COMBINING COMMON BEAN RUST RESISTANCE AND HEAT TOLERANCE IN SNAP BEANS (Phaseolus vulgaris L.) FOR EASTERN AFRICA

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COMBINING COMMON BEAN RUST RESISTANCE AND HEAT TOLERANCE IN SNAP BEANS (*Phaseolus vulgaris* L.) FOR EASTERN AFRICA

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Common bean rust caused by *Uromyces appendiculatus* (Per.:Pers) Unger, and heat stress limit snap bean production in many tropical and temperate regions. Snap beans that combined broad-spectrum rust resistance with heat tolerance in the same genetic backgrounds were developed and selected for tropical agroecosystems. Eight breeding populations were developed from combination of 'BelJersey-RR-15' and 'BelFla-RR-1' (each containing the *Ur-4* and *Ur-11* rust resistance genes) and heat tolerant snap bean breeding lines 'HT601', 'HT603', 'HT608' and 'HT611'. Three heat tolerant F_5 lines which were homozygous for the *Ur-4* and *Ur-11* genes were selected.

The three selected F_5 lines and a rust resistant but heat sensitive control were together with 12 cultivars evaluated in 2009 for reaction to natural rust infection and yield at six contrasting field sites in East Africa and response to heat stress verified in Puerto Rico. Rust incidence and severity was high at three sites. Three of the four breeding lines and only two of the 12 cultivars were rust resistant. The breeding lines showed stable yields in East Africa compared to cultivars currently grown in the region. Yield in Puerto Rico strongly correlated (R^2=0.71, P<0.001) with that of the hottest site in East Africa, highlighting similarity in genotypic response to high temperatures at the two distinct sites. This research demonstrated the effectiveness of *Ur-4* and *Ur-11* rust gene combinations in tropical environments, and effective selection for heat tolerance correlating across multiple environments.

The three breeding lines with proven rust resistance and heat tolerance were utilized in crosses with cultivars currently grown in East Africa with the aim of improving snap beans for the region for the two traits. Twenty breeding lines were selected from the populations developed, evaluated and selected at four distinct field sites in East Africa in 2010. Four high yielding breeding lines (‘L5’, ‘L9’, ‘L13’ and ‘L17’) that showed high promise with rust resistance and heat tolerance in desired plant types were selected. Advancement and subsequent release of these selections as cultivars and utilization in breeding programs will improve production of snap bean in eastern Africa and other tropical environments with similar production constraints.

BIOGRAPHICAL SKETCH

Charles Juma Wasonga was born in 1973 in Migori, Kenya. He was raised in Obama Village of Central Sakwa in Migori from where he attended Komolorume Primary School (1980-87), Rapogi High School (1988-1991) and later Egerton University, Njoro-Kenya. He graduated from Egerton with a BS in Horticulture in 1998 and MS Soil Science in 2004. He enrolled into the PhD program at Cornell in Fall 2006 as a Cornell Assistantship for Horticulture in Africa (CAHA) fellow. Prior to joining Cornell he coordinated for five years (2001-6) an adaptive research program with a Kenyan NGO – Environmental Action Team (EAT) which focused on research and training of rural farming communities in western and North Rift regions of Kenya on farm-level strategies for achieving household food security.

To God be the glory

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CHAPTER ONE

COMMON BEAN, RUST, AND HEAT STRESS

1.1 COMMON BEAN

1.1.1 Economic Importance and Market Classes

Common bean (*Phaseolus vulgaris* L.) is a short season crop that typically matures at 65-110 days after planting. It is the most important legume grown for direct human consumption in the world (Singh, 1999). World production of common beans exceeds 23 million metric tons, of which more than 30% is produced in Latin America and Africa (Broughton et al., 2003; Kelly, 2004). Currently, it is the second most important source of human dietary protein and the third most important source of calories for over 100 million people in rural and poor urban communities in Africa (CIAT, 2001). The crop is typically consumed as dry beans or as immature green pods, called snap beans (Smith and Goenaga, 2005), but is also harvested for its leaves in some regions. Snap beans are grown extensively in temperate regions for fresh-market and processing, and also in tropical and sub-tropical regions. Snap bean cultivars are developed for fresh-pod harvest and can be referred to as garden beans, French beans, stringless beans, wax beans, whole beans, Romano beans, bush beans, and green beans. They are characterized by succulent pods with reduced fiber (Myers and Baggett 1999). As such, seed and pod characteristics, which are broadly classified into dry beans (grown for the mature seed) and snap beans, define common bean market classes (Skroch and Nienhuis, 1995). In the case of dry beans, consumer preferences for seed types, color, shape, and brilliance or seed coat luster of dry bean vary significantly even within countries. However, many consumers also place high value on sweet taste and fast cooking attributes, and varieties that rank high in these attributes are in high demand and sometimes attain higher prices.

In terms of nutritional quality, dry bean is often characterized as a nearly perfect food because of its high protein content, high fiber content, complex carbohydrates and other nutritional benefits. A single serving (1 cup) of beans provides at least half of USDA's recommended daily allowance of folic acid, 25-30% of recommended levels of iron, 25% daily requirement of magnesium and copper as well as 15% of potassium and zinc (CIAT, 2001). Snap beans similarly have many nutritional benefits including being an excellent source of vitamin C, vitamin K and manganese and also a good source of vitamin A, dietary fiber, potassium, folate, iron, magnesium, thiamin, riboflavin, niacin, copper, calcium, phosphorus, protein, and omega-3 fatty acids (Netzer, 1992; USDA, 2007).

1.1.2 Domestication and Genetic Diversity

There are over 30 species in the genus *Phaseolus* but of these only five, namely *P. acutifolius* A. Gray (tepary bean), *P. coccineus* L. (scarlet runner bean), *P. lunatus* L. (Lima bean), *P. polyanthus* Greenman (year-long bean), and *P. vulgaris* L. (common bean), have been domesticated (Singh, 2001). Among these domesticated species, common bean is the most widely grown, accounting for more than 85% of production area sown to all *Phaseolus* species in the world (Singh, 2001). The common bean was domesticated more than 7000 years ago at two centers of origin – Mesoamerica/Middle America (Mexico and Central America) and the Andean region of South America. The domestication is believed to have started when common beans grew as weeds in fields planted with cassava and sweet potatoes in Central America. Over the millennia that followed, farmers grew complex mixtures of common bean types as a hedge against drought, disease and pests. This process resulted in a broad genetic array of beans with large variations in colors, textures, and adaptation to different regions and environments.

Common bean genetic resources consist of a complex array of major and minor gene pools, races and intermediate types, with occasional introgression between wild and domesticated-types (Broughton et al., 2003). These gene pools are composed of genotypes drawn from two centers of diversity of common bean: Middle American and Andean regions (Gepts 1990). Generally, Middle American forms comprise beans with small-sized seeds that are less variable in seed-coat color. In contrast, Andean types are larger seeded, have considerable range of seed-coat color, and are more widely distributed globally as commercial cultivars (Singh et al., 1991). Cultivars from the two gene pools were independently domesticated from biologically and geographically distinct wild populations (Sandlin et al., 1999). The separation of Andean and Middle American forms has been reconfirmed using various molecular markers (Becerra et al., 1994; Haley et al., 1994). Based on phaseolin protein patterns, snap beans appear to be of Andean origin while dry bean land races are of Middle American origin (Brown et al., 1982; Gepts et al., 1986; Skroch and Nienhuis, 1995).

There is a vast array of genetic diversity in *Phaseolus* which consists of about 65,000 accessions held in major germplasm banks. More than 90% of these accessions are *P. vulgaris.* The Centro Internacional de Agricultura Tropical (CIAT) collections, the largest in the world and held in trust for the Food and Agriculture Organization (FAO), includes over 36,000 entries, of which 26,500 are cultivated *P. vulgaris*, about 1300 are wild types of the common bean, and the rest are distant relatives of the common bean (CIAT, 2001). Core collections are more manageable and have been created to reduce the complexity that the large number of accessions poses to detailed evaluations of germplasm for useful traits. The core collections of domesticated common bean contain about 1400 accessions (Gomez, 2004). The USDA core collection contains 406 accessions representing both Andean and Middle American collections (USDA-GRIN). This genetic diversity makes it possible to improve beans

for quality, yield, pest and disease resistance and various environmental stresses that limit yield and quality.

1.1.3 Production Constraints

Abiotic and biotic constraints limit common bean production (Schwartz and Pastor-Corrales, 1989; Singh, 1992; Wortmann et al., 1998). Among the most widely distributed abiotic constraints to common bean production are edaphic factors such as low soil fertility, particularly deficiency of nitrogen, phosphorus, and zinc, as well as toxicities of aluminum and manganese. Drought is also an endemic abiotic constraint that affects bean production in many regions around the world including Latin America and Africa. High temperatures (>30°C day and/or >20°C night) in tropical lowlands (especially below 650 m elevation) and at higher latitude areas (e.g., California, Colorado, Idaho, Nebraska, Washington, and Wyoming in the USA) also severely limit bean production (Singh, 2001).

In the case of biotic stresses, bacterial diseases such as common bacterial blight (CBB), halo blight and brown spot are widespread problems from tropical to temperate bean growing environments. In relatively cooler and wetter areas, halo blight [caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkh.)] and bacterial brown spot (caused by *Pseudomonas syringae* pv. *syringae* van Hall) may cause severe yield losses. With respect to fungal diseases, angular leaf spot [caused by *Phaeoisariopsis griseola* (Sacc.) Ferr.], anthracnose [caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib.], and common bean rust (*Uromyces appendiculatus*) are considered among the most widely distributed foliar fungal diseases that cause severe yield losses of common bean in the Americas, Africa, and other parts of the world (Singh, 2001; Schwartz et al., 2005). Root rots caused by *Pythium* spp., Rhizoctonia spp., *Fusarium solani* f. sp. *phaseoli* (Burkh.) Snyd. &

Hansen and other soil borne pathogens are also found in most bean growing environments. Web blight [caused by *Thanatephorus cucumeris* (Frank) Donk.] in the warm humid tropics, and white mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] and ascochyta blight [caused by *Phoma exigua* var. *diversispora* (Bub.) Boerma] in cool wet regions, also occasionally become severe on common bean (Singh, 2001). Among the viral diseases, *Bean common mosaic virus* (BCMV, a potyvirus) causes severe yield losses in most bean production regions of the world.

Insect pests are also among the biotic constraints that affect common bean. Among the insect pests that cause significant yield losses, are leafhoppers and aphids, which apart from actually inflicting damage on the crop also transmit viruses which further compound losses in yield. Bean fly which is also known as the bean stem maggot (*Ophiomyia* spp) is by far the most damaging insect pest of common bean in Africa (Abate and Ampofo, 1996; Wortmann et al., 1998). Bean weevil *Zabrotes subfasciatus* (in warm tropical and subtropical environments) and *Acanthoscelides obtectus* (in cool and temperate environments) also causes severe postharvest losses when dry beans are not properly stored (Singh, 2001).

1.2 COMMON BEAN RUST

1.2.1 Biology and Host Interactions

The common bean rust fungus *Uromyces appendiculatus* (Per.:Pers) Unger is a Basidiomycete of the order Uredinales (Agrios, 2005). As with many other rust pathogens, species within the genus *Uromyces* are obligate parasites that establish biotrophic relationships with their host plants. Host plant infection by *Uromyces* can be divided into three phases: penetration phase, followed by a biotrophic phase in which it lives parasitically in the host, and then sporulation phase (Mendgen and Hahn 2002). The penetration phase begins with a uredospore landing on host leaf surface

and forming an adhesion pad that enables its attachment to the leaf cuticle. Depending on water availability on the leaf surface, the uredospore germinates to produce a germ tube that grows toward a stoma guided by physical cues on the leaf surface and, upon contact with stomatal guard cell lips forms an appressorium (Hoch and Staples 1987). The appressorium drives a penetration hypha into the substomatal cavity. Morphogenesis of the infection hypha continues until it comes into contact with a mesophyll cell when it differentiates a haustorial mother cell at the hyphal tip. The haustorial mother cell produces a haustorium that penetrates host cell to commence the biotrophic phase. Through the haustorium, the pathogen secretes into the host cell cytoplasm effector proteins that may alter host metabolism and defense pathways (Catanzariti et al., 2007). The pathogen also absorbs nutrients from the host through the haustorium which is surrounded by an extrahaustorial matrix (Mendgen et al., 2006). The fungus continues to grow through the host tissues and, as it matures it develops sporogenous tissue that marks the beginning of a sporulation phase in which new spores are formed. The growth of rust on the plant reduces photosynthetic competence of infected leaves, which negatively affects yield (Lopes et al., 2001).

1.2.2 Life Cycle and Epidemiology

Common bean rust has an autoecious, macrocyclic life cycle that completes all stages on the bean host (McMillan et al., 2003; Agrios, 2005). The life cycle begins with overwintering teliospores from the previous growing season's rust infected host debris germinating to produce metabasidia (Stavely, 1994; McMillan and Schwartz, 2003). Meiosis occurs in the metabasidium and results in production of basidiospores that infect the new season's bean plants. Basidiospore infection produces spermagonia (pycnia) on the upper (adaxial) leaf surface. Pycniospores contained in a pycnium move to pycnia of the opposite mating type and cross-fertilize with compatible

pycniospores resulting in development of snowy white aecial horns on the lower surface of the leaf below the pycnia. Aeciospores released from the white aecial horns then infect new bean leaves, erupting in the (reddish-orange) uredinial bean rust that can travel long distances and infest other bean fields. The uredinial rust stage can infect new plants, and release new spores in repeating cycles several times during the growing season. At the end of the growing season as bean plants begin to change color, rust infected plants produce teliospores (black rust) that overwinter in bean debris and initiate the cycle again in the following spring season. Even though *U. appendiculatus* has a mixed reproduction system, there is less occurrence of the sexual stage in the tropics and subtropics where live host tissue is always available (Taylor et al., 1999). Low frequency of occurrence of the sexual stage under such conditions has been suggested as a contributor to evolution of asexual populations of the pathogen (Taylor et al., 1999).

Moderate temperatures (17-25^{o}C) and high relative air humidity (>95%) over long periods of time provide the most favorable conditions for *U. appendiculatus* incidence (Souza et al., 2007). Moderate temperatures and duration of plant-surface moisture for 10-18 hours favor infection by urediniospores (Stavely and Pastor-Corrales, 1989). Germination of urediniospores occurs in 6-8 hours at optimal temperatures of 16-25^{o}C. High humidity below the saturation point, long day lengths and succulent host tissue favor abundant urediniospore production. Urediniospores are dispersed by wind currents and can be carried long distances.

1.2.3 Population Biology and Host Interactions

Rust is highly variable with more than 300 races that vary in pathogenicity on bean cultivars having been reported worldwide (Mbaga et al., 1996; Araya et al., 2004; Avecedo et al., 2006). Little is known about the geographic distribution of

recombining populations in the pathogen (Henk et al., 2006). Virulence diversity of rust varies in time and space and, as such bean, varieties that are resistant in one year or location may be susceptible in another. Understanding the virulence and genetic diversity and the evolution of the rust pathogen, as well as the diversity resistance in its common bean host, is essential to the development and selection of bean cultivars with durable resistance.

Two distinct groups of bean rust isolates have been distinguished using differential host cultivars and molecular techniques including random amplified polymorphic DNA (RAPD) analysis (Sandlin et al., 1999; Araya et al., 2004; Pastor-Corrales, 2006; Henk et al., 2006). One group is identified as Andean and is made up of isolates that have narrow and specific host range; being compatible only with or mostly with Andean bean cultivars. It occurs in areas like Equador and Mozambique where Andean bean cultivars predominate. The other group is called Middle American and has isolates with broad and nonspecific host range; being compatible with Andean and Middle American beans and often found in Central America, Mexico and other countries where Middle American beans predominate. Differential distribution of resistance to *U. appendiculatus* across host populations in these regions underscores the influence of host diversity on the evolution of virulence of the pathogen in these areas (Avecedo et al., 2006). Considerable variation in races of rust also occurs in Africa where the different bean genotypes are presently grown (Beaver et al., 2003; Jochua et al., 2004; Liebenberg et al., 2006).

1.2.4 Control of Rust

On a worldwide scale, appropriate control measures are needed to mitigate the high yield losses attributed to rust. Rust disease management practices include crop rotation, soil incorporation of bean debris, timely planting, timely irrigation, timely

spraying of fungicides, and use of resistant cultivars (Stavely and Pastor-Corrales, 1989). However, growing resistant cultivars remains a more sustainable strategy for managing the disease and hence there is a need to continuously improve cultivars for resistance.

Major gene resistance of the race-specific type and partial or quantitative resistances have been reported in beans (Ochoa et al., 2007). There are nine rust resistance genes that have been identified, characterized, and named (Basset, 2004; Kelly et al 1996; Liebenberg et al. 2006). All of these genes, including *Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, *Ur-7*, *Ur-9*, *Ur-11*, *Ur-12*, and *Ur-13*, are dominant. Some of the genes originate from the Andean gene pool (*Ur-4*, *Ur-6*, *Ur-9*, *Ur-12* and *Ur-13*), while others (*Ur-3*, *Ur-5*, *Ur-7*, and *Ur-11*) are from the Middle American gene pool (Pastor-Corrales 2007; Steadman et al 2002). However, high diversity of pathogenic races that also vary in time and space coupled with frequent changes in virulence that is associated with race-specific type of resistance in the cultivars remains a major challenge and calls for strategic deployment of resistance genes in ways that ensure durable resistance in cultivars of interest (Jung et al., 1998; Hillocks et al., 2006; Miklas et al., 2006).

Resistance genes in Andean originated beans tend to fail to confer resistance against Andean races of rust pathogen; however, these genes are often very effective against many important Middle American races (Pastor-Corrales, 2006). Resistance genes from Middle American bean cultivars are susceptible to many Middle American races but particularly resistant to most Andean races. For example, *Ur-11* a rust resistance gene of Middle American origin is resistant to all known races of the rust pathogen except race 108, which is of Middle American origin. The Andean *Ur-4* rust resistance gene is susceptible to most Andean races of the rust pathogen, but is resistant to race 108 and to many other Middle American races. The *Ur-3* and *Ur-5*

rust resistance genes of Middle American origin are susceptible to several Middle American races of the rust fungus but provide resistance to most Andean races.

1.2.5 Sources of Rust Resistance

Many of the rust resistance genes confer resistance against multiple races of rust and are found in different bean cultivars (Correa et al., 2000). Bean cultivars or breeding lines with the different rust resistance genes include: Ouro Negro which is a black type has resistance gene *Ur*-Ouro-Negro (Correa et al., 2000); BelMiDak-RMR-10-12, a Navy type has *Ur-4* and *Ur-11* (Pastor-Corrales, 2003); BelDakMi-RMR-19-23, a Pinto bean has *Ur-3, Ur-4, Ur-6* andUr-*11* (Pastor-Corrales, 2003); BelMiNeb-RMR-9-13, a Great Northern bean has *Ur-3, Ur-6 and Ur-11* (Pastor-Corrales, 2003); BelNeb-RR-1, a Great Northern bean has *Ur-5, Ur-6* and *Ur-7* (Stavely et al., 1989); Merlot, a Red Mexican or a small red type has *Ur-3* (Hosfield et al., 2004); Rosada Nativa, a Pink or a Cranberry type has *Ur-5*; PC-50 a Red mottled type or a Dark red kidney type has *Ur-9* and *Ur-12*; BelDade-RGMR-4-6, a Snap type has *Ur-3+* and *Ur-4* (Stavely et al., 1997). Great northern bean cultivars: BelMiNeb-RMR-8, -9, -10, -11, -12, and -13 and pinto bean types: BelDakMi-RMR-19, -20, -21, -22, and -23 that combine two Middle American (*Ur-3* and *Ur-11*) and two Andean (*Ur-4* and *Ur-6)* rust genes are resistant to all known races of rust (Pastor-Corrales et al., 2007).

1.2.6 Detection of Rust Resistance Genes

Presence of these rust resistance genes can be tested in genotypes of interest by utilizing greenhouse and laboratory screening techniques that involve plant evaluation for rust symptom development after inoculation with different races of the pathogen, field screening in which the cultivars are evaluated for resistance to rust under field conditions or by utilizing molecular markers linked to the genes as selection tools (Correa et al., 2000; Araya et al., 2004; Jochua et al., 2004; Park et al., 2004;

Liebenberg et al., 2006; Miklas et al., 2006). For example, to identify bean genotypes that contain both *Ur-4* and *Ur-11* rust resistance genes through the greenhouse evaluation method, inoculation with race 67 tested the presence of *Ur-11* while race 108 on the other hand tested for *Ur-4* (Pastor-Corrales, 2006).

Various limitations are associated with these different screening techniques. Greenhouse/laboratory screening requires highly controlled conditions for successful testing including inoculation with the right isolate of the pathogen, maintaining separate distinct cultures of the pathogen and suitable conditions for disease symptom development and evaluation. Field evaluation on the other hand may be less efficient because disease development, which needs to be uniform and severe, is highly dependent on the existence of environmental conditions that favor rust development. Escapes may result during field evaluations under unfavorable environmental conditions for rust and lead researchers to the wrong conclusions (Sillero et al., 2006).

Molecular markers linked to some of the bean rust genes have been identified and published for utilization in breeding programs to enhance efficiency by which rust resistance genes are identified and deployed into bean genotypes, (Johnson et al., 1995; Correa et al., 2000; Kelly et al., 2003; Park et al., 2004; Miklas 2006; Swart et al., 2006). To improve reproducibility of RAPD markers, sequence characterized amplified region (SCAR) markers, derived from corresponding RAPD markers, have become the basis for the indirect selection of economically viable traits in bean breeding (Kelly, 2004). For instance, markers linked to race specific disease resistance genes form the basis for indirect selection for major gene resistance (Hillocks et al., 2006). Marker-assisted selection offers a way to overcome problems of masking of hypostatic genes and inadequate inoculation techniques, resulting in disease escape in conventional screening. It has also been possible to identify linkages between markers and quantitative trait loci controlling complex traits such as stress tolerance (Schneider

et al., 1997). Some of the rust genes with linked RAPD markers include *Ur-3* that is found in NEP II and PI 181996 of Middle American gene pool; *Ur-4* that is found in Early Gallatin in the Andean gene pool; *Ur-5* in Mexico 309 in the Middle American gene pool; and *Ur-9* in Pompadour from the Andean gene pool (Kelly and Miklas, 1998). RAPD and SCAR markers have been linked to the *Ur-6* Andean gene that controls specific rust resistance in common bean (Park et al., 2004). SCAR markers have also been developed for *Ur-11* and *Ur-13* (Boone et al., 1999; Souza et al., 2003; Queiroz et al., 2004; Liebenberg et al., 2006).

There are at least two published SCAR markers for the *Ur-11* gene: SAE19 (Queiroz et al., 2004) and UR11-GT2 (Miklas et al., 2002). However, a limitation with some of the current molecular markers is low utility across different genetic backgrounds or gene pools of the common bean (Steadman et al., 2002). For example the published markers for the *Ur-11* gene have not been reproducible across different bean genetic backgrounds (Steadman et al., 2002). Close linkage of *Ur-11* to *Ur-3* possibly explains the lack of reproducibility of its published markers (Miklas et al., 2002; Steadman et al., 2002).

1.3 HEAT STRESS

Heat stress may be defined as exposure to temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development (Wahid et al., 2007). Heat stress inducing temperatures exert negative effects on plant growth and development, which reduces yield and quality of many crops (Maestri et al., 2002; Sato et al., 2006). Exposure of plants to heat stress induces a variety of physiological and molecular changes that negatively affect plant growth and development, which translate into reduced yield and quality in various crop species (Wahid et al., 2007).

On a global scale, the negative effects of heat stress on crop yield and quality is a major concern in many areas. Adverse effects of high temperatures on plant growth and productivity in natural and managed ecosystems is of concern on a global scale in light of current predictions that average global atmospheric temperature will rise 2.6 °C by 2050 relative to 1990, and 5.8 °C by 2100 (IPCC, 2001). On a regional scale, many areas around the world presently experience periods of heat stress that occur within crop production seasons and cause significant reductions in yield especially when the hot periods coincide with critical stages of plant development. Furthermore, there is a need to expand the world's agriculturally productive areas into warmer climates so as to increase food supplies to meet nutritional needs of the world's rapidly growing population. Crops that have the ability to maintain yield even when exposed to heat stress (heat tolerant crops) are therefore needed for such areas. Strategies for enhancing crop tolerance to heat stress are needed in order to increase and sustain high crop productivity.

In order to identify or develop functional and sustainable strategies for enhancing crop tolerance to heat stress, it is important to understand the mechanisms by which heat stress inducing temperatures cause losses in yield and quality in crop plants in general and snap beans in particular. Both transitory and constantly high temperatures cause a range of morpho-anatomical, physiological and biochemical changes that adversely affect overall plant growth and development (Wahid et al., 2007). These changes include physiological damage at different levels of the plant's structural organization. Heat stress induces morphological changes such as reduction in dry matter production and partitioning that lead to reduced pod and seed formation and hence low yield in snap bean (Omae et al., 2007; Rainey and Griffiths, 2005b). Various anatomical changes at cellular and sub cellular levels have also been attributed to heat stress. At the cellular level, heat stress has been shown to affect cell

membrane thermostability by increasing cell membrane permeability in various plant species including common bean (Li et al., 1991), cowpeas (Ismail and Hall, 1999), grape (Zhang et al., 2005), and cotton (Rahman, 2006). At the sub cellular level, effects of heat stress include: water loss from chloroplasts (McCain et al., 1989) and damage to primary photosynthetic processes and thylakoid membranes (Wahid et al., 2007; Petkova et al., 2007). These sub cellular level effects reduce photosynthesis and lower accumulation of photosynthates by plants.

In the case of common bean when average maximal day and minimal night temperatures exceed 30°C and 20°C, respectively, during reproductive development, yield reductions occur (Rainey and Griffiths, 2005a). High temperatures disrupt fertilization and ovule development which leads to reduced seed set and deformed pods (Porch and Jahn, 2001; Omae et al., 2007). In snap bean, heat stress during reproductive development can cause floral abscission resulting in reduced pod number (Li et al., 1991; Rainey and Griffiths, 2005a). Bean genotypes have also been shown to differ in cell membrane stability following exposure to heat stress (Li et al., 1991). Thus, genetic variability in terms of heat tolerance exists in common bean. Genotypes with tolerance are used in crosses to develop heat tolerant lines (Porch and Jahn, 2001; Rainey and Griffiths, 2005b; Petkova et al., 2007). It is therefore possible to breed snap bean for increased.

Various physiological changes that condition plant tolerance to heat stress are induced following exposure to the stress. Plant production of antioxidants including ascorbic acid and glutathione that scavenge reactive oxygen species (ROS) produced in response to heat stress and which would otherwise cause oxidative damage on the plant is a case in point (Alscher, 1989). Genetic variability in antioxidant content and activity has been reported in several species including common bean (Guzy and Heath, 1993; Gonzalez et al., 1998; Zheng et al., 2000; Burkey and Eason, 2002). However, it

is not known if bean tolerance to heat stress is correlated with antioxidant capacity and/or activity and the extent to which this (antioxidant production and/or activity) may account for bean genotype differences in heat tolerance is presently less understood. Cell membrane thermostability (CMT) as indicated by leaf electrolyte leakage measurements following exposure to heat stress has been used to predict variations in heat tolerance among genotypes within different plant species including cowpea, cotton and common bean (Li et al., 1991; Thiaw and Hall, 2004; Rahman, 2006). A highly significant correlation between CMT and yield was demonstrated among cowpea genotypes following exposure to heat stress during the reproductive development stage indicating an association between CMT and heat tolerance (Ismail and Hall, 1999). Genotype variation in CMT, which influences heat tolerance, may possibly arise from differences in cell membrane structure and/or ability to protect and maintain integrity of cell membrane structure and associated ion/metabolite transport proteins. Plant production and maintenance of activity of antioxidants, which are known to scavenge ROS generated under heat stresses, and which would otherwise damage the integrity of the cell membrane, may serve this protective function on the cell membrane. However no studies have been conducted to demonstrate the extent to which antioxidant production and activity may account for genotype differences in cell membrane thermostability and hence heat stress tolerance.

1.4 COMMON BEAN IN AFRICA

Beans are grown on over four million hectares each year in Africa and provide food for more than 100 million people in the region (Wortmann et al., 1998). It is the second most important source of dietary protein and the third most important source of calories for lower income African households after cassava and maize (Broughton et al., 2003). The two main environments for bean production are the cool highlands

of East and Central African countries (including Kenya, Uganda, Tanzania, Rwanda and Burundi) and the warmer mid-elevation areas of DR Congo, Ethiopia, and several countries of Southern Africa (Asfaw et al., 2009). The bean cropping systems in Africa includes production as individual crops or as intercrops with maize, banana, root or tuber crops. The crop is suited to these cropping systems because of its rapid maturity and shade tolerance. Production is primarily by smallholder farmers, especially by women, traditionally for home consumption and now increasingly for income generation (CIAT, 2001).

There is variation in growth habit of beans grown in Africa which ranges from determinate bush to indeterminate and vigorous climbing bean types. Bush beans are the most predominant types grown in the region. However, climbing beans, which were originally restricted to small pockets of higher and more fertile soils in northern Rwanda, northeast DR Congo and Malawi, are now spreading to other areas and countries, particularly to those areas where land is limiting and human population density is high. In terms of market classes, there are about nine commercial seed types grown in Africa. Of these types, the Calima (Rosecoco or mottled red) and the reds (large and small) account for about 50% of the production, primarily because of their high market demand. Other market classes include the navy beans, cream-colored, brown tan, yellow types, purples, white and blacks.

1.5 SNAP BEANS IN EAST AFRICA

Snap beans are probably the most important high value beans grown in East and Central Africa. In Eastern and Southern Africa, snap beans are grown mainly for export to regional and international markets especially Europe and the Middle East and are a significant source of income for growers in Kenya, Tanzania, Uganda, and Sudan (Silbernagel et al., 1993; CIAT, 2004; Okello and Roy, 2007). Even though

they are grown mainly for export markets, domestic markets especially in the urban areas are growing rapidly. However, snap bean productivity is presently low in many areas in the region due to diseases such as rust and abiotic stresses including nutrient deficiency, drought and high temperatures (Wortmann et al., 1998; CIAT, 2004, 2008; Kelly, 2004). Common bean rust and heat stress often occur within the same production regions, such as Eastern Africa, and significantly reduce snap bean yield.

In Eastern Africa as in many other areas in sub-Saharan Africa, there is high rust pressure on snap beans as a result of mixed and intensive nature of the cropping systems in which live tissues of both dry and snap beans are available in farms all year round and sustain propagation of the rust pathogen. Bean genotypes from Andean and Middle America gene pools are grown in the Eastern Africa though distribution of genotypes from these gene pools varies by country (Asfaw et al., 2009). For example, a recent study on common bean landraces in the region showed that Middle American genotypes are predominant in Ethiopia while Andean genotypes are predominant in Kenya (Asfaw et al., 2009). The growing of bean genotypes from the different gene pools has, over time, led to a proliferation in different races of the bean rust pathogen with which the bean genotypes coevolved at the centers of origin (Beaver et al., 2003; Jochua et al., 2004; Liebenberg et al., 2006; Pastor-Corrales, 2006). Furthermore, new races of the rust pathogen may have possibly arisen from recombination of populations within the intensive bean production systems. However, little is presently known about the geographic distribution of recombining populations (Henk et al., 2006).

Genetic resistance is a practical, efficient, and cost-effective strategy for managing bean rust (Stavely and Pastor-Corrales, 1989). However, deployment of rust resistance genes in common bean market classes and cultivars of interest to ensure sustainable management of the disease has remained a challenge due to the high

variability in races of the bean rust pathogen coupled with lack of information on prevalent races of the pathogen in places such as East Africa. A large number of bean genotypes, previously improved for resistance to rust have not remained resistant across all sites and seasons and most snap bean cultivars grown in Eastern and Southern African countries are very susceptible to rust (Kimani et al. 2002; Mutunga et al. 2002; Jochua et al. 2004; Hillocks et al., 2006). Recent studies have shown that combinations of *Ur-4* and *Ur-11* rust resistance genes in bean genotypes confer resistance against all races of the rust pathogen (Pastor-Corrales, 2006). However, bean genotypes with the *Ur-4* and *Ur-11* gene combinations have not been widely tested, documented or adopted in the snap bean growing regions in East Africa.

In the East African region snap bean production is presently limited to cool highland areas above 1500m as higher temperatures that prevail at lower altitudes reduce yield of the available cultivars. This situation prevents a large number of farmers with landholdings in the warmer environments from effectively engaging in the production of snap beans. Moreover, even in the cooler highland areas snap bean production is vulnerable in the longer term especially if anticipated global changes in climate inevitably result in high temperatures that adversely affect plant growth and productivity within agro-ecosystems (IPCC, 2001; Challinor et al., 2007; Wahid et al., 2007; Tubiello et al., 2008). Genetic improvement of snap bean cultivars grown in the East Africa region for rust resistance and heat tolerance is needed to minimize yield loss attributed to rust disease and increase production in areas and seasons with higher than optimal growth temperatures. Development of snap bean cultivars adapted to higher temperatures will expand the set of crop choices for growers in such areas and would also enable farmers to moderate effects of or adapt to spatial and temporal variability in climatic parameters such as temperature.

1.6 RESEARCH OBJECTIVES

The combined challenges that common bean rust and high ambient temperatures (heat stress) pose to snap bean production in East Africa and similar environments which calls for the development of cultivars with the ability to perform well under these two stresses formed the focus of this study. The goal of this study was to evaluate and improve snap beans to enable increased production in East Africa and similar environments through the targeted combination of rust resistance genes and heat tolerance traits in the same genetic background. Specific objectives of the study were to:

1) Develop and select snap bean breeding lines with combinations of rust resistance and heat tolerance traits in the same genetic background.
2) Evaluate the selected breeding lines combining the two traits under field conditions in East Africa for yield and quality.
3) Evaluate and document the reaction of the target rust resistant and heat tolerant breeding lines to prevalent races of the rust pathogen and other important common bean diseases in the East African region
4) Utilize heat tolerant and rust resistant breeding lines with the best performance in greenhouse and field environments to genetically improve current cultivars for the East African region for the two traits while maintaining important quality attributes.

CHAPTER TWO

COMBINING COMMON BEAN RUST RESISTANCE AND HEAT TOLERANCE IN SNAP BEANS

2.1 Introduction

Common bean rust, caused by the basidiomycete fungus, *U. appendiculatus* (Per.:Pers) Unger, is a destructive disease of dry beans and snap beans worldwide. It is a particularly endemic and severe disease of snap beans in Eastern and Southern Africa (Wortmann et al. 1998; Kimani et al. 2002; Mutunga et al. 2002; Jochua et al. 2004; Kelly 2004; Liebenberg 2003). Common dry bean market classes including small red, pinto, navy and snap beans, are typically susceptible to bean rust. Yield losses attributed to bean rust range from 18-100% and damage is particularly high in humid and tropical areas where severe epidemics are frequent (Stavely and Pastor-Corrales 1989; Liebenberg 2003; Sillero et al. 2006). Snap bean cultivars with resistance gene combinations targeted to protect crops against common bean rust could reduce or eliminate the heavy dependence on fungicides while simultaneously lowering production costs and improving crop quality.

While genetic resistance is a cost-effective, practical, and environmentally sound strategy for managing bean rust, effective resistance over time and space has been difficult to achieve due to the high diversity of virulence in *U. appednidculatus* (Stavely and Pastor-Corrales 1989). The bean rust pathogen is highly-variable with more than 300 strains, known as races, that differ in virulence (Stavely 1984; Stavely and Pastor-Corrales 1989; Mbaga et al. 1996; Pastor-Corrales 2001; Araya et al. 2004; Acevedo et al. 2006). Due to the vast virulence diversity of the pathogen, common bean cultivars that are resistant to bean rust in one location or year may be susceptible in another. Moreover, resistance conferred by single resistance genes has often failed

due to the appearance of new races of the bean rust pathogen. Effective genetic resistance strategies to manage bean rust should therefore aim to combine multiple rust resistance genes in order to provide a broader and longer-lasting, or more durable resistance (Pastor-Corrales, 2006; Pastor-Corrales et al., 2007).

Genetic variation within the common bean rust pathogen mirrors the genetic variation for resistance in common bean. There are nine rust resistance genes that have been identified, characterized, and named (Kelly et al 1996; Liebenberg et al. 2006). All of these genes: *Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, *Ur-7*, *Ur-9*, *Ur-11*, *Ur-12*, and *Ur-13*, are dominant. The genes *Ur-4*, *Ur-6*, *Ur-9*, *Ur-12* and *Ur-13* originate from beans of the Andean gene pool, while *Ur-3*, *Ur-5*, *Ur-7*, and *Ur-11* are from beans belonging to the Middle American gene pool (Pastor-Corrales 2007; Steadman et al 2002). The *Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, and *Ur-11* genes provide resistance to 44, 30, 70, 22, and 89 races, respectively, of the 90 races maintained at the United States Department of Agriculture (USDA)-Agriculture Research Service (ARS)-Beltsville Agricultural Research Center (BARC) (Stavely 2000; Pastor-Corrales 2001). The *Ur-11* gene is the most effective of all rust resistance genes known and is susceptible only to the Middle American (*U. appendiculatus*) race 108 from Honduras (Pastor-Corrales et al., 2007). The Guatemalan plant introduction (PI) 'PI 181996' which is one of the original sources of *Ur-11* was resistant to approximately 250 different isolates of the rust pathogen from Eastern and Southern Africa (Liebenberg, 2003). The Andean *Ur-4* resistance gene which is susceptible to many known Andean races of the rust pathogen is resistant to race 108 that overcomes *Ur-11* and to many other Middle American races (Pastor-Corrales 2006). The combination of *Ur-4* and *Ur-11* genes provides resistance to all races of the pathogen maintained at the BARC (Pastor-Corrales et al. 2007).

Adding to the challenge of bean rust in many tropical snap bean production environments is heat stress. Exposure of plants to heat stress reduces the yields and quality of many crops (Maestri et al. 2002; Sato et al. 2006; Wahid et al. 2007). The adverse effects of heat stress on plant growth and productivity is of concern on a global scale in light of the current predictions that the average global atmospheric temperatures will rise 2.6 °C by 2050 relative to 1990, and rise 5.8 °C by 2100 (IPCC 2001; Challinor et al., 2007; Tubiello et al., 2008). Many areas around the world presently experience periods of heat stress that coincide with crop production seasons and significantly reduce yields. The production of crops, including common bean, are being expanded into more marginal, and warmer zones to meet nutritional needs of the world's growing human population (Porch et al. 2007).

In common bean, average maximum (day) and minimum (night) temperatures exceeding 30 °C and 20 °C, respectively during reproductive development reduces yield (Rainey and Griffiths 2005a). Heat stress affects pollen development, and disrupts fertilization and ovule development in common bean, which leads to bud and flower abscission, development of deformed pods, and reduced pod number (Omae et al. 2007; Porch and Jahn 2001). There is genetic variability in tolerance to heat stress within the common bean (Petkova et al. 2007; Porch et al. 2004; Rainey and Griffiths 2005b). Snap beans with tolerance to heat stress could increase productivity in existing high temperature environments, as well as expand production areas and/or enable production during higher temperature seasons of the year.

The objectives of the research were: 1) to develop and evaluate snap bean populations that combine the *Ur-4* and *Ur-11* rust resistance genes with heat tolerance, 2) to select from subsequent generations of these populations breeding lines that combine rust resistance (based on the *Ur-4* and *Ur-11* gene combination) and heat tolerance in the same genetic background for use in future cultivar improvement

2.2 Materials and Methods

2.2.1 Plant materials and population development

Two USDA-ARS-BARC bean breeding lines, 'BelFla-RR-1' and 'BelJersey-RR-15', both of which have the *Ur-4* and *Ur-11* rust resistance genes, were used as sources of resistance. 'BelFla-RR-1' (BF) was a fresh-market bush type green bean with the *Ur-11* gene introgressed from 'PI 151385', and the *Ur-4* gene from 'Early Gallatin'. 'BF' also contains the *I*-gene that confers resistance to Bean Common Mosaic Virus (BCMV) (Stavely and McMillan, 1991). 'BelJersey-RR-15' (BJ) was a processing bush type green bean with the *Ur-11* gene introgressed from 'PI 181996' and the *Ur-4* gene from 'Early Gallatin'. 'BJ' also contains the *I*-gene for BCMV resistance (Stavely and Steinke, 1992). Four snap bean breeding lines, 'HT601', 'HT603', 'HT608' and 'HT611', were used as sources of heat tolerance. The heat tolerant lines were developed at Cornell University, Geneva, New York (Rainey and Griffiths, 2004; Rainey and Griffiths 2005c). The four heat-tolerant parents were early maturing; had fleshy, straight, long and smooth textured pods; and a bush-type growth habit with pod set concentrated in the upper two-thirds of the canopy.

Eight populations were developed in March and April of 2007 from crosses involving the two rust resistant lines ('BF' and 'BJ') with each of the four sources of heat tolerance ('HT601', 'HT603', 'HT608' and 'HT611'). The parents were crossed using a hooking method without emasculation (Bliss 1980). The eight F_1 hybrids were self-pollinated and subsequent generations of each of the eight populations were evaluated for rust resistance or yield under heat stress in greenhouse comparisons.

2.2.2 Selection for heat tolerance in the greenhouse

The F_2 generations were grown under heat stress for preliminary selection of plants under high temperature conditions. The two sets of parents and also two snap

bean cultivars: ‘Hystyle’ and ‘Juliet’ were used as controls. Two seeds (later thinned to one plant after germination) were planted in each pot on October 7, 2007 in 20 cm diameter and 20 cm deep round plastic pots filled with ‘Cornell mix’ growth medium (Broodley and Sheldrake 1972). Forty-eight plants were grown from each of the eight F_2 populations and eight plants were grown for each control. Sets of plants of each of the eight populations and controls were randomly assigned to separate benches within the greenhouse in a completely randomized design (CRD). The plants were uniformly irrigated and fertigated. Plants were staked and tied to ensure upright growth within the pots. Greenhouse temperature was maintained at 24°C/21°C for the first three weeks after which it was raised and maintained at 32°C/27°C until the end of the crop cycle in order to ensure heat stress commenced several days before anthesis.

The F_2 plants were visually selected for pod set, pod quality and growth habit. The data collected at harvest included pod number per plant, seed weight per plant, seed number per plant, seeds per pod and single seed weight. Single seed weight was recorded to guide selection for pod size, since seed weight and pod size are highly correlated.

Seed was harvested from the F_2 selections and five progeny from each selection were tested in February 2008 high temperature stress as described for the F_2 experiment. For the F_3 heat stress evaluation, cultivar controls included ‘Bronco’, ‘CT70’, ‘HB1880’, ‘Masai’, ‘Spartacus’, ‘Venture’, and those used in the F_2 screen. Twenty one breeding lines derived from additional crosses were also evaluated. Plants were randomized throughout the greenhouse and maintained as previously described. Means of four yield components: pod number per plant, seed number per plant, seed number per pod and seed weight per plant were independently ranked and mean rankings were calculated.

2.2.3 Selection for rust resistance

F_4 lines selected for tolerance to heat stress at the F_2 and F_3 generations were evaluated for homozygosity of two dominant rust genes, *Ur-4* and *Ur-11*, following inoculation of bean seedlings with races 67 and 108 of the bean rust pathogen under controlled greenhouse conditions at USDA-ARS-BARC. The inoculations were conducted between May 6 and June 18, 2008. To distinguish the reaction of the breeding lines to the two races of the bean rust pathogen, primary leaves of each seven-day-old bean seedling inoculated with race 67 were cut at the tip while the leaf inoculated with race 108 was left intact. The inoculum (urediniospores), at a concentration of 60,000 spores ml^{-1}, was dispersed in water using Tween 20. To promote pathogen development, inoculated plants were placed in the dark in a Percival dew chamber set at $19^{o}C$ and programmed to deliver very light dew that resulted in a relative humidity of approximately 95%. The plants were kept in the dew chamber for 16 hours and then moved to a greenhouse where they were kept for 14 days before the host reaction was recorded. For each of the lines, 14 plants were scored for rust and those that were fixed (homozygous) for the two rust genes were selected.

Pustule size and type on the foliage was visually evaluated using the standard grading scale of 1-6 for rust evaluation (Stavely et al. 1983). The grading scale was as follows: 1, leaves without any visible rust symptoms; 2, leaves with necrotic spots without sporulation, 2^{+}, necrotic spots 0.3-1 mm in diameter, 2^{++}, necrotic spots 1-3 mm in diameter; 3, tiny pustules (uredinia) less than 0.3 mm in diameter; 4, uredinia 0.31-0.5 mm in diameter; 5, uredinia 0.51- 0.8 mm in diameter; and 6, uredinia larger than 0.8 mm. Plants with a grade of 1-3 (pustules absent, necrotic spots without sporulation, or tiny sporulating pustules with diameters under 0.3 mm) were considered resistant, whereas those with large sporulating pustules with grades of 4 or higher were considered susceptible. The resistance reaction conditioned by the *Ur-4*

rust gene in response to race 108, or to any other race of common bean rust where *Ur-4* conditions resistance is expressed as necrotic non-sporulating spots (referred to as grades 2, 2^{+}, 2^{++}), also known as the hypersensitive response or HR. The resistant reaction of the *Ur-11* rust gene to race 67, or to any other race to which *Ur-11* is resistant, is expressed as faint necrotic, non-sporulating spots accompanied by tiny sporulating uredinia (rust pustules) that is referred to as grade 3. The susceptible reactions of *Ur-4* and *Ur-11* are expressed as large or very large sporulating uredinia classified as grades 4 to 6. Five check cultivars were included in the rust evaluation: Pinto 114, with no known rust resistance genes and susceptible to races 67 and 108; 'Early Gallatin', with *Ur-4* and susceptible to race 67 and resistant to race 108; 'PI 181996', with *Ur-11* and resistant to race 67 and susceptible to race 108; and the snap bean germplasm lines 'BF' and 'BJ', both with *Ur-4* and *Ur-11* and both resistant to races 67 and 108.

2.3 Results

2.3.1 Population development, evaluation and selection for heat tolerance

The four breeding lines, 'HT608', 'HT611', 'HT601', and 'HT603,' chosen as heat tolerant parents were significantly higher yielding under the high temperature stress of 32°C/27°C compared to the two sources of rust resistance 'BelFla-RR-1' and 'BelJersey-RR-15' (Table 2.1). The heat tolerant parents had superior performance under heat stress based on the mean values for each of four yield components evaluated: number of pods per plant, total seed weight per plant, number of seeds per plant, and number of seeds per pod. The heat tolerant parents also had significantly higher mean values for the four yield components as compared to the two snap bean cultivars, 'Hystyle' and 'Juliet'. The performance of the heat tolerant parents relative

to the rust resistant parents and to the two snap bean cultivars confirmed their suitability as sources of heat tolerance.

Table 2.1. Means of four yield components evaluated during a heat test, using 32°C/27°C (day/night) temperatures, of two rust resistant parents, 'BelFla-RR-1' and 'BelJersey-RR-15', four heat tolerant parents (HT), and two controls, 'Hystyle' and 'Juliet'.

Line[a]	No. of pods per plant	Seed weight per plant (grams)	No. of seeds per plant	No. of seeds per pod	Single seed weight (grams)
BelFla-RR-1	2.5 ± 1.63	1.73 ± 1.12	7.2 ± 4.52	1.6 ± 0.74	0.12 ± 0.05
BelJersey-RR-15	3.2 ± 1.40	1.10 ± 0.64	6.3 ± 3.31	1.2 ± 0.47	0.09 ± 0.04
HT608	7.3 ± 0.67	4.86 ± 0.47	27.0 ± 4.42	3.6 ± 0.27	0.19 ± 0.01
HT611	11.3 ± 0.99	6.44 ± 0.46	38.3 ± 3.56	3.4 ± 0.15	0.17 ± 0.01
HT601	6.2 ± 1.25	4.13 ± 0.90	26.3 ± 5.40	3.6 ± 0.71	0.13 ± 0.03
HT603	9.8 ± 0.85	5.43 ± 0.97	26.8 ± 6.61	2.6 ± 0.41	0.22 ± 0.02
Hystyle	1.0 ± 0.68	0.25 ± 0.17	1.7 ± 1.09	0.6 ± 0.37	0.05 ± 0.03
Juliet	1.0 ± 0.45	0.18 ± 0.08	1.8 ± 0.91	0.9 ± 0.45	0.05 ± 0.03

[a] 'BelFla-RR-1' and 'BelJersey-RR-15' were used as rust resistant parents and carry the *Ur-4* and *Ur-11* genes
± = standard error of mean, n = 48.

Eight populations were developed from crosses involving the four heat-tolerant and two rust resistant snap bean breeding lines (Figure 1). Quantitative information under greenhouse conditions on four yield components: number of pods per plant, number of seeds per pod, number of seeds per plant, and weight of seed per plant, were utilized together with selection based on the agronomic traits to make preliminary selections of superior plants from each population under heat stress. The number of plants selected from each of the populations during the F_2 evaluation

differed depending on the frequency of superior plants based on the selection criteria (Table 2.2).

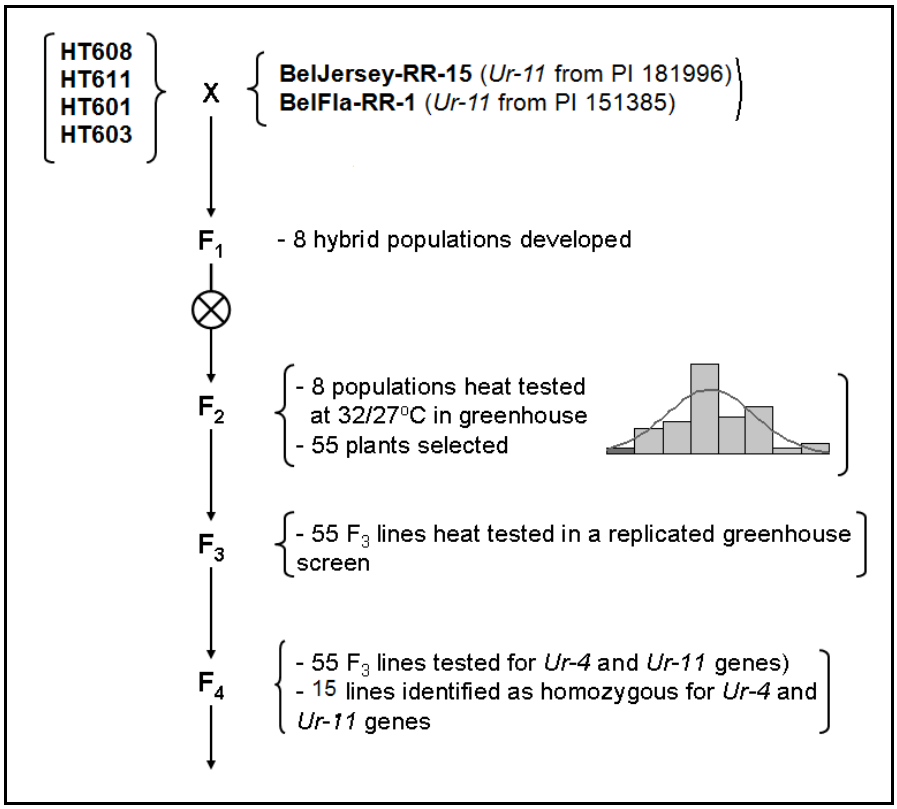

Figure 2.1. Development and evaluation of snap bean populations segregating for heat tolerance (HT) and rust resistance genes (*Ur-4, Ur-11*).

Seed from 55 F_2 plants yielding well under heat stress were grown out in the greenhouse and the resultant F_3 progeny lines were re-tested for heat tolerance alongside 35 controls: six parental lines, seven cultivars, and 22 other heat tolerant breeding lines. Variation was observed in yield and quality attributes among plants within the F_3 families indicating variability with respect to heat tolerance. Mean rankings calculated for the 90 lines evaluated under heat stress. The lines varied for

the four yield components: pod number per plant, seed number per plant, seed number per pod, and seed weight per plant.

Table 2.2. Yield components of 48 plants of each of eight segregating F_2 populations evaluated during a heat test using 32°C/27°C (day/night) temperatures. The populations were developed from crosses involving rust resistant and heat tolerant lines.

Line	Pod no. plant^{-1}	Seed weight plant^{-1} (g)	Seed no. plant^{-1}	No. of seeds pod^{-1}	Single seed weight, (g)	No. of selected plants
(HT601xBJ)F2	3-22	1.8-13.3	8-78	2-5.0	0.1-0.29	12
(HT603xBF)F2	0-22	0-14.8	0-51	0-4.5	0-0.30	3
(HT608xBF)F2	0-15	0-11.5	0-50	0-4.5	0-0.36	2
(BFxHT601)F2	5-20	3.9-12.3	18-70	2.4-5.3	0.1-0.29	8
(BFxHT611)F2	0-21	0-15.6	0-81	0-5.1	0-0.28	11
(BJxHT608)F2	0-23	0-18.3	0-96	0-4.8	0-0.34	7
(BJxHT611)F2	0-19	0-14.9	0-88	0-5.3	0-0.26	7
(BJxHT603)F2	0-22	0-15.0	0-68	0-4.9	0-0.29	5

2.3.2 Evaluation and selection for rust resistance under greenhouse conditions

Single plant progeny of the 55 lines selected for heat tolerance in the F_3 generation were evaluated in the F_4 for the presence of the *Ur-4* and the *Ur-11* rust resistance genes following inoculation with races 108 and 67 of the bean rust pathogen *U. appendiculatus*. Lines were simultaneously increased for field evaluation. Rust inoculation results on check genotypes, 'PI 181996', 'Early Gallatin', 'Pinto 114', and 'BF' and or 'BJ', were as expected and confirmed that the appropriate races were used

to identify the selected rust resistance genes and that viable spores of the races were used (Figure 2.2). 'PI181996', which has *Ur-11* but not *Ur-4*, did not develop rust symptoms after inoculation with race 67, but did with race 108. 'Early Gallatin', which has *Ur-4* but not the *Ur-11* resistance genes, developed rust symptoms following inoculation with race 67 but not with race 108. 'Pinto 114', which has neither of the two genes, developed rust symptoms after inoculation with both rust races. 'BF' and 'BJ', both of which have *Ur-4* and *Ur-11*, developed tiny pustules accompanied by faint and tiny non-sporulating necrotic spots after inoculation with race 67, and necrotic non-sporulating spots after inoculation with race 108. Occurrence of rust-resistant reactions, identical to that of 'BF' and 'BJ', on all plants inoculated with rust races 67 and 108 indicated that the line was fixed (homozygous) for *Ur-4* and *Ur-11* rust resistance genes and therefore rust resistant.

The distribution of lines homozygous for the *Ur-4* and *Ur-11* genes among the eight populations was as follows: three homozygous lines each from HT601xBJ, BFxHT601, and BFxHT611, while from BJxHT611 and BJxHT603, there were two homozygous lines each; from HT603 x BF and BJ x HT608, there was one homozygous line, and there were no homozygous lines from the HT608xBF population (Table 2.3).

Fifteen of the 55 lines were found to be homozygous for *Ur-4* and *Ur-11* (Table 2.4). These lines had the typical reaction of common bean plants with the *Ur-4* and *Ur-11* genes when inoculated with races 67 and 108. The 15 lines that were homozygous for the two rust genes translated to 27% of the 55 lines tested. This percentage was high because the two sets of parents used were both fixed for one of the rust resistance (*Ur-4*) genes and this increased the frequency and efficiency of generating broad rust resistance with *Ur-4* and *Ur-11*.

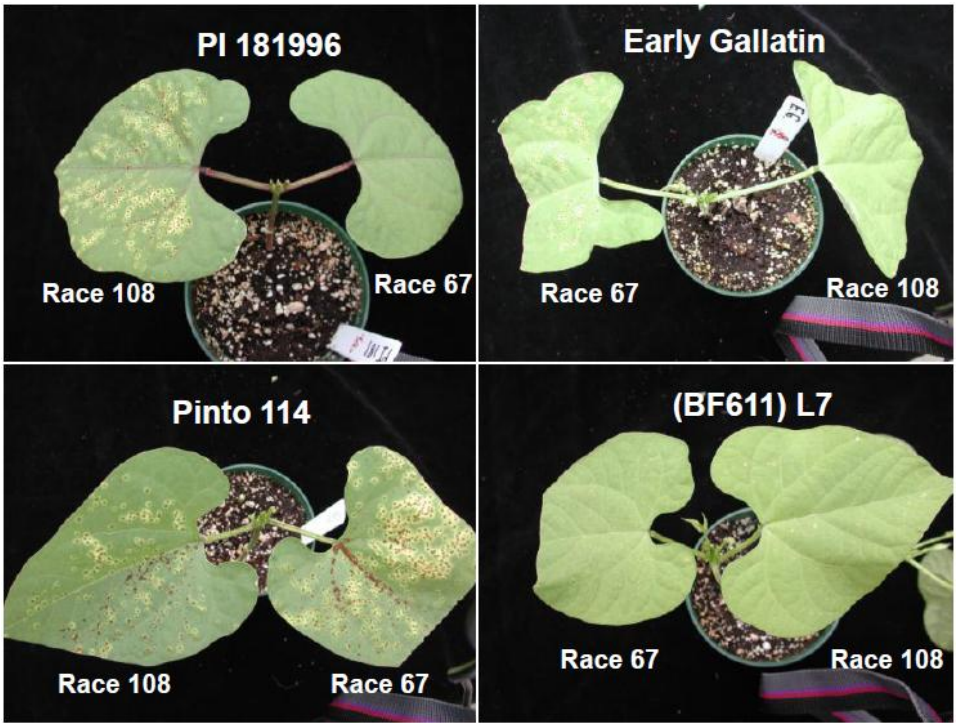

Figure 2.2. Rust disease symptoms on three common bean check genotypes and on a heat tolerant and rust resistant line '(BF611)L7' following inoculation with rust races 108 and 67 to test for the presence of *Ur-4* and *Ur-11* genes, respectively. Early Gallatin (with the *Ur-4* rust resistance gene) is susceptible to race 67 and resistant to race 108; 'PI 181996' (with *Ur-11*) is resistant to race 67 and susceptible to race 108; '(BF611)L7' with *Ur-4* and *Ur-11* is resistant to races; 'Pinto 114' does not have any known resistance genes and is susceptible to both races.

Table 2.3. Distribution of *Ur-4* and *Ur-11* rust resistance genes in 55 F_3 lines selected from eight populations developed from crosses involving rust resistant and heat tolerant parents.

Line	Number of Lines				Total no. of lines tested
	Ur-4+Ur-11 **homozygous**	***Ur-4+Ur-11*** **heterozygous**	***Ur-4*** **only**	***Ur-11*** **only**	
(HT601xBJ)F3	3	6	3	0	12
(HT603xBF)F3	1	1	1	0	3
(HT608xBF)F3	0	1	1	0	2
(BFxHT601)F3	3	3	2	0	8
(BFxHT611)F3	3	8	0	0	11
(BJxHT608)F3	1	4	2	0	7
(BJxHT611)F3	2	5	0	0	7
(BJxHT603)F3	2	2	1	0	5
Total	**15**	**30**	**10**	**0**	**55**

The 15 rust-resistant lines homozygous for the *Ur-4* and *Ur-11* genes were highly variable in their reaction to heat stress. Tolerance to heat was evaluated through ranking of four yield components: pod number per plant, seed number per plant, seed number per pod and seed weight per plant (Table 2.4). Twenty five percent, or 23 lines, of the 90 lines tested at the F_3 generation were heat-tolerant based on their high ranking for the yield components measured and among these, three lines, '(BF601)L4', '(601BJ)L9', and '(BF611)11'hereafter referred to as 'HT1', 'HT2', and 'HT3', respectively, were confirmed at the F_4 generation as homozygous for the two rust resistance genes.

2.4 Discussion

The snap bean genotypes developed in this study were ranked for heat tolerance by averaging individual ranks for four yield components: number of seeds

Table 2.4. Mean values and rankings (R) of four yield components in 15 F_3 lines determined to be homozygous for *Ur-4* and *Ur-11* rust resistance genes, and a subset of five controls, in a trial where 90 lines were tested in a greenhouse under high temperature, 32/27 ºC (day/night), conditions.

Line [y]	Seeds plant^{-1}		Seeds pod^{-1}		Pods plant^{-1}		Seed wt. plant^{-1} (g)		Av
	Value [z]	R	Value	R	Value	R	Value	R	R
(BJ611)L3	95.8 a	1	4.6 a-c	4	20.8 a-e	7	16.0 ab	2	**4**
(601BJ)L9	90.8 ab	2	4.0 a-h	20	21.0 a-d	6	17.8 a	1	**7**
(BF601)L4	72.4 a-k	13	4.6 a-d	5	16.0 a-l	32	14.5 a-e	6	**14**
Bronco	82.6 a-d	4	3.6 a-n	51.5	22.6 ab	2	14.0 a-h	9	**17**
(BF611)L11	66.8 a-m	20	4.1 a-h	17	16.4 a-k	29	13.2 a-i	16	**21**
(BF611)L2	58.3 a-n	38	4.4 a-f	8	13.3 b-l	62	12.1 a-j	26	**34**
(BJ603)L3	56.0 a-n	43	3.6 a-n	53.5	15.6 a-l	37	11.9 a-j	30	**41**
(BF601)L5	48.6 b-n	56	4.1 a-h	15.5	11.2 d-l	73	10.2 a-k	49	**48**
Spartacus	53.2 b-n	48	3.6 a-n	56	14.2 a-l	54	10.3 a-k	46	**51**
(BF611)L7	48.0 c-n	59.5	3.4 a-o	61.5	14.3 a-l	51	11.1 a-j	36	**52**
(BJ611)L1	46.8 c-n	65.5	4.2 a-h	11	11.0 e-l	74.5	6.7 e-k	77	**57**
Juliet	57.4 a-n	40	3.1 c-o	75	14.2 a-l	54	7.1 c-k	75	**61**
Venture	37.4 e-n	77	4.2 a-g	11	9.0 i-l	87	6.1 g-k	84	**65**
(BF601)L6	36.3 e-n	79	3.6 a-n	50	10.3 f-l	80.5	8.3 b-k	62	**68**
(603BF)L1	32.0 h-n	82	2.0 o	90	15.0 a-l	44	8.0 b-k	65	**70**
(BJ603)L4	40.6 e-n	73	3.1 d-o	76	12.8 c-l	63	7.3 c-k	72	**71**
(BJ608)L1	46.6 c-n	67	3.2 b-o	71.5	11.4 d-l	72	7.1 c-k	74	**71**
(601BJ)L5	28.2 l-n	88	3.6 a-n	51.5	7.8 j-l	88	6.4 e-k	80	**77**
(601BJ)L4	35.3 e-n	80	3.4 a-o	61.5	10.3 f-l	80.5	4.7 j-k	88	**78**
(603BF)L2	28.8 l-n	86	2.2 m-o	88	12.5 c-l	64	7.3 c-k	73	**78**
Masai	20.8 n	90	2.5 k-o	86	6.3 l	90	2.3 k	90	**89**

[y] 'Bronco', 'Spartacus', 'Juliet', 'Venture' and 'Masai' were a subset of the controls used.

[z] For each of the yield components columns, means followed by the same letter were not significantly different according to student's t test ($P \leq 0.05$, n = 5). The mean groupings and rankings (R) shown are based on a total of 90 lines tested.

per plant, number of seeds per pod, weight of seeds per plant, and number of pods per plant. Averaging the ranks of the four yield components giving equal weight to each minimizes possibilities of inaccuracies that may have arisen from selections based on only a single yield component. For instance, selections based only on pod number per plant may not accurately reflect tolerance to heat stress as some genotypes have the ability to form numerous pods under hot conditions but fail to form viable seed. Other genotypes were able to form more seeds per plant even under hot conditions but experienced reductions in seed weight and thus a yield reduction. Seed weight is also highly correlated with pod sieve size, so small-sieve selections yield higher seed numbers per plant. Total seed weight per plant is a better indicator of the plants ability to yield under heat stress. The mean of the rankings of the different yield components is an effective method for selection of heat tolerance; however, the yield information for total seed weight and seeds per pod are the best indicators of a breeding lines ability to yield under heat stress and fill pods under heat stress, respectively.

This research has effectively combined resistance to common bean rust, involving the *Ur-4* and *Ur-11* rust-resistance genes, with tolerance to heat stress. Both traits were combined in a snap bean genetic background. The targeted introgression of these traits into a snap bean genetic background is unique and offers an innovative approach to increasing snap bean yields in the tropics. This is the first documented effort to address the challenge created by the co-occurrence of rust disease and heat stress across common bean production environments.

Results from this study are significant for regions such as Eastern and Southern Africa where rust and heat stress are constraints to snap bean production. Productivity of snap bean is limited in high temperature environments where there is poor pod and seed set due to heat stress at flowering. Snap bean production regions that experience heat stress are also frequently exposed to common bean rust. Development and

selection of snap beans with targeted combinations of genes for tolerance to heat stress and resistance to rust could increase the yield potential in these areas as well as expand potential production areas and/or seasons. Thus, specific classes of common bean that are grown widely, such as small sieve snap bean cultivars grown in East Africa and dry beans, are potential candidates for targeted improvement using the germplasm developed in this study.

Utilization of the breeding lines developed in this study could contribute to reduced reliance on fungicides in the mitigation of yield losses from the rust disease. The reduction of fungicide use in the control of rust would reduce the costs of producing snap beans and hence increase profitability for growers. In addition, potential risks to growers and consumers caused by pesticide exposure during application or through exposure to fungicide residues in the harvested products would be reduced. Also the buildup of residues of applied fungicides in the environment would be reduced. These factors could contribute to achieving more efficient and sustainable snap bean production and positively impact farmers' incomes and food security.

CHAPTER THREE

ADAPTATION OF NOVEL RUST RESISTANT AND HEAT TOLERANT SNAP BEANS TO CONTRASTING TROPICAL ENVIRONMENTS

3.1 Introduction

Common bean genotypes from Andean and Middle America gene pools are grown in Eastern Africa although the distribution of genotypes from these gene pools varies among countries (Gepts, 1990; Asfaw et al., 2009). The growing of diverse bean genotypes from the different gene pools has led to the proliferation of compatible races of the bean rust pathogen with which these bean genotypes coevolved at the centers of origin (Beaver et al., 2003; Jochua et al., 2004; Liebenberg et al., 2006; Pastor-Corrales, 2006). New races of the rust pathogen have likely arisen within intensive bean production systems, but little is presently known about the geographic distribution of populations and races of the rust pathogen in most countries of Eastern and Southern Africa (Henk et al., 2006). Recently, the virulence diversity of some 248 isolates of the bean rust pathogen from various African countries was reported (Liebenberg, 2003).

Deployment of rust resistance genes in common bean market classes and cultivars of interest to ensure sustainable management of the rust disease has remained a challenge due to the diversity of common bean rust races coupled with the lack of information on prevalent races of the pathogen in many locations including East Africa. A large number of bean genotypes previously released with single genes for resistance to rust have not remained resistant across all sites and seasons. Most snap bean cultivars grown in Eastern and Southern African countries are, as a result, highly susceptible to rust (Kimani et al. 2002; Mutunga et al., 2002; Jochua et al., 2004; Hillocks et al., 2006). While studies have shown that combinations of *Ur-4* and *Ur-11*

rust resistance genes confer resistance against all known races of the rust pathogen in common bean, snap beans with the combination of these two genes have not been widely tested, documented, or adopted in Eastern Africa.

In addition to bean rust, heat stress is another challenge to snap bean production in Eastern Africa. The agroecological zones in which snap beans are grown in the region are at present mostly restricted to high and mid-altitudes where temperatures are cooler and where current cultivars are well adapted. The altitude in these regions is typically over 1500 m. At the lower and mid-altitude regions where temperatures are higher, particularly during night-time, snap bean yield is low. High ambient temperatures constrain a large number of farmers in the warmer environments from effectively engaging in the production of snap beans. The combined challenges that common bean rust disease and high temperatures pose to snap bean production in Eastern Africa, call for better understanding of the trait responses and combinations, and development of cultivars with the ability to perform effectively under these two stresses. The objective of the research was to evaluate the selected breeding lines with combinations of the two traits at field sites in East Africa, and to verify their performance in a high temperature environment in Puerto Rico.

3.2 Materials and methods

3.2.1 Design of field trials in East Africa and Puerto Rico

Three selected rust resistant and heat tolerant breeding lines ‘HT1’, ‘HT2’, and ‘HT3’, were evaluated together with a rust resistant but heat sensitive control, ‘HS1’ and an additional set of 12 cultivars adapted to different geographical regions (Table 3.1). ‘Amy,’ ‘PV712,’ ‘Teresa,’ and ‘PV 698’ are cultivars currently grown in the East African region; ‘Barrier’ and ‘Juliet’ are grown in Southern Africa; ‘Palati’ is grown in Northern Africa; ‘Opus’ and ‘Brio’ are grown in the Southern United States as

fresh-market cultivars; and ‘Bronco’, ‘Hystyle’ and ‘Masai’ are grown in the Northeastern United States.

Seed for the field evaluation was increased in a climate controlled greenhouse with day and night temperatures maintained at 24°C and 21°C, respectively. Seeds were coated with the fungicide ‘Captan’ (Bayer Crop Science, NC USA) to satisfy seed import requirements and to enable a more uniform stand.

The trials were laid out in a randomized complete block design (RCBD) with four replications planted in East Africa trials and with five replications in Puerto Rico. Six sites were planted in East Africa, five in Kenya and one in Tanzania; and one site in Juana Diaz, Puerto Rico. Single rows of 25 plants were planted per block for each of the 16 lines. A planting density was adopted in which single plants were planted at a spacing of 0.5 m between rows in East Africa and 1 m between rows in Puerto Rico with 0.1 m seed spacing within rows at all locations. The spacing between rows was wider in Puerto Rico to accommodate mechanized operations in the management of the research plot.

3.2.2 Location and description of field trials

Field trials were undertaken between March and June 2009 during the long rain season at the sites in East Africa and from June to September 2009 in Puerto Rico. The East African sites were selected on the basis of differences in soils and climate. Table 3.2 summarizes information on the locations, standard geographic coordinates, climatic conditions, and soil chemical properties at the six sites in Africa. The site in Tanzania was located at the World Vegetable Center-Regional Center for Africa (AVRDC-RCA) in Arusha while sites in Kenya were in Homabay, Kibos, Maseno, Sabatia and Kitale. Altitude at the sites ranged from 1172 m at Homabay to 1829 m at Kitale (Table 3.2).

Table 3.1. Snap bean genotypes evaluated in 2009 at field sites in East Africa and Puerto Rico.

Genotype	Source	Characteristics
HT1	Cornell, USA	Heat tolerant, rust resistant (has *Ur-4* and *Ur-11*)
HT2	Cornell, USA	Heat tolerant, rust resistant (has *Ur-4* and *Ur-11*)
HT3	Cornell, USA	Heat tolerant, rust resistant (has *Ur-4* and *Ur-11*)
HS1	Cornell, USA	Heat sensitive, rust resistant (has *Ur-4* and *Ur-11*
Amy	Seminis, USA	Bush bean, small sieve
Barrier	Alpha Seed, S. Africa	Bush bean, medium sieve
Brio	Seminis, USA	Bush bean, BCMV resistant, medium sieve
Bronco	Seminis, USA	Heat tolerant, BCMV resistant, medium sieve
Hystyle	Harris Moran, USA	Bush bean, persistent green color, BCMV resistant
Juliet	Alpha Seed, S. Africa	Bush bean, small sieve
Masai	Syngenta, USA	Rust susceptible, small sieve
Opus	Seminis, USA	Bush bean, BCMV resistant, medium sieve,
Palati	Syngenta, USA	Bush bean, rust resistant, medium sieve
PV698	Pop Vriend, Holland	New variety, rust resistant, small sieve
PV712	Pop Vriend, Holland	New variety, rust resistant, small sieve
Teresa	Seminis, USA	Bush bean, rust resistant, small sieve

*HT1, HT2 and HT3 were developed and selected for rust resistance and heat tolerance. HS1 was a rust resistant and heat sensitive control from a genetic background similar to that of HT2. The rest of the genotypes are commercial cultivars selected for adaptability to different geographical areas.

The field trial in Puerto Rico was carried out at the Experiment Station of the University of Puerto Rico in Juana Diaz, located in south central Puerto Rico, at 18.01º N and 66.22º W, and at an elevation of 21 m. The Puerto Rico site differed significantly from the others in terms of latitude, soil and climatic conditions.

There were temperature differences between the sites in East Africa especially during the March to June period of the study (Table 3.2). Homabay, which was at the

lowest altitude of the six, sites had temperatures exceeding those at the high altitude site at Kitale by 2-3°C and was more stressful for common bean reproductive development. Arusha had the lowest mean temperatures due to cooling effects of nearby Mount Meru. Puerto Rico had the highest mean temperatures among the trial sites with mean daily temperatures of 22.9º C (minimum) and 33.8º C (maximum) during the period that coincided with the reproductive phase of development.

Soils at the sites differed in type, chemical properties and fertility status (Table 3.2). Soil pH at the sites ranged from 4.7 to 7.3. Soils at Homabay were heavy clays, high in phosphorus (P) and potassium (K) and moderate in nitrogen (N) content and were the most fertile of the sites. Soils at Arusha were neutral in pH and had the highest K content compared to the other sites

The sites were tractor ploughed and harrowed to a fine tilth prior to planting. Planting in East Africa was undertaken in late March and early April 2009 at the onset of the rainy season while in Puerto Rico the planting was completed on June 25, 2009. A compound inorganic fertilizer, containing: N-10%, P-26%, K-10%, was row applied at planting at a rate of 200 kg ha^{-1} at all the sites in East Africa except Arusha. In Puerto Rico, inorganic fertilizer (containing N-10%, P-10%, and K-10%) was applied at two weeks after planting at a rate of 400 kg ha^{-1}. Furrow irrigation supplemented rainfall at Arusha while the trial plots at the other East African sites were rain fed. The site in Puerto Rico was drip irrigated. Standard agronomic practices were followed in managing the trial plots.

3.2.3 Data collection and analysis

Each of the entries was monitored for symptoms of rust and other diseases. Dates on which first disease symptoms were observed at the sites were noted so as to account for possible site differences in rust severity and its effect on yield. The genotypes

Table 3.2. Location, standard geographic coordinates, climatic and soil chemical properties at six field sites in East Africa where 16 snap bean genotypes were evaluated in 2009.

	Site [a]					
	Arusha	**Homabay**	**Kibos**	**Kitale**	**Maseno**	**Sabatia**
Country	Tanzania	Kenya	Kenya	Kenya	Kenya	Kenya
Altitude, m	1235	1172	1185	1829	1526	1583
Latitude	3.37º S	0.53º S	0.037º S	1.00º N	0.00º	0.12º N
Longitude, ºE	36.81	34.47	34.82	34.99	34.60	34.76
Rainfall, mm/(Temperature, ºC)[b]						
March	0 (27)	40 (31)	52 (32)	24 (29)	52 (-)	127 (-)
April	66 (25)	175 (29)	205 (29)	260 (27)	205 (-)	154 (-)
May	53 (23)	211 (28)	103 (29)	142 (26)	103 (-)	123 (-)
June	13 (22)	44 (28)	29 (30)	33 (25)	29 (-)	66 (-)
Soils [c]						
pH (H_2O)	7.30	5.54	5.15	5.30	4.70	5.03
Carbon, %	2.93	2.05	0.41	1.22	1.08	1.17
N, %	0.14	0.45	0.22	0.37	0.33	0.44
P, ppm	60.71	324.32	49.53	39.35	17.60	6.72
K, m.e. %	2.70	1.15	0.31	0.76	0.15	0.13
Ca, m.e. %	8.44	29.60	4.40	8.57	5.00	5.99
Mg, m.e. %	2.61	2.54	1.48	0.93	nd	0.56
Mn, ppm	nd	0.31	0.89	1.01	3.08	3.24
Cu, ppm	nd	2.64	0.84	1.62	5.39	11.40
Fe, ppm	nd	16.22	28.98	38.75	38.29	33.12
Zn, ppm	nd	3.58	4.19	9.64	9.47	11.70

[a] Site position details: altitude, latitude and longitude were measured using a Global Positioning Tool, GPS 12XL (1998, Garmin, Olathe, USA).
[b] Site rainfall and temperature data are shown together. Temperature is bracketed.
[c] Temperatures at Maseno and Sabatia were not recorded. Soils within the study plots were randomly sampled at a depth of 0-30 cm. The soil analytical values presented for N and P are total N and available P, respectively. At Arusha P was determined following Olsen extraction method while for the other sites Melhich extraction method was used. Micronutrient content in the soils at Arusha was not determined.

were scored for common bean rust at flowering (R6) and at pod filling (R8) developmental stages. Genotype reaction to rust (severity) was evaluated following the CIAT common bean evaluation scale ranging from 1-9 (van Schoonhoven and Pastor-Corrales, 1987). According to this system of evaluation, a severity score of 1 represented a highly resistant response with no visible rust pustules present; a score of 3 represented a resistant reaction with few, generally small pustules covering approximately 2% of foliar area; a score of 5 represented an intermediate reaction with presence of generally small or intermediate pustules covering approximately 5% of foliar area; a score of 7 represented a susceptible reaction with large pustules often surrounded by a chlorotic halo and covering approximately 10% of foliar areas; and a score of 9 represented a highly susceptible reaction with large and very large pustules surrounded by chlorotic halos that covers more than 25% of leaf tissue and cause premature defoliation.

To obtain more quantitative information on genotypic reaction to rust, rust incidence data was obtained by counting the number of rust infected plants per plot. The number of rust pustules formed per leaflet determined rust severity. The sets of rust incidence and severity data were used in a regression analysis to obtain a simultaneous quantification of genotype differences in reaction to rust. In the regression plot, genotypes that had no rust were considered highly resistant while those with high rust incidence and severity were considered highly rust susceptible. The genotypes that had high, moderate, or low rust severity on a few plants (low incidence) were considered either partially rust resistant or heterozygous for the rust resistance genes. To understand the genetic basis that accounted for the observed genotypic reactions to rust at the field sites, the presence of rust resistance genes in the cultivars included in the field test was determined at USDA-ARS, Beltsville. To distinguish the different rust genes, plants of the different bean genotypes were

inoculated with rust races 49 and 108 to identify *Ur-4*, race 47 to identify *Ur-5*, and race 67 to identify *Ur-11*.

Information on yield was obtained by recording the number of pods produced per plant. The total number of pods produced per plot was counted, while excluding plants at the end of the rows, and then divided by the total number of plants examined within the plot. The data was statistically analyzed using regression, analysis of variance and the Tukeys HSD test for separating means. The statistical analyses were completed using JMP 7 software (SAS, 2008).

3.3 Results

3.3.1 Reaction to common bean rust under field conditions

High rust incidence and severity were observed at Arusha, Homabay and Kitale. No rust was observed in Sabatia and Puerto Rico. No data was collected from the Kibos site due to damage of trial plots by a wild animal or at the Maseno site due to heavy bean fly (*Ophiomyia* spp) infestation. The Arusha site experienced a longer duration of lower temperatures compared to Kitale and Homabay (Table 3.2). The lower temperatures of 22-25^{o}C at Arusha in April to June promoted development of rust early in the season and the conditions remained favorable for rust development until the end of the crop cycle. Rust symptom development at the Arusha site commenced on ‘Hystyle’, ‘Barrier’, ‘Brio’, ‘Masai’, ‘Amy’, ‘Bronco’, ‘HT1’, ‘Opus’, and Juliet approximately two weeks after planting, when the plants were at the second trifoliate leaf growth (V2) stage. At Kitale and Homabay, disease development delayed until the sixth and seventh weeks after planting when most of the genotypes were at the pod formation and pod filling stages.

Similar trends in rust incidence on the 16 genotypes were observed across the three sites (Arusha, Homabay and Kitale), and genotypes differed significantly in the

frequency of rust infected plants (Figure 3.1). Three of the four breeding lines: HT1, 'HT2', and 'HS1', all of which combine the *Ur-4* and *Ur-11* rust resistance genes, had no visible rust symptoms at the three sites where common bean rust occurred.

Approximately 25% of the 'HT3' plants had rust, indicating that this breeding line was still segregating for one of the *Ur-4* and *Ur-11* rust genes (most likely *Ur-11* since other genotypes that had only *Ur-11* were rust resistant at all the three mentioned sites). The cultivars 'PV698' and 'PV712', both of which have the *Ur-11* rust resistance gene, had no rust at the three sites. 'Teresa', which has the *Ur-5* rust resistance gene, had no rust symptoms at Arusha and Kitale, while at Homabay approximately 30% of its plants had rust symptoms. 'Palati', which has the *Ur-4* and *Ur-5* rust resistance genes, exhibited rust symptoms on 42% of the plants at Homabay and on 10-12% of plants at Arusha and Kitale. The remainder of the genotypes tested: 'Amy', 'Barrier', 'Brio', 'Bronco', 'Hystyle', 'Juliet', 'Masai' and 'Opus' showed rust on more than 60% of plants at each of the three sites (with the exception of 'Opus' that showed rust on approximately 40% of plants at Arusha) indicating that they were susceptible to rust at these sites. Many of these genotypes have the *Ur-4* rust resistance gene while others have no known rust resistance genes.

The genotypes differed significantly in rust severity as indicated by different pustule sizes and counts observed on plant leaves (Figure 3.2). According to the CIAT standard system of evaluation, the breeding lines 'HT1', 'HT2', 'HS1' and the cultivars 'PV698', 'PV712', and 'Teresa' were classified as highly resistant as they had no visible rust pustules present at any of the three sites (Table 3.3). 'Teresa' was also highly resistant at Arusha and Kitale but not at Homabay where some plants had rust symptoms, albeit with low severity.

'Palati' was classified as resistant, as only a small number of plants had small pustules. There was intense rust pustule formation on leaves of some 'Juliet' plants

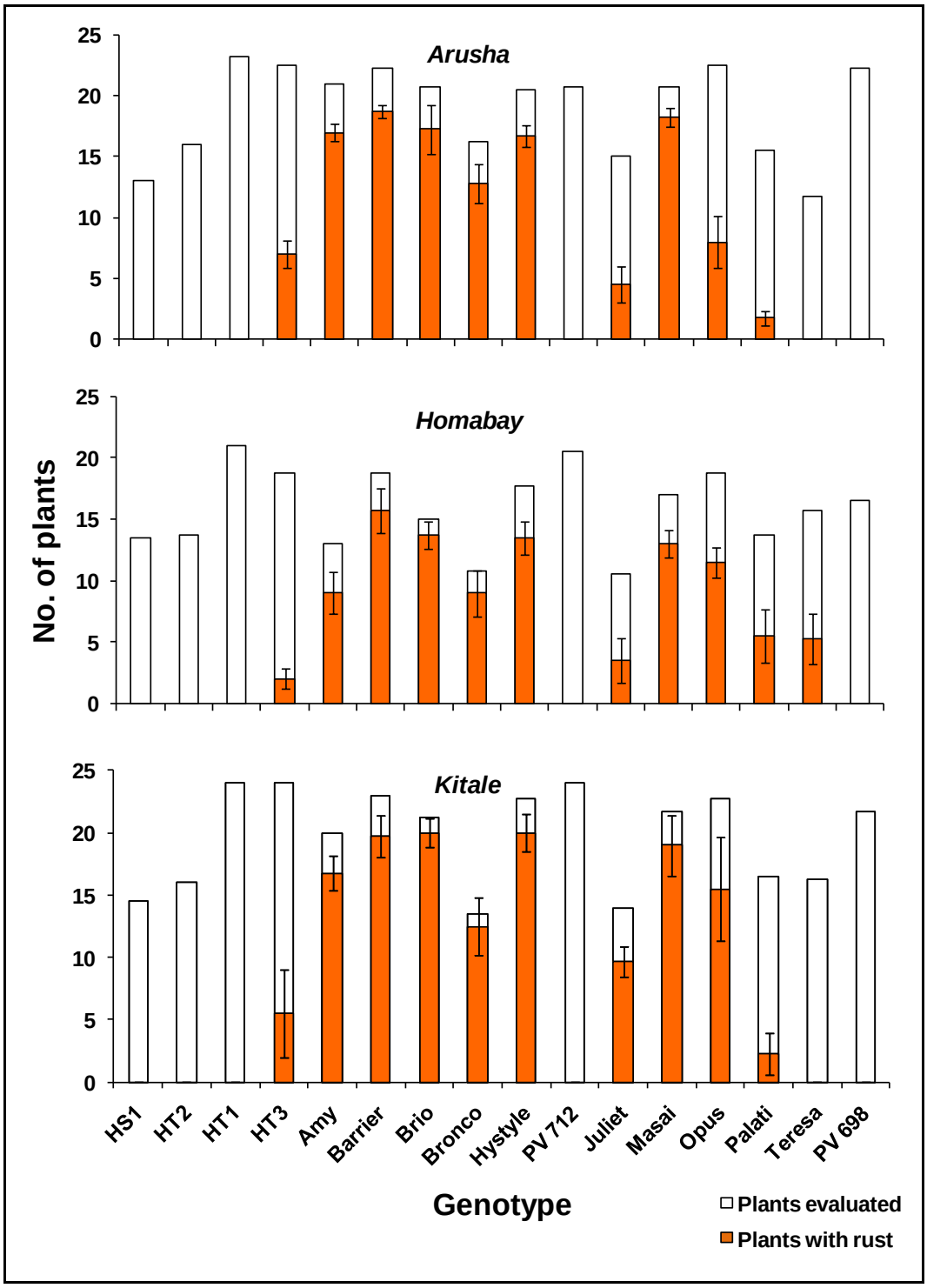

Figure 3.1. Incidence of common bean rust on 16 snap bean genotypes grown at Arusha, Homabay and Kitale field sites in East Africa during the 2009 wet season. The significance bars indicate standard errors (n=4).

indicating that its seed was inadvertently mixed. ‘Opus’ was intermediate at Arusha and susceptible at Homabay and Kitale. ‘Amy’, ‘Barrier’, ‘Brio’, ‘Bronco’, ‘Hystyle’, and ‘Masai’ were highly susceptible at all three locations where rust was present (Arusha, Homabay and Kitale) and had a high density of very large pustules surrounded by chlorotic halos that covered more than 25% of the leaf tissue and caused premature defoliation. Very large pustules were also observed on the pods of ‘Amy’, ‘Barrier’, ‘Brio’ and ‘Hystyle’ at the Arusha site, confirming that these cultivars were highly susceptible to the bean rust pathogen.

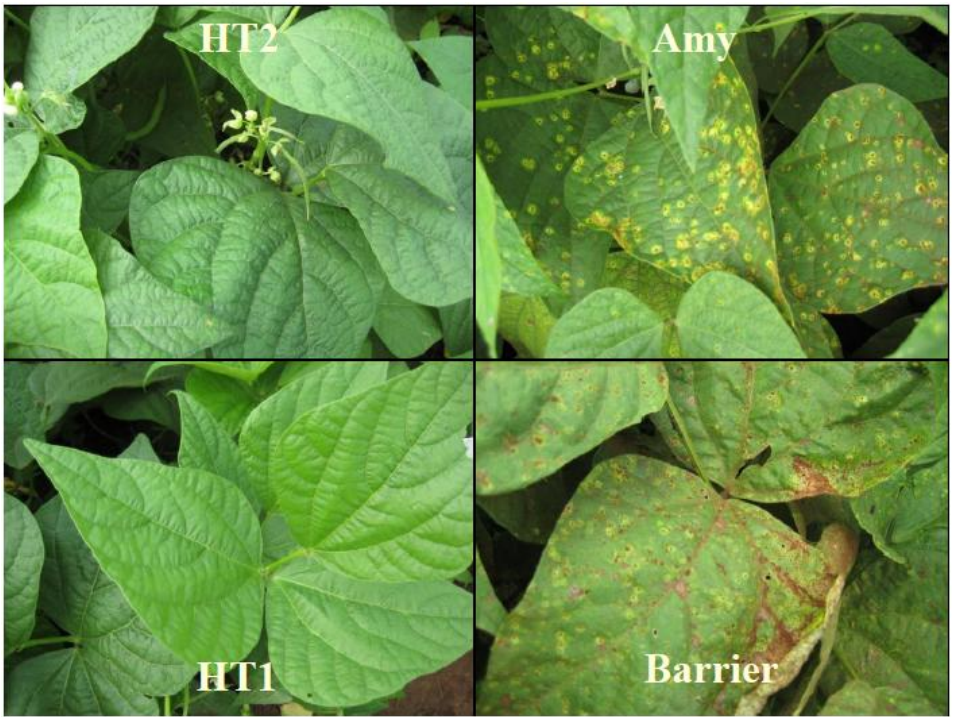

Figure 3.2. Severity of common bean rust on two snap bean breeding lines, ‘HT1’ and ‘HT2’, that combine the *Ur-4* and *Ur-11* rust resistance genes in a heat tolerant genetic background and two rust susceptible snap bean cultivars, ‘Amy’ and ‘Barrier’, at Arusha, Tanzania during 2009 wet season.

Table 3.3. Snap bean rust scores at three East African field sites. The scores were made following the CIAT standard system for evaluating bean germplasm in which a score of 1 represents a highly resistant/immune genotype; 3, resistant; 5, intermediate; 7, susceptible; and 9, highly susceptible.

Genotype	Rust score at sites		
	Arusha	Homabay	Kitale
HT1	1	1	1
HT2	1	1	1
HS1	1	1	1
HT3*	1,7	1,7	1,3
Opus	3	7	7
Palati	3	3	3
Hystyle	9	9	9
Barrier	9	9	7
PV698	1	1	1
Amy	9	9	9
Juliet**	1,9	1,3	1,7
Masai	7	7	7
Brio	9	9	9
PV712	1	1	1
Teresa	1	1,3	1
Bronco	9	7	9

*These results, showing some plants without and others with rust suggest that 'HT3' was still segregating for the *Ur-4* and *Ur-11* rust resistance genes.
**These results suggest that the cultivar 'Juliet' seed might have been inadvertently mixed.

Regression analysis of rust incidence and severity data showed that rust was more severe on genotypes that had the highest rust incidence (Figure 3.3). The observed relationship between rust incidence and severity within genotypes underscores the contribution of different rust resistance genes and gene combinations on snap bean genotype response to rust. Genotypes that had no known rust resistance genes, including 'Amy' and 'Masai', or only had the Ur-*4* gene, including 'Opus', 'Brio', 'Barrier', 'Bronco', and 'Hystyle', had high rust incidence and severity (Figure 3.3). The high rust incidence and severity observed on *Ur-4* genotypes indicates that

this gene is not effective against the race(s) of the bean rust found at the three sites (Arusha, Homabay and Kitale).

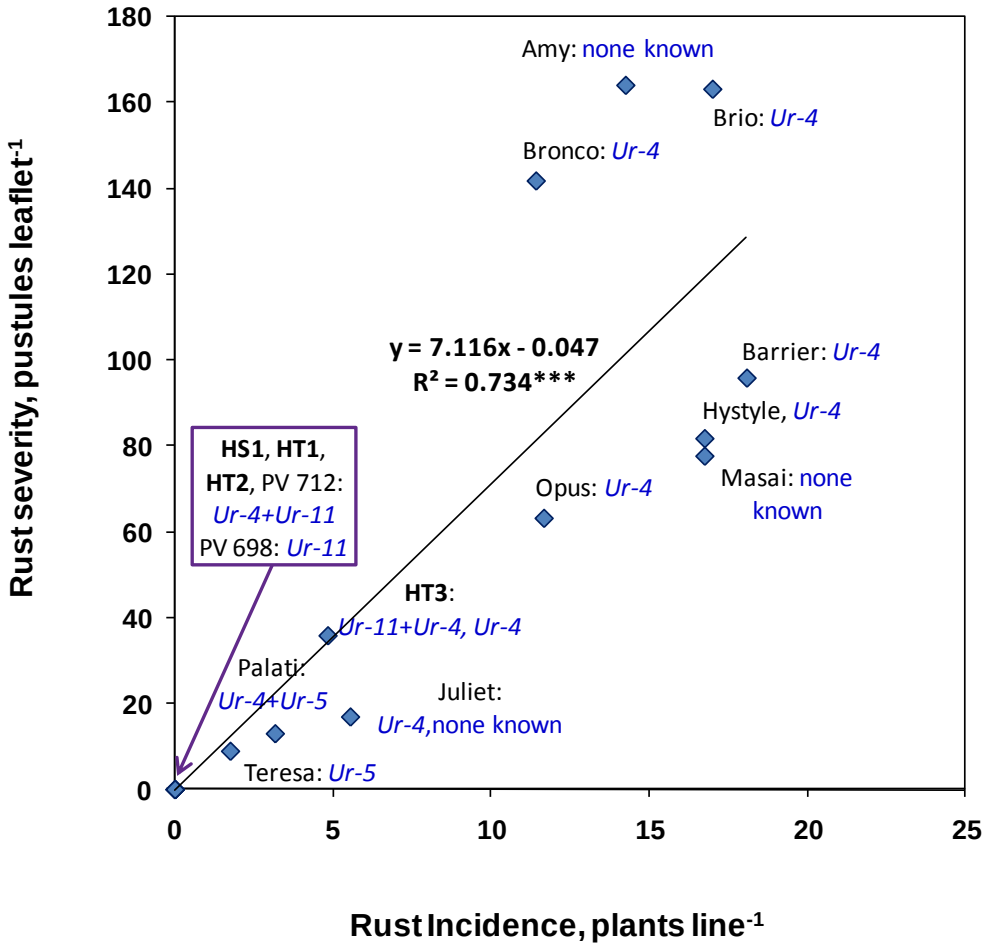

Figure 3.3. Effect of different rust resistance genes and gene combinations on rust disease incidence and severity in 16 snap bean genotypes at Arusha, Homabay and Kitale field sites in East Africa during 2009 wet season.

Among the rust susceptible genotypes that had no rust resistance genes or had the less effective *Ur-4* gene, there were differences in incidence and severity of rust (Figure 3.3). Among the genotypes with only *Ur-4*, 'Opus' had relatively low rust incidence and severity (Figure 3.3). This observation may be attributed to the presence

of other unidentified, but less effective, rust resistance genes within this group of genotypes, and to the extent to which these less effective rust resistance genes interact with different races of the rust pathogen found at the sites.

'Teresa' and 'Palati', which have the *Ur-5* rust gene, were resistant to rust as they had low rust incidence and severity at the three sites (Table 3.3 and Figure 3.3). Effectiveness of the *Ur-5* gene was particularly notable at Kitale and Arusha, where there were no symptoms of rust on all 'Teresa' plants and no symptoms on 88-90% of 'Palati' plants, indicating that the *Ur-5* gene was effective against races of the rust pathogen at these sites (Figure 3.1). The *Ur-5* gene, which is of Middle American origin, has broad resistance against many races of the bean rust pathogen especially races that are Andean in origin (Pastor-Corrales, 2006). Deployment of the *Ur-5* gene in cultivars targeted to regions where predominantly Andean beans are grown increases the success rate of conferring resistance against the bean rust pathogen. However, the *Ur-5* conferred rust resistance in 'Teresa' and 'Palati' was not complete at the Homabay site where approximately 30% of 'Teresa' plants and 42% of 'Palati' plants had rust symptoms (Figure 3.1). This observation could be indicative of the possible presence, at the Homabay site, of a bean rust isolate that overcomes the *Ur-5* gene although there was not 100% infection of 'Teresa' and 'Palati' plants with the *Ur-5* overcoming isolate.

'PV698', which has the highly effective *Ur-11* rust resistance gene and 'HS1', 'HT1', 'HT2', and 'PV712' that have the *Ur-11* and *Ur-4* rust gene combinations, were highly rust resistant and had no rust at the three sites. The observation that 'PV698' was rust resistant at the sites implies that the *Ur-11* gene conferred resistance against all races of the bean rust pathogen found at these sites and that the Middle American rust race 108, which is known to overcome the *Ur-11* gene (Pastor-Corrales, 2006), was not present at the study sites.

3.3.2 Yield in East Africa and Puerto Rico

Yield data was collected from four East African field sites: Arusha, Homabay, Kitale and Sabatia, and also from Puerto Rico. In terms of performance of all the genotypes combined at each site, pod yield was highest at Kitale followed by Arusha and Homabay, which were not significantly different from each other, followed by Puerto Rico and was lowest at Sabatia (Figure 3.4). The very low yield at Sabatia was the result of a combination of factors including poor soil fertility, soil acidity, potential Al and Mn toxicity, as well as a root rot disease complex. The data from Sabatia was excluded from further analysis due to absence of common bean rust at the site coupled with confounding effects due to various soil and disease factors that resulted in very low yields at the site.

Bean rust infection was the most important yield-influencing factor at Arusha, while at Homabay yield was largely influenced by high temperature stress and to a lesser extent by bean rust. Rust had less impact on yield at the Homabay site as rust symptoms did not appear until six weeks after planting when the bean genotypes were at the pod formation and pod filling stages. At the Puerto Rican site, high day (34°C) and night (23°C) temperature stress during reproductive development resulted in significant symptoms of heat stress, including bud, flower and pod abortion, poor pod fill, curved pods, and the excessive production of pin pods, thus significantly affecting yield response.

Analysis of variance of yield data from Arusha, Homabay, Kitale, and Puerto Rico indicated significant effects due to genotype, site, and genotype by site interaction (Table 3.4). Across the four sites, the breeding lines ‘HS1’and ‘HT2’ were the highest yielding among the genotypes tested, while ‘PV 712’, ‘Masai’ and ‘Palati’ had the lowest yields (Table 3.5). The high yields of the two breeding lines relative to the other genotypes tested underscores the contribution that the combined

introgression of rust resistance and heat tolerance traits had in stabilizing performance of the breeding lines across the different sites.

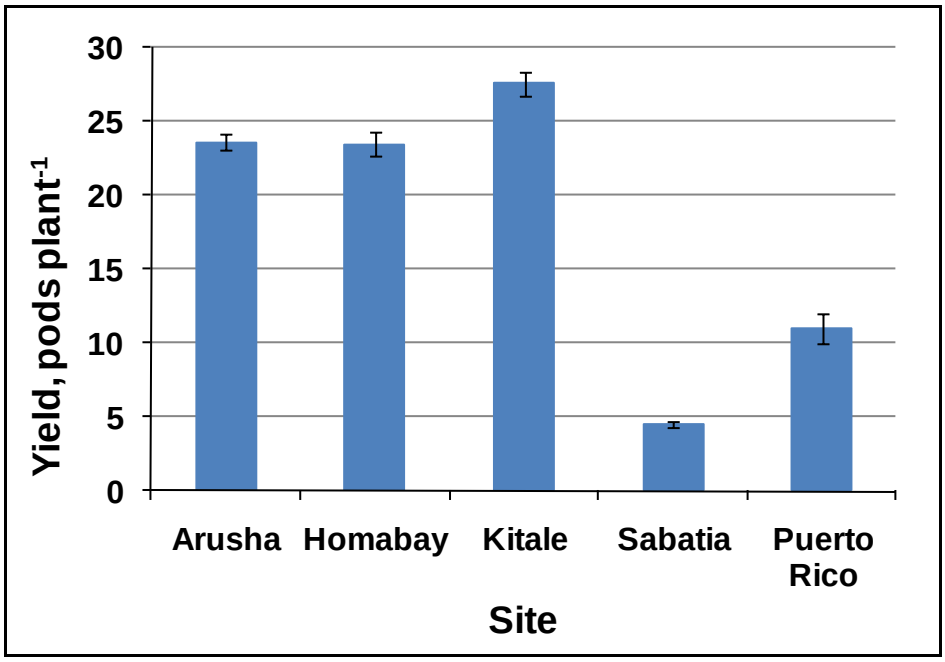

Figure 3.4. Mean site yields of 16 snap bean genotypes from a field trial during the 2009 March to June rainy season. The significance bars indicate standard errors (n=64).

Table 3.4. Analysis of variance of pod yield of 16 snap bean genotypes grown in the field in 2009 at three bean rust infested and temperature variable sites in East Africa and at one high temperature site in Puerto Rico.

Source	df	Sum of Squares	F Ratio	*P*
Genotype	15	1828.0	3.76	<0.0001
Site	3	10415.5	107.11	<0.0001
Genotype x Site	45	3777.8	2.59	<0.0001

Table 3.5. Pod yield of 16 snap bean genotypes at field sites in East Africa and Puerto Rico.

Genotype*	Yield, pods plant^{-1}				
	Arusha	**Homabay**	**Kitale**	**Puerto Rico**	**All sites**
HS1	24.4 b-e	27.4 ab	29.3 a-c	17.2 a	24.5 ab
HT2	26.0 bc	31.4 a	30.4 a-c	18.4 a	26.5 a
HT1	24.1 b-e	26.0 a-c	25.9 a-d	15.3 ab	22.8 ab
HT3	21.2 d-f	24.8 bc	23.3 cd	13.5 a-c	20.7 a-c
Amy	23.6 c-e	20.3 c-e	29.5 a-c	5.3 cd	19.7 a-c
Barrier	21.4 c-f	25.0 bc	24.4 b-d	10.0 a-d	20.2 a-c
Brio	17.0 f	26.5 ab	31.7 ab	17.7 a	23.2 ab
Bronco	20.7 ef	27.1 ab	32.1 ab	18.1 a	24.5 ab
Hystyle	21.0 ef	28.5 ab	19.4 d	13.6 a-c	20.6 a-c
PV712	25.7 b-d	11.3 f	22.6 cd	1.4 d	15.3 c
Juliet	28.5 ab	23.5 b-d	27.0 a-d	6.5 b-d	21.4 a-c
Masai	21.9 c-e	17.7 de	32.2 ab	6.7 b-d	19.6 bc
Opus	22.7 c-e	28.8 ab	28.3 a-c	12.8 a-c	23.1 ab
Palati	22.9 c-e	18.0 de	23.5 cd	11.5 a-c	19.0 bc
Teresa	24.5 b-e	23.0 b-d	32.9 a	11.9 a-c	23.1 ab
PV698	32.4 a	16.9ef	29.5 a-c	1.0 d	19.9 a-c
Mean	**23.6**	**23.5**	**27.6**	**11.3**	**21.5**

*Within a site/column, genotype means followed by the same letter are not significantly different according to Tukeys HSD.

From the analysis of variance table, it is notable that there was a highly significant genotype by site interaction effect (Table 3.4). This significant interaction effect implies that factors influencing yield were unique to each of the sites and that they differentially affected genotypic performance. Given the uniqueness of the sites, genotypic performance at the four sites is better interpreted on a site by site basis. At

Arusha, where an early outbreak of the common bean rust was the most important yield influencing factor, 'PV698', 'Juliet' and the breeding line 'HT2' were the highest yielding while the rust susceptible cultivars, 'Brio', 'Bronco' and 'Hystyle' were the lowest yielding (Table 3.5).

In Kitale, where there was a late season bean rust outbreak and higher temperatures compared to Arusha but mild compared to Homabay, the highest yield was obtained from 'Teresa' followed closely by 'Masai', 'Bronco', 'Brio' and 'HT2', while the lowest yield was from 'Hystyle' (Table 3.5). At Homabay, where there was high temperature stress and a late season rust outbreak, the highest yield was from 'HT2', followed by 'Opus', 'Hystyle', 'HS1', 'Bronco' and 'Brio', and the lowest yields were from 'PV712' followed by 'PV698', 'Masai' and 'Palati' (Table 3.5). In Puerto Rico, the highest yields were from HT2, Bronco, HS1 and Brio while the lowest yields were from 'PV698', 'PV712', 'Amy', 'Juliet' and 'Masai' (Table 3.5).

To understand how the different site conditions, including high rust pressure from an early season outbreak at Arusha and high temperature stress at Homabay and Puerto Rico, influenced genotypic performance, yield at the four sites were compared using contrasts (Table 3.6) and regression analyses.

Yield data from Kitale and Arusha were contrasted using rust resistant and susceptible genotypes to test whether the difference in the duration of rust infection at the two sites significantly affected yield. There was no significant yield difference between the two sites for the highly rust resistant genotypes: 'HS1', 'HT1', 'HT3', 'PV712', 'Teresa' and 'PV698'. Non-significant differences were also observed for 'Juliet', 'Palati' and 'Opus' on which mild symptoms of rust were observed (Table 3.6).

Among the highly rust susceptible genotypes, two types of yield responses were observed between the early, or longer duration, rust infected Arusha site and the

late or shorter duration, rust infected sites, especially Kitale. In the first response, yield was significantly reduced at the early rust infected site at Arusha, compared to the late rust infected site at Kitale (Tables 3.5, 3.6 and Figure 3.5). The cultivars that manifested this yield response were ‘Brio’, ‘Bronco’ and ‘Masai’, as the highly significant contrasts of their yields at these two sites illustrate (Table 3.6 and Figure 3.5). The cultivars that showed this first type of yield response illustrate the magnitude of yield loss that a rust epidemic within bean production regions may cause on susceptible cultivars depending on the stage in the crop growth cycle at which the disease begins.

In the second yield response, in which the other highly susceptible cultivars ‘Amy’, ‘Barrier’ and ‘Hystyle’ were grouped, there were no significant yield differences between the early and late rust infected sites (Tables 3.5 and 3.6). This second group of cultivars could therefore be considered as rust tolerant in terms of yield. However, there were large rust pustules on pods which resulted in deformed pods and therefore reduced quality even in these rust tolerant cultivars.

Yield data from Kitale and Homabay was contrasted for all the genotypes grown to test whether the difference in temperature at the two sites significantly affected yield. The effect of rust infection on yield at these two sites was assumed to be similar but minimal, since rust outbreak at both sites was late in the season. Homabay and Kitale sites differ in both altitude and average temperature, with Kitale being cooler and in the highlands and Homabay hotter and at a lower altitude. The yields of ‘Amy’, ‘Teresa’, ‘PV712’, ‘Masai’ and ‘PV698’ were significantly reduced by the higher ambient temperature at Homabay, while the yields of ‘HS1’, ‘HT1’, ‘HT2’, and ‘HT3’ did not significantly differ at the two sites (Table 3.6; Figure 3.6). Similar results were obtained when Homabay yield data was compared to that of Arusha (Table 3.6).

Table 3.6. Site contrasts of pod yield for 16 snap bean genotypes at one Puerto Rican and three East African field sites.

Genotype*	Site yield contrasts, (*P* value)					
	Arusha *vs.* Homabay	**Arusha *vs*. Kitale**	**Arusha *vs*. Puerto Rico**	**Homabay *vs*. Kitale**	**Homabay *vs*. Puerto Rico**	**Kitale *vs*. Puerto Rico**
HS1	0.361	0.133	0.099	0.553	0.021	0.006
HT2	0.096	0.168	0.061	0.772	0.002	0.003
HT1	0.563	0.579	0.022	0.981	0.006	0.006
HT3	0.271	0.522	0.046	0.645	0.004	0.012
Amy	0.300	0.067	<0.001	0.004	<0.001	<0.001
Barrier	0.268	0.357	0.003	0.851	<0.001	<0.001
Brio	0.003	<0.001	0.845	0.101	0.023	<0.001
Bronco	0.048	<0.001	0.502	0.118	0.020	<0.001
Hystyle	0.019	0.617	0.090	0.004	<0.001	0.184
PV712	<0.001	0.337	<0.001	<0.001	0.010	<0.001
Juliet	0.118	0.659	<0.001	0.312	<0.001	<0.001
Masai	0.188	0.001	<0.001	<0.001	0.005	<0.001
Opus	0.056	0.081	0.010	0.863	<0.001	<0.001
Palati	0.127	0.845	0.003	0.086	0.090	0.002
Teresa	0.645	0.009	0.001	0.002	0.004	<0.001
PV698	<0.001	0.369	<0.001	<0.001	<0.001	<0.001

*For a given genotype within a row $P < 0.05$ (in shaded cells) indicate significant yield differences between the sites contrasted.

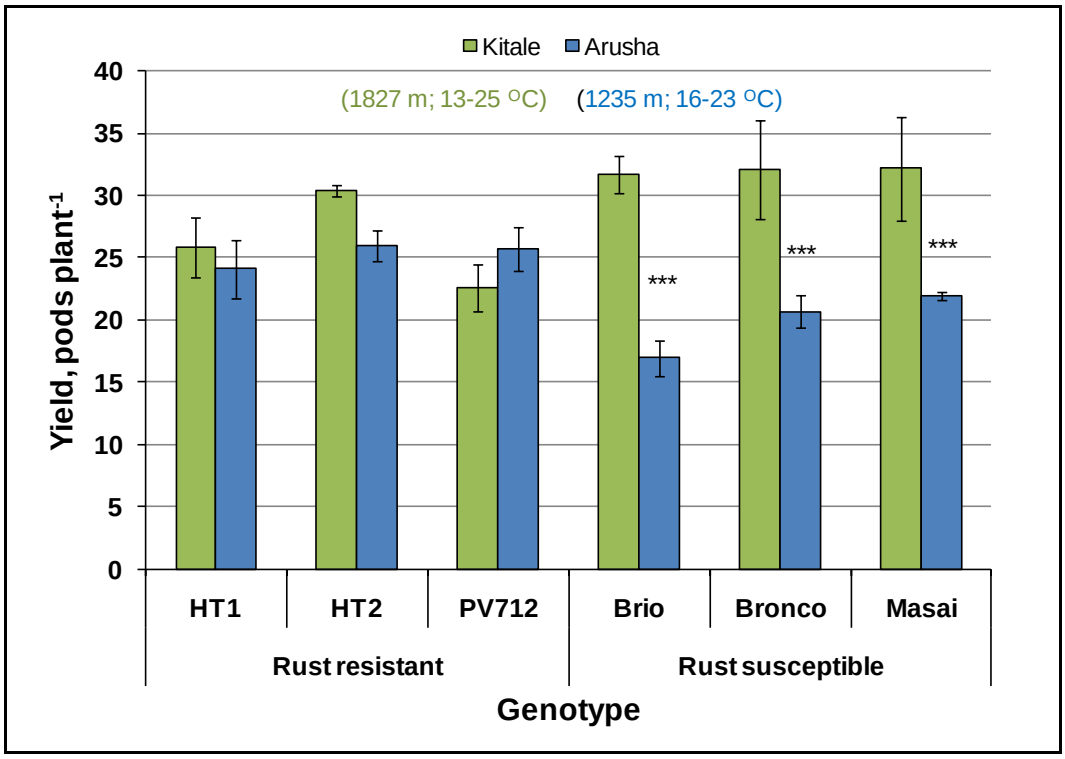

Figure 3.5. Effect of early season and late season rust disease outbreak at Arusha and Kitale respectively, on yield of rust resistant and rust susceptible snap bean genotypes. Bars indicate standard errors, *** *P* <0.001, n=4.

The non-significant difference in yield of the breeding lines between the higher temperature and cooler temperature sites in East Africa confirmed that they were tolerant to the higher temperatures at least to the temperature levels at Homabay. In these yield comparisons, it was also notable that the breeding line HS1, which was included in the trial as a rust resistant but heat sensitive check, did not significantly differ at any of the three sites in East Africa (Table 3.6).

Regression analyses of data from the four sites revealed a strong positive correlation (R^2=0.71, *P*<0.001) of pod yield of the 16 genotypes under high temperature stress in Puerto Rico when compared with Homabay, the highest

temperature African trial site (Figure 3.7). There was a weak negative correlation of yield data between Puerto Rico and the Arusha site (R^2=0.34, *P*=0.0188) and no correlation with the Kitale site (R^2=0.03, *P*=0.525) (Figure 3.7). The significant but weak negative correlation between Arusha and Puerto Rico yield data is attributed to contrasting responses of two sets of genotypes: 'PV698' and 'PV712' that are rust resistant and heat sensitive, and which yielded well in Arusha but poorly in Puerto Rico. 'Brio' and 'Bronco' two cultivars that are rust susceptible but heat tolerant, yielded well in Puerto Rico and poorly in Arusha (Figure 3.7). Statistical contrasts of 'Brio' and 'Bronco' showed insignificant differences in yield between the two sites (Table 3.6). The contrasting response of these two sets of genotypes contributed significantly to the observed negative correlation.

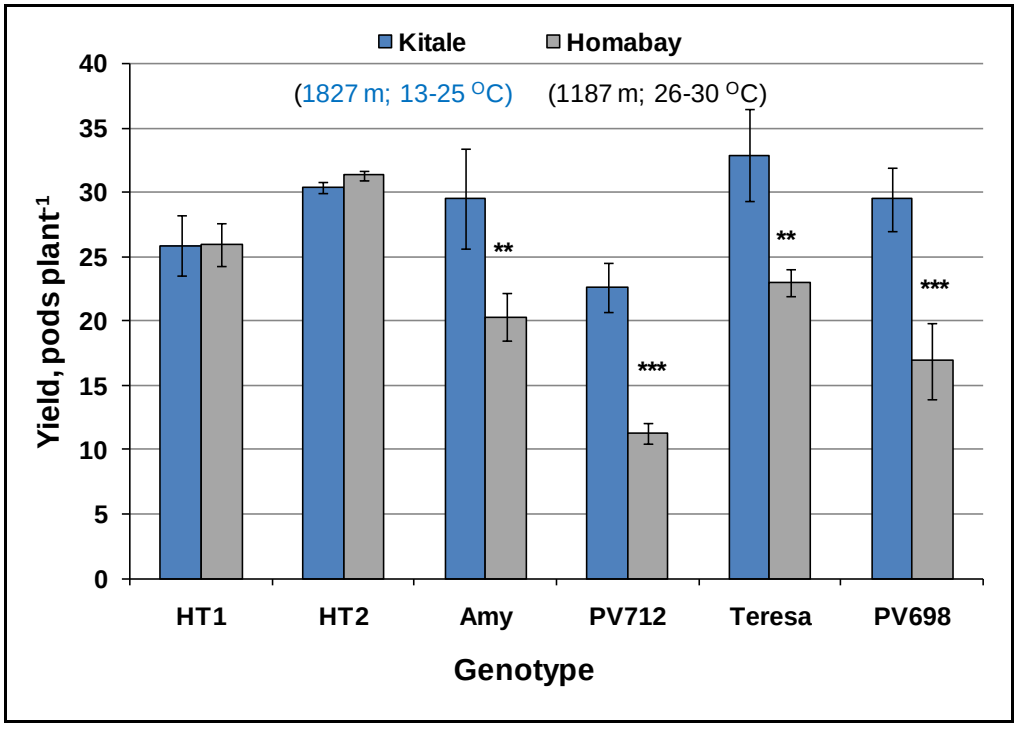

Figure 3.6. Effect of temperature differences between Homabay and Kitale sites on yield of two rust resistant and heat tolerant snap bean breeding lines: 'HT1' and 'HT2', and four snap bean cultivars, 'Amy', 'PV712', 'Teresa' and 'PV698'. Bars indicate standard errors, *** *P* <0.001, ** *P* <0.01, n=4.

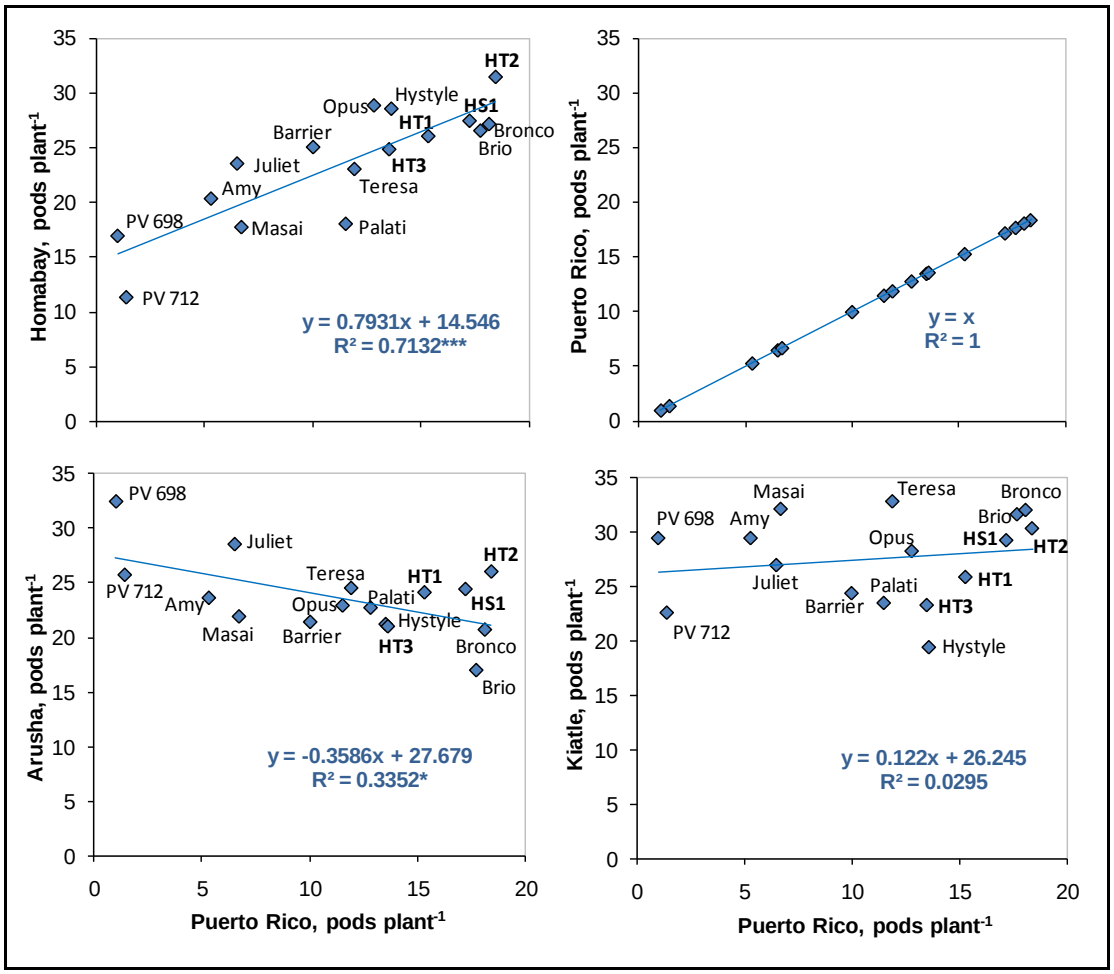

Figure 3.7. Regression analysis of pod yield of 16 selected snap bean genotypes from three East African sites: Arusha, Homabay and Kitale with yield from Puerto Rico. The data points are means of 4 replicates for the East African sites and 5 replicates for the Puerto Rican site. R^2 values followed by *** and * indicate $P<0.001$ and $P<0.05$, respectively.

At the Homabay and Puerto Rico sites ‘Amy’, ‘Masai’, ‘PV698’, and ‘PV712’ were the lowest yielding genotypes while ‘HT2’, ‘Bronco’, ‘HS1’, and ‘Brio’ were among the highest yielding, with ‘HT2’ having the highest yield at both sites (Table 3.5, Figure 3.7). The highly significant correlation of the results for Homabay and Puerto Rico indicate that the high temperatures at the two sites similarly influenced

genotype response across two very distinct agroecological zones. The lowest yielding genotypes at the two sites were therefore the most heat sensitive while the highest yielding genotypes were the most heat tolerant. This observation confirmed that the genotype difference in yield responses at Homabay and Kitale were due to ambient temperature differences.

Of the two high temperature sites, Homabay and Puerto Rico, Puerto Rico was more stressful as the values of the y-intercept in the regression equation indicate (Figure 3.7) and as the contrasts of genotypic yield at the two sites confirm (Table 3.6). In the regression equation, the y-intercept value for pod yield implies that a genotype that had a yield of 14.5 pods per plant at Homabay would not yield any pods in Puerto Rico. Contrasts of yield at the two sites revealed that of the 16 genotypes evaluated, 15 had significantly lower pod yields in Puerto Rico, further supporting the observation that Puerto Rico was more stressful (Tables 3.6 and Figure 3.7). However, it is notable that pod yield of the rust resistant and heat tolerant breeding lines: 'HT2', 'HS1', 'HT1', and 'HT3' ranked 1, 4, 5, 6, respectively, out of the 16 genotypes evaluated in Puerto Rico (Table 3.6). These results confirm the high temperature tolerance of these breeding lines under consistent heat stress in these tropical field environments.

3.4 Discussion and Conclusions

This is the first documented study under field conditions in the East African region comparing heat and rust resistant snap bean breeding lines with commercial snap bean cultivars grown in the region. The rust and heat tolerance traits are of great significance and have the potential to increase snap bean production in Eastern Africa as well as in other areas of the tropics and subtropics where heat stress and the common bean rust disease are constraints to production.

Results from the present study reveal stable rust resistance in three of the snap bean breeding lines, 'HS1', 'HT2' and 'HT1' with combinations of the *Ur-4* and *Ur-11* rust resistance genes, at three distinct field sites in Kenya and Tanzania. The fourth breeding line, 'HT3', appears heterozygous for the *Ur-11* rust resistance gene. The results obtained with the three breeding lines (with *Ur-4* and *Ur-11* genes) concur with the findings of Liebenberg (2003) in an earlier study in which this combination of rust resistance genes provided effective rust resistance to all known races of the bean rust pathogen from Eastern and Southern Africa. We have further demonstrated that selection of these breeding lines for heat tolerance traits under controlled environmental conditions conferred yield stability in their performance at the three rust infected, but variable temperature sites, and at the high ambient temperature site in Puerto Rico.

The observation that 'HS1', which was selected as a heat sensitive control was able to yield well even at the stressful high temperature field sites in East Africa and Puerto Rico implies that day and night greenhouse temperatures of 32°C and 27°C, that were used during the selection process, were more stressful than the conditions at the highest temperature field sites used in this study. The large yield reductions in 'Amy', 'Teresa', 'PV712' and 'PV698' that were observed at the Homabay and Puerto Rico sites (Tables 3.5, 3.6 and Figure 3.6) indicate that these cultivars, which are presently grown in or are targeted for production in the Eastern Africa region have high-sensitivity to relatively small increases in ambient temperature. This explains the current scenario in which snap bean production in the region is restricted to the cooler mid and higher altitude regions and supports the need to improve cultivars for high temperature tolerance. The large reduction in yield of these cultivars at the higher temperature sites illustrates the magnitude of the increase in production in warmer areas that genetic improvement for heat tolerance can bring. This observation together

with the observation that there was significant rust occurrence at the high temperature Homabay site, confirms the need to combine rust resistance and heat tolerance traits in snap bean cultivars for the East African region.

The high correlation of pod yield response from two very distinct sites, Homabay and Puerto Rico, indicates possible broad geographical effectiveness of the breeding lines selected for heat tolerance and thus the potential for broad applicability of heat tolerant cultivars across broad agro-ecological zones. This result also confirms the effectiveness of greenhouse high temperature tolerance selection.

The observation that Teresa was rust resistant at the Kitale site and, especially at the Arusha site where rust pressure was high, indicated that the *Ur-5* gene, present in Teresa, is effective against many races of rust found in the East African region. The finding that *Ur-5* also confers resistance against many races of the bean rust may be utilized to enhance durability of the resistance on *Ur-4* and *Ur-11* gene combination when *Ur-5* genotypes such as Teresa are crossed to genotypes that combine *Ur-4* and *Ur-11* resulting in cultivars that pyramid *Ur-4, Ur-5, and Ur-11*. These pyramided lines, carrying the Middle American resistance alleles, *Ur-5* and *Ur-11*, would be particularly effective against Andean races of rust that predominate in Eastern Africa.

The observation that only a portion of Teresa and Palati plants had symptoms of bean rust at Homabay may further imply that a race that overcomes *Ur-5* is present at this site at relatively low frequency or that it is less aggressive in nature. Possible differences in the structure of the genetic diversity of common beans grown in the Homabay region and hence a corresponding difference in the diversity of compatible races of the bean rust pathogen may also account for this observation.

Results from this study are significant for tropical regions such as Eastern and Southern Africa where common bean rust and heat stress are common constraints to snap bean production. The development and selection of snap bean breeding lines that

combine tolerance to heat stress and resistance to rust could increase the yield potential in these areas as well as expand potential production areas and/or seasons. Specific classes of common bean that are grown widely, such as small sieve snap bean cultivars grown in East Africa, are potential candidates for targeted improvement using the germplasm developed in this study. Utilization of the breeding lines developed in this study would also reduce reliance on fungicides that are used to mitigate yield losses from the rust disease. The reduction of fungicide use in the control of rust would reduce the costs of producing snap bean and hence increase profitability for growers.

CHAPTER FOUR

DEVELOPING SNAP BEAN GENOTYPES WITH COMMON BEAN RUST RESISTANCE AND HEAT TOLERANCE FOR EASTERN AFRICA

4.1 Introduction

Snap beans (*P. vulgaris* L.) are an important source of income to growers especially smallholder farmers in East Africa where production is mainly for export markets but demand for domestic consumption is also rapidly increasing (Okello and Roy, 2007; CIAT, 2008). Common bean rust, caused by *Uromyces appendiculatus*, and heat stress, caused by high ambient temperature, constrain snap bean production in many areas in tropical and temperate zones. Bean rust and heat stress often occur within the same production regions, for instance Eastern Africa, and significantly reduce snap bean yield. Yield losses to common bean rust range from 18-100% and damage is particularly high in areas where moderate mean ambient temperatures of 17-25^{o}C and high relative humidity over long periods of time during bean growth promote development of bean rust (Stavely and Pastor-Corrales 1989; Liebenberg 2006). In Eastern Africa high intensity of production of rust-susceptible dry and snap bean cultivars exacerbates severity of common bean rust. Most snap bean cultivars grown in Eastern and Southern African countries are highly susceptible to rust (Kimani et al. 2002; Mutunga et al., 2002; Jochua et al., 2004; Hillocks et al., 2006). The high severity of rust has led to excessive use of fungicides to control the disease, a practice that increases production costs and also has negative effects on produce quality, human health and the environment.

Exposure of common bean to day and night temperatures exceeding 30°C and 20°C, respectively, during reproductive development reduces yield and quality through floral abscission, disrupted fertilization and ovule development and pod

deformation (Porch and Jahn, 2001; Rainey and Griffiths, 2005a; Omae et al., 2007). In the East African region, snap bean production is presently limited to cool highland areas above 1500m as higher temperatures that prevail at lower altitudes reduce yield of available cultivars. This situation prevents a large number of farmers with land-holdings in the warmer environments from effectively engaging in the production of snap beans. Moreover, even in the cooler highland areas snap bean production is vulnerable in the longer term especially when considered in the light of global changes in climate that will result in high temperatures that adversely affect plant growth and productivity within agro-ecosystems (IPCC, 2001; Challinor et al., 2007; Wahid et al., 2007; Tubiello et al., 2008).

Genetic resistance is a cost-effective, practical, and environmentally sound strategy for snap bean farmers to manage bean rust (Stavely and Pastor-Corrales, 1989). However, since there are many races (>100) of rust pathogen that affect the common bean and none of the 13 known rust resistance genes are able to confer resistance against all the races, complete resistance is achieved through combined deployment of specific rust resistance genes. Combination of *Ur-4* and *Ur-11* rust resistance genes has been shown to confer resistance against all known races of the rust pathogen (Pastor-Corrales, 2006).

Similarly genetic improvement of snap beans for tolerance to high ambient temperatures is a cost effective and practical approach for increasing production and quality in high temperature environments. This approach is practical in that there is genetic variation in common bean for tolerance to heat stress and heat tolerant genotypes could be utilized to improve cultivars (Porch and Jahn, 2001; Rainey and Griffiths, 2005a; Petkova et al., 2007). Development of snap bean cultivars adapted to higher temperature settings will expand the set of crop choices for farmers in areas including East Africa and would also enable farmers to moderate effects of, or adapt to

spatial and temporal variability in climatic parameters such as increase in ambient temperature.

The overall strategy of this project was to develop snap beans with simultaneous introgressions of rust resistance and heat tolerance traits while selecting lines adapted to East African production environments. Additional goal was to select yield and quality attributes that are acceptable to growers and consumers. Genetic improvement of snap bean cultivars for the two traits is needed for the East African region to minimize yield and quality loss attributed to common bean rust, and to increase production in areas and seasons characterized by higher than optimal ambient temperatures.

To achieve this initial work focused on development and evaluation of snap bean populations that concurrently segregated for rust resistance (involving *Ur-4* and *Ur-11* gene combinations) and heat tolerance traits. Eight populations segregating for the two traits were developed from crosses between two USDA-ARS-BARC bean breeding lines, 'BelFla-RR-1' and 'BelJersey-RR-15', (both of which have the *Ur-4* and *Ur-11* bean rust resistance genes) as sources of rust resistance and four heat tolerant lines 'HT601', 'HT603', 'HT608' and 'HT611'. The four heat tolerant lines were from a previous diallel study conducted at Cornell University, Geneva, New York (Rainey and Griffiths, 2004; Rainey and Griffiths 2005c). From the populations developed, heat tolerant lines were selected at the F_2 and F_3 generations following exposure to greenhouse temperature settings of 32°C (day) and 27°C (night). Heat tolerant breeding lines that were homozygous for the *Ur-4* and *Ur-11* genes were selected at the F_4 generation following inoculation with rust races 67 and 108 (Chapter 3). Three breeding lines which were both heat tolerant and rust resistant were selected from three of the eight populations. The three selected breeding lines together with a rust resistant but heat sensitive control and 12 cultivars (selected for adaptation to

regions) were evaluated in East Africa and Puerto Rico at field sites with contrasting ambient temperatures. The breeding lines had superior performance (in terms of rust resistance and yield at the sites) relative to the cultivars including those currently grown in the East African region. This highlights their potential utility in genetic improvement of the cultivars.

The objectives of the present study were to: 1) utilize rust resistant and heat tolerant breeding lines with the best performance in greenhouse and field environments as parents in crosses with cultivars currently grown in East Africa to genetically improve them for the two traits while maintaining important yield and quality attributes; 2) evaluate the breeding lines developed from crosses with the East African cultivars at ecologically diverse field sites in East Africa; and 3) select breeding lines to target for further advancement and subsequent registration and release.

4.2 Materials and Methods

4.2.1 Plant materials

Three heat tolerant and rust resistant breeding lines: 'HT1', 'HT2', and 'HT3', together with the one more heat sensitive but rust resistant breeding line - HS1 - from the same genetic background as 'HT2' were planted in June 23, 2008 and crossed to selected snap bean cultivars 'Amy', 'Teresa' and 'PV712' which are currently grown in or targeted to the East African region but lack resistance to common bean rust, tolerance to heat stress or both (Table 4.1).

In making crosses with the East African cultivars key quality attributes were targeted in the selections including small sieve sized pods for which growers and consumers have high preference. 'PV712' is a new cultivar targeted to East Africa and has rust resistance based on the *Ur-4* and *Ur-11* genes but is highly heat sensitive.

Teresa also has rust resistance based on the *Ur-5* gene but is also heat sensitive. 'Amy' is both rust susceptible and heat sensitive. Crosses with the heat sensitive line 'HS1' were aimed at developing populations from which heat sensitive lines could be selected for use as checks during subsequent evaluations. The breeding lines were also crossed to 'Masai' and 'Bronco' which are cultivars that are grown in the USA but have plant types and quality attributes that could potentially be adopted in East Africa. The four breeding lines were the female parents and the cultivars were the males. The parents were grown in a greenhouse with temperature settings of 24°C/21°C day/night. The breeding scheme and characteristics of the parents are shown in Figure 4.1.

Table 4.1. Sources and characteristics of snap bean genotypes used in crosses to develop rust resistant and heat tolerant snap beans for East Africa.

Genotype	Source	Characteristics
HT1	Cornell University, Geneva, NY, USA	Heat tolerant, rust resistant (has *Ur-4* and *Ur-11*)
HT2	Cornell University, Geneva, NY, USA	Heat tolerant, rust resistant (has *Ur-4* and *Ur-11*)
HT3	Cornell University, Geneva, NY, USA	Heat tolerant, rust resistant (has *Ur-4* and *Ur-11*)
HS1	Cornell University, Geneva, NY, USA	Heat sensitive, rust resistant (has *Ur-4* and *Ur-11*
PV712	Pop Vriend Seeds, Netherlands	New variety, heat sensitive, rust resistant (has*Ur-4*, *Ur-11*)
Amy	Seminis, Saint Louis, MO, USA	Heat sensitive, rust susceptible, small sieve
Teresa	Seminis, Saint Louis, MO, USA	Heat sensitive, has rust resistance based on *Ur-5* gene, small sieve,
Bronco	Seminis, Saint Louis, MO, USA	Heat tolerant, rust susceptible BCMV resistant, medium sieve
Masai	Syngenta, Golden Valley, MN, USA	Heat sensitive, rust susceptible, small sieve

4.2.2 Population development and breeding line selection

Twenty F_1 hybrid combinations were generated from crosses involving the four selected snap bean breeding lines and five cultivars. The F_1s hybrids were self-pollinated to produce 20 F_2 populations. From each of the 20 F_2 populations, 40 plants were grown January 2009 in a greenhouse with temperature settings similar to those used during the crossing and selfing of the F_1 hybrids. A total of 800 F_2 plants were grown in a randomized complete block design (RCBD) with four replications. The F_2 populations were evaluated and selected for small sieve sized pods. Four lines that had high numbers of pods and seeds per plant in addition to having small sieve sized pods (also characterized by small seeds weighing 0.14-0.20 g) were selected from each of 16 populations derived from crosses with the three small sieve cultivars ('Amy', 'Masai', 'Teresa' and 'PV712'. An additional set of four lines each that had high numbers of pods and seeds per plant in addition to having medium sieve sized pods (with single seeds weighing 0.20-0.35 g) were selected from four of the populations that were derived from 'Bronco' which has large sieve sized pods. A total of 80 selections were made.

The 80 selected lines (F_3s) were planted in April 2009 in a temperature controlled greenhouse for evaluation and selection for heat tolerance and pod quality. Four plants each of the nine parental lines were also grown alongside the four lines selected from the 20 populations giving a total of 356 plants in the greenhouse. The plants were grown within the greenhouse in a completely randomized design (CRD) with four replications each represented by a single plant. Three weeks after planting (just before the plants switched to reproductive phase) greenhouse temperature settings were adjusted to 32/28°C day and night to ensure heat stress until end of the crop cycle.

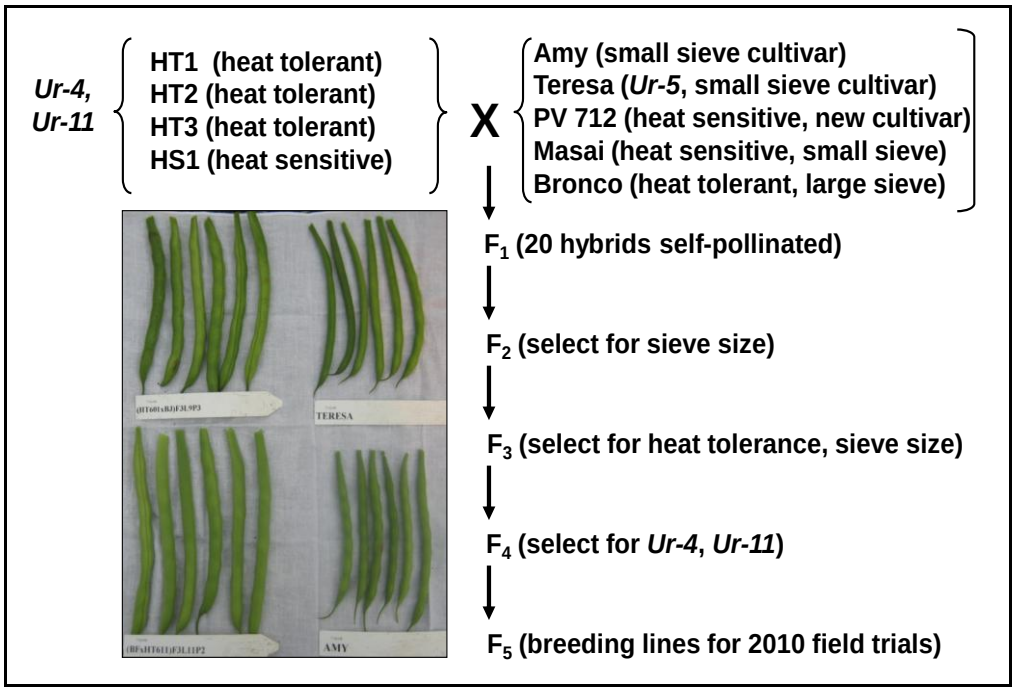

Figure 4.1. Breeding scheme for the development and selection of rust resistant heat tolerant snap beans for East Africa.

Harvest data analysis focused on five yield components: number of pods per plant, number of seeds per plant, number of seeds per pod, total seed weight per plant and single seed weights. The best performing plants were picked from each of the 80 F_3 lines. For each of the lines, plants that had the highest number of seeds per pod, total seed weight per plant, number of pods per plant and total number of seeds per plant were selected. An additional set of 26 lines were selected from the 20 populations as backup to the 80 best performing lines. The 80 best lines together with the 26 backup lines were concurrently increased for seed and also tested for the presence of *Ur-4* and *Ur-11* rust resistance genes to identify lines fixed for the two rust genes. For the seed increase, four plants of each of the 106 lines were grown in a greenhouse with temperatures settings of 24°C/21°C day/night. The procedure followed in testing for the two rust genes is described in Chapter 2. The F_4 lines that

were fixed for the two rust resistance genes were selected. For each of the four cultivar backgrounds, four lines that were fixed for *Ur-4* and *Ur-11* rust genes and also ranked high in heat tolerance were selected. Heat tolerant lines that were fixed or heterozygous for the *Ur-11* gene were additionally selected in situations where less than four heat tolerant breeding lines were fixed for the two rust genes to enable equal cultivar background representation during subsequent field evaluations preceding final selections. Twenty breeding lines were selected for subsequent field testing (Table 4.2).

4.2.3 Field Trials

4.2.3.1 Plant materials and design of trials

Twenty rust resistant and heat tolerant breeding lines were field-evaluated at the F_5 generation together with the parents: 'HT1', 'HT2', 'HT3', 'HS1', 'Amy', 'PV 712', 'Teresa', 'Masai' and, 'Bronco' as controls. Additional controls in the field evaluation were other cultivars adapted to various geographical regions involved: 'PV 698' (East Africa); 'Barrier' and 'Juliet' (Southern Africa); 'Palati' (Northern Africa); 'Opus' and 'Brio' (Southern USA); and 'Hystyle' (Northeastern USA). There were 36 entries in total. The entries were grown in a randomized complete block design (RCBD) with four replications per site on a total of four field sites.

Twelve differential cultivars were also planted at each of the four sites with the aim of determining the virulence diversity of the bean rust pathogen at the sites. Six of the differential cultivars are from the Andean gene pool and included 'Early Gallatin', 'Redlands Pioneer', 'Montcalm', 'P.C.50', 'Golden Gate Wax', and 'PI 260418' while those from the Middle American gene pool included 'Aurora', 'Compuesto Negro Chimaltenango' (CNC), 'PI 181996', 'Mexico 309', 'Great Northern 1140', and 'Mexico 235'.

4.2.3.2 Location and description trials

The field trials were carried out between March and June 2010 during the long rain season at four sites in East Africa: Homabay, Kakamega and Kitale in Kenya and Arusha in Tanzania. The sites were selected on the basis of differences in altitude, soils and climate. Three of the sites: Arusha, Homabay, and Kitale were used in an earlier study that involved the current set of 16 controls and characteristics of these sites are detailed in Chapter 3. Briefly, altitude at the sites ranged from 1172 m at Homabay to 1829 m at Kitale. Kakamega is located at an altitude of 1585 m, latitude 00º 16'N and longitude 34º 45'E. Homabay had the highest mean temperatures among the trial sites with mean daily temperatures of 27º C (minimum) and 33.5º C (maximum).

The sites were tractor ploughed and harrowed to a fine tilth prior to planting. The planting dates were March 10 (Arusha), March 22 (Kakamega), March 24 (Kitale) and March 26 (Homabay). Single rows of 25 plants were planted per block for each of the 36 entries. A planting density in which single plants were planted at a spacing of 0.5 m between rows and 0.1 m within rows was used at all the sites. A compound inorganic fertilizