polymorphic bands of 170 bp and 150 bp in *Amy* susceptible parent and in *MU#13* resistant parent, respectively. 170 bp and 150 bp bands were observed in the susceptible bulks and resistant bulks, respectively (Plate 4.7).

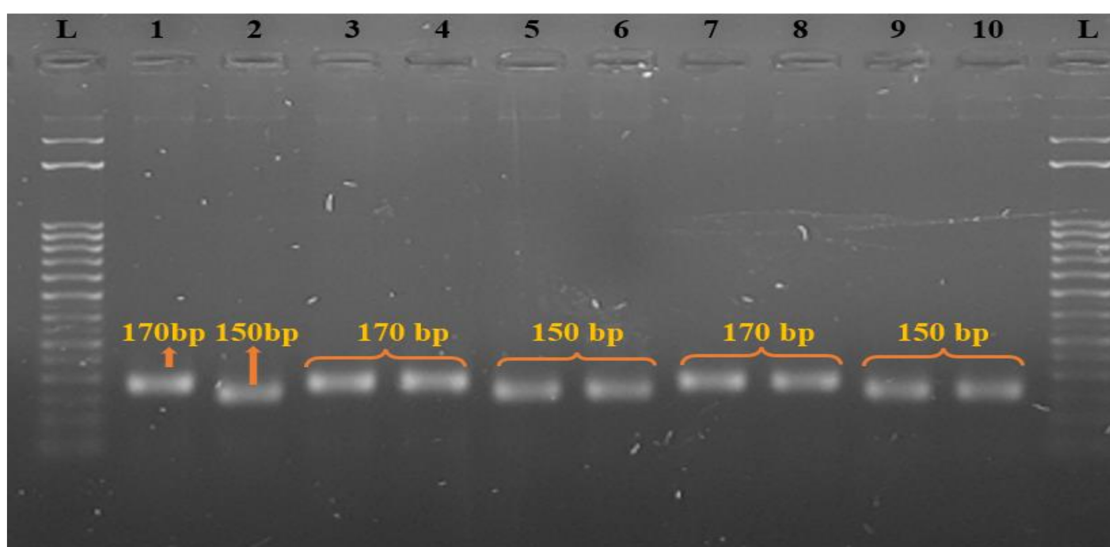

Plate 4.7. *PV-ctt001* marker amplification. L= 50 bp ladder, 1=*Amy*, 2=*MU#13*, 3= Susceptible bulk 1 RILs, 4= Susceptible bulk 2 RILs, 5= Resistant bulk 1 RILs, 6= Resistant bulk 2 RILs, 7= Susceptible bulk 1 F_2s, 8= Susceptible bulk 2 F_2s, 9= Resistant bulk 1 F_2s, 10= Resistant bulk 2 F_2s.

CHAPTER FIVE

DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

5.1 DISCUSSION

5.1.1 Prevalence and severity of bean rust

Bean rust disease is widespread in western and central Kenya, as revealed in this study. A moderate occurrence of bean rust disease has been observed in Nyanza, the Rift Valley, Eastern mid- and high-altitude areas, as well as Central and Western highland regions in Kenya (Wortmann, 1998). Normally, these areas receive rainfall >1230 mm and a mean annual temperature of 19.6 °C (Jaetzold *et al*., 2007), which are conducive for bean rust disease development (Singh and Gupta, 2019). The high incidence of bean rust may be attributed to the farming practices adopted in the production areas among smallholder farmers, for instance, the use of susceptible cultivars and poor bean debri management (Sanyang *et al*., 2019). Rust incidences and severities were high in Bungoma, Kakamega, and Uasin Gishu counties, which could be explained by specific cultural practices compounded by high relative humidity due to high rainfall received in 2020 (KMD, 2020). Low bean rust incidences and severities were observed in low-altitude areas, especially in lower parts of Embu County that occasionally receive low rainfall and experience high temperatures.

The occurrence and severity of common bean diseases may vary by location depending on environmental conditions and crop husbandry practices. The prevalent common bean fungal diseases, including angular leaf spot, anthracnose, and bean rust, thrive in conditions prevailing in high-altitude areas in Kenya characterized by elevated rainfall and humidity levels. High rainfall increases relative humidity, which is favourable for the infection and development of fungal diseases (Liebenberg and Pretorius, 2010; Mohammed, 2013; Singh and Gupta, 2019). This is consistent with findings of high severity of angular leaf spot (Mwang'ombe *et al*., 2007), anthracnose (Mogita *et al*., 2017), and bean rust (Fininsa and Yuen, 2001) in areas above 1,200 m a.s.l in Kenya and Ethiopia. These findings support the need for multi-year and multi-season evaluation for bean rust resistance across different altitudinal ranges in central and western Kenya for targeted deployment of resistance genes.

Farming practices and the crop production environment influenced the prevalence of bean rust, which is consistent with previous findings that show that the environment is a major factor influencing the occurrence and distribution of biotic stressors on pulse crops in production areas (Egho, 2011; Ogecha *et al*., 2019). The incidence and severity of rust disease were not significantly influenced by the cropping system, which is contradictory to the findings by Odogwu *et al*. (2016), and this may be attributed to the fact that plant diseases occur as a result of the interaction of a set of factors such as ideal environmental conditions, host plant susceptibility, and the high virulence of the pathogen over time (Scholthof, 2007). Further, Paparu *et al*. (2014) found significantly low rust severities for all fungicide-treated plots than plots without fungicide treatment. The occurrence of rust in some fields in the surveyed counties in the central region despite fungicide treatment suggests ineffective application of fungicides or possibly that the pathogen in those areas has developed resistance to the fungicides being used. This finding emphasizes the need to evaluate the effectiveness of the available fungicides and inform the use of fungicides in the management of bean rust among smallholder farmers.

Cultivar selection among common bean farmers in Kenya is limited (Opole *et al*., 2003), resulting in the use of susceptible cultivars, contributing to the high incidence and severity of bean rust disease in the surveyed counties. Odogwu *et al*. (2016) similarly observed that the common bean cultivar being grown had a considerable effect on both the occurrence and severity of bean rust, with higher disease severity observed in fields where landraces and commercial cultivars were present. Common bean cultivars have a wide spectrum of resistance to bean rust disease depending on their inherent genetic structure under field conditions (Acevedo *et al*., 2013; Odogwu *et al*., 2017).

Uromyces appendiculatus cannot survive without its common bean host, being an obligate parasite (Souza *et al.*, 2008). This could explain the significant influence of different strategies used by farmers in managing volunteer plants and bean debri on bean rust incidence and severity. Bean plant debris may bear viable rust spores, and this influences the occurrence and severity of bean rust (Souza *et al*., 2008). Using bean debri in making trash lines, preparing compost manure, and leaving it on the soil surface significantly

contributed to the high incidence and severity of bean rust in farmers' fields compared to those who reported practicing soil incorporation and had significantly low rust. These findings agree with the recommendation for the elimination of bean debri through strategies such as soil incorporation to aid in the control of bean rust (Souza *et al*., 2008). Furthermore, high severity and incidence were observed at close spacing. Under close spacing, there tends to be increased relative humidity and enhanced pathogen spread, which could favour bean rust development (Manjesh *et al*., 2018; Souza *et al*., 2008).

5.1.2 Germplasm resistance to bean rust

Field and greenhouse screening of the common bean germplasm in Kenya revealed high variability in response to rust. In this study, variability in host resistance to different races of bean rust indicates the possibility of varying types of *Ur* genes inherent in the genotypes (Odogwu *et al*., 2017; Wahome *et al*., 2011). Genotypes *MU#13*, *UN2-Darkgreen, UN6-Nakholo*, *Kat X56,* and *KMR-11* (*Angaza*) exhibited high resistance and therefore are potential parental genotypes in common bean breeding. According to Wagara and Kimani (2007), genotype variability in response to bean rust can be exploited as a source of resistance. *MU#13*, a local French bean breeding line, exhibits resistance against several races of bean rust and anthracnose in Kenya (Arunga, 2012; Kamiri *et al*., 2021). This genotype can be used as valuable stock for disease resistance to counter local races. However, this underscores the necessity to characterize these resistance sources and develop closely linked molecular markers. These markers are vital for aiding in marker-assisted breeding strategies to enhance rust resistance as emphasized by Souza *et al*. (2013). Furthermore, this study demonstrated that the consistent reaction of *Ur-3,* $Ur\text{-}3^{+}$, *Ur-5*, *Ur-11*, *Ur-14,* and *Ur-CNC* to rust under field and greenhouse conditions emphasizes their importance in breeding for resistance in Kenya (Arunga *et al*., 2012). Most genotypes exhibited a susceptible reaction to rust, and this may be attributed to the broad pathogenic variability of *Uromyces appendiculatus,* as similarly reported by Hillocks *et al*. (2006) and Kimani *et al*. (2002). Therefore, this necessitates the need for pyramiding *Ur* genes into the common bean background to aid in the control of rust. Low disease pressure under field conditions compared to greenhouse screening with mixed isolates may be due to low initial

inoculum, high chances of disease escapes, and unfavourable environmental conditions in the field (Sillero *et al*., 2006).

Generally, Mesoamerican genotypes exhibited high resistance to bean rust compared to those of the Andean gene pool, supporting probable pathogen coevolution with the common bean host, as reported by Acevedo (2008). Furthermore, the Andean genotypes as well as some Mesoamerican genotypes were susceptible to the Andean races used in this study, complementing the findings by Acevedo *et al*. (2013). High resistance among the Mesoamerican genotypes emphasizes their usefulness in gene introgression to aid in the integrated management of bean rust. The races 29-1, 29-3, and 61-1 identified in this study were previously reported in Kenya by Arunga *et al*. (2012) and Nyang'au *et al*. (2016), and this highlights their predominance and importance in genotype screening for resistance in breeding programs. Race 63-1, identified in this study, has not been previously documented in Kenya.

5.1.3 Inheritance and co-segregation of rust resistance in *MU#13*

The segregation pattern of 3 resistant (R_):1 susceptible (rr) in the F_2 population and the 1R:1S ratio in the RILs population, resulting from crosses between *Amy* and *MU#13*, provided strong confirmation that the *MU#13* French bean line possesses at least one major dominant *Ur* gene conferring resistance to races 63-1 and 61-1. A similar conclusion can be drawn from the 3R_:1rr segregation ratio observed in the crosses involving *MU#13* with *Early Gallatin*, *Montcalm*, *PC-50*, *Golden Gate Wax*, and *Great Northern 1140*. The resistance profiles against bean rust displayed by these lines indicate that *Ur-MU#13* is distinct from *Ur-4*, *Ur-6*, *Ur-7*, and the unnamed locus (*Ur-?*) found in cultivar *Montcalm*. These results are synonymous with earlier studies that suggested that a single dominant gene mainly conditions resistance to bean rust (Hurtado-Gonzales *et al*., 2017; Souza *et al*., 2013; Souza *et al.*, 2011; Pastor-Corrales *et al*., 2008).

The allelic relationship study indicated that *MU#13* carries at least one dominant major gene segregating autonomously from the *Ur-3, Ur-3*$^{+}$, *Ur-5*, *Ur-11*, *Ur-13, Ur-14,* and

CNC (*Ur-?*) resistance gene loci. The segregation ratio 63R:1S observed in the F_2 mapping population for the cross between *CNC* and *MU#13* indicates that three independent dominant genes conditioned resistance in this population. This is expected as cultivar *CNC* was reported to carry two independent major *Ur* genes (Grafton *et al*., 1985), and the third gene was contributed from genotype *MU#13*. The *Ur* gene in *MU#13* confers resistance to bean plants at the seedling stage; therefore, it is distinct from *Ur-12* in cultivar *PC-50*. In genotype *PC-50*, the *Ur-12* gene confers host resistance to adult plants at the V4 growth stage, which corresponds to the fourth trifoliate leaf stage. Constant identification and characterization of novel resistance sources is imperative to effectively combat the extensive virulence diversity and variability existing within the population of the bean rust pathogen (Liebenberg and Pretorius, 2010; Souza *et al*., 2013). Thorough characterization of *Ur* genes originating from both the Mesoamerican and Andean gene pools holds vital significance within the domain of common bean breeding, as this broadens the spectra of *Ur* genes for introgression (Liebenberg and Pretorius, 2010).

The *Ur* gene in *MU#13* does not have an allelic relationship with known genes tested in this study; potentially, it is a new source of resistance to rust. Despite the fact that *Ur* genes have been mapped and tagged in common beans, most of them are found among dry bean cultivars, as reviewed by Souza *et al*. (2013). Additionally, French bean cultivars produced in Kenya are mainly imported and often have their resistance overcome by local races due to the variability of the bean rust pathogen across the globe. In this regard, *MU#13*, a locally adapted French bean line resistant to local races of the rust pathogen, would essentially be useful in dry and French bean improvement programs.

5.1.4 SSR molecular markers linked to rust resistance in *MU#13*

Genetic linkage mapping through bulk segregant analysis (BSA) can significantly reduce time and hasten efficiency in identifying molecular markers compared to analysis of the entire mapping population (Michelmore *et al.*, 1991). The BSA approach using the two extreme bulk samples was followed in mapping resistance to rust in *MU#13,* and this provided crude simulation in the F_2 and RIL mapping populations. The BSA approach was highly efficient in the elimination of unlinked markers while identifying candidate SSRs

linked to the resistance gene with less DNA samples for testing. Eleven of the SSRs used in this study produced a non-polymorphic band between *Amy* and *MU#13* parental genotypes. Low genetic divergence due to selection for specific quality traits among French bean genotypes (Arunga *et al.*, 2015) could explain the low levels of polymorphism observed. The SSR marker *BM161* was not informative between the parental genotypes, complementing the findings by Arunga (2012). The polymorphic fragments observed between *Amy* and *MU#13* parental genotypes for SSRs *Bmb583* and *PVM156-P3* with dissimilar alleles in the resistant and susceptible bulks denote the presence of a differential trait other than resistance to race 63-1.

The informative marker identified through BSA was SSR *PV-ctt001,* which was polymorphic between *Amy* and *MU#13* with similar alleles in the resistant bulk and susceptible bulk. The SSR *PV-ctt001* is located on linkage group (LG) 4, a region reported to harbour a complex cluster of genes providing resistance against common bean diseases including rust, angular leaf spot, and anthracnose (Valentini *et al*., 2017b). The LG-4 region is poorly covered by molecular markers (Meziadi *et al.*, 2016). The marker *PV-ctt001* was identified using both the RILs and the F_2 population and therefore emphasizes the occurrence of the resistance gene locus in genotype *MU#13,* located on LG 4. Arunga (2012) reported loose linkage confirmed by linkage analysis between *PV-ctt001* and the resistance gene, *Ur-MU#13*. Therefore, high-throughput molecular markers could be mapped on chromosome 4 of the genotype *MU#13* genome using better techniques such as SNP genotyping and genome-wide association studies (GWAS).

5.2 Conclusion

Bean rust disease is widespread in western and central Kenya's common bean production areas. Additionally, host plant resistance, crop management practices, environmental factors, and pathogen characteristics influence the prevalence and severity of common bean rust disease. Common bean production practices such as selection of resistant cultivars, elimination of bean debri and volunteer plants through soil incorporation, and informed and timely spraying of fungicides can aid in reducing the occurrence of bean rust disease.

Host plant resistance occurs in some common bean germplasm utilized by farmers for bean production. Such genetic materials are particularly useful in breeding for resistance against local common bean rust races. The resistant genotypes such as *Embean 14*, *Kat X56*, *KMR-11,* and *Enclave* can be desirably produced by farmers, considering their high resistance to bean rust. These genotypes can also serve as valuable reservoirs of rust disease resistance in the surveyed areas. This would particularly aid in eliminating the need to use fungicides, which are expensive and potentially hazardous to the environment. *Ur* genes from both gene pools should be deployed through gene pyramiding for durable resistance to rust. This can be achieved by utilizing one or more of the Mesoamerican genes (*Ur-3, Ur-3*$^{+}$, *Ur-5*, *Ur-11*, *Ur-14,* and *Ur-CNC)* in common bean improvement. Bean rust resistance in *MU#13* is a potentially novel locus, and this is a considerable step forward towards the development of elite French bean and dry bean resistant cultivars. Further, the resistance gene in *MU#13* is located on LG 4, a region that can be evaluated for high-throughput molecular markers to aid in MAS for rust resistance.

5.3 Recommendations

5.3.1 Recommendations derived from this study

i. There is a need to adjust the common bean breeding focus and include the incorporation of multiple *Ur* genes to aid in the integrated management of bean rust.

ii. Proper choice of resistant cultivars for production, management of crop debri, and use of fungicides can desirably be used in managing bean rust disease. Additionally, farmers need to be informed on the appropriate cultural practices to employ to reduce the incidence and severity of common bean rust.

iii. Bean rust can be controlled by resistant cultivars rather than fungicides, which can be harmful to the environment.

iv. Genotypes *Embean 14*, *Kat X56, KMR-11,* and *Enclave* can be desirably produced by farmers, considering their high resistance to bean rust.

v. Breeding for resistance can utilize local germplasm such as *MU#13*, *Kat X56,* and *KMR-11*, as well as one or more of the Mesoamerican genes (*Ur-3, Ur-3*$^{+}$, *Ur-5*, *Ur-11*, *Ur-14,* and *Ur-CNC)* in common bean improvement.

5.3.2 Recommendations for further research

i. It would be necessary for the comprehensive collection and characterization of bean rust isolates into physiological races for targeted introgression and deployment of resistant cultivars in Kenya.

ii. Inheritance studies, allelism tests, and the identification of molecular markers tagging resistance in the genotypes *Kat X56,* and *KMR-11* would be necessary in the understanding of the inherent genetic basis for resistance and their utility in common bean improvement.

iii. Further allelic relationship analysis between *Ur-MU#13* and other known *Ur* genes would be key in determining the novelty of the resistance in genotype *MU#13*.

iv. Fine mapping of the resistance in *MU#13* and development of robust, tightly linked SSR markers co-segregating with the *Ur* gene using better techniques such as SNP genotyping would be desirable in facilitating MAS.

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APPENDICES

Appendix 1. Modified Mahuku DNA extraction protocol (Mahuku, 2004).

1. Transfer DNA young leaves of 150 mg to a mortar and pestle and add acid washed sand. Macerate the leaves for 2 minutes and transfer them into a 1.5-mL Eppendorf (micro-centrifuge) tube. Add 500µL of TES extraction buffer (0.2 M Tris-HCl [pH 8], 10mM EDTA [pH 8], 0.5 M NaCl, 1% SDS).
2. Vortex the samples for 30 seconds to thoroughly mix and place the tubes in a water bath at 65 °C for thirty minutes.
3. Add one-half the volume (250µL) of 7.5M Ammonium acetate.
4. Mix and incubate the samples at -0.5 °C in a refrigerator for 10 minutes.
5. Centrifuge the samples at 15,000rpm for 15 minutes.
6. Transfer the supernatant to a new micro-centrifuge tube and add an equal volume (500µL) of ice-cold isopropanol.
7. Incubate the samples for 1-2 hours at -20 °C.
8. Centrifuge the samples at 15,000rpm for 10 minutes to pellet the DNA. Decant the supernatant and wash the DNA pellet with 800µL of cold 70% ethanol.
9. Turn the tubes upside-down on a clean sterile paper towels for 10-15 minutes to air-dry the DNA.
10. Elute the DNA from the pellet by adding 250µL 1xTE buffer (10mM Tris-HCl [pH 8], 1mM EDTA) centrifuging each time for 5 minutes at 15,000rpm to avoid collecting pelleted polysaccharides.
11. Transfer the DNA solution to a 1.5-mL micro-centrifuge tube and add 2µL of RNAse (10mg/mL) and incubate at 37~ for 60 minutes.
12. Recover the DNA and air-dry as described above. Elute the DNA in 50µL and store at -20°C.

Appendix 2. Gel electrophoresis (Green and Sambrook, 2019)

1. Prepare a 1.5% agarose gel by adding 100ml of 1X Sodium Borate ($Na_2[B_4O_5(OH)_4]\cdot 8H_2O$) buffer to 1.5g of Agarose in a conical flask.
2. Heat the mixture in a microwave for 3 minutes to dissolve the agarose.
3. Allow the molten gel to cool and add 5 µM ethidium bromide ($C_{21}H_{20}BrN_3$) (visualization dye). Mix the gel solution thoroughly by swirling gently.
4. Position the comb 0.5-1.0 mm above the plate so that a complete well is formed when the agarose solution is added into the gel casting plate. Pour the warm agarose solution into the gel casting plate.
5. Allow the gel to completely polymerize (20-45 minutes at room temperature) then pour a small amount of electrophoresis buffer and carefully remove the rubber stopper. Mount the gel in the electrophoresis tank and add enough electrophoresis buffer to cover the gel to a depth of approximately 1mm.
6. Mix the DNA sample with a loading dye in a ratio 1:5.
7. Load the sample mixture into the wells of the submerged gel using disposable micropipette tips. Load size standards (DNA ladder) into the wells on the right and left sides of the gel.
8. Close the lid of the gel tank and attach the electrical leads such that the DNA will migrate towards the positive anode.
9. Apply a voltage of 100 volts for a specified time frame depending on the PCR product.
10. View the DNA bands under ultraviolet light (UV) trans-illuminator.
11. Estimate the DNA quantity and quality using the band size and intensity of the standards.

Appendix 3. Data collection sheet

Data Collection Sheet: Common bean rust study

General Information:

County: ______________________ Subcounty: ______________________

Farmer name: ____

Farm coordinates: _________________________ Altitude: ____________

Contact information: ______________________

Date of data collection: ______________________

Size of the farm: ______________________

1. Cropping system:

☐ Sole crop (only common beans grown in the field)

☐ Intercrop

If intercrop, specify the crops: ____

2. Common bean cultivar under production:

Name of bean cultivar: ______________________

3. Seed source:

☐ Farmer saved seeds

☐ Local market seeds

☐ Certified seeds from merchants

4. Previous Crop Planted:

Name of previous crop: ______________________

5. Cultural practices:

a) Fungicide use:

Yes / No

If yes, specify the name of the

i. Fungicide name: ______________________

ii. Crop stage at which spraying is done and: ______________________

b) Crop debri management:

Removal of crop debri after harvest (Yes / No)

If yes, describe the method of crop debri management: ____
____

c) Crop spacing:

- Row-to-row spacing (in cm): _______
- Plant-to-plant spacing (in cm): _______

d) Management of Volunteer Plants:

Yes / No

If yes, describe the method of volunteer plant management: ____
____

Additional Notes: (Provide any additional information related to bean rust disease)

____
____
____
____
____

Note: The data collected from farmers will be used for research and educational purposes only. The information provided will be kept confidential and will not be shared with any third parties without prior consent.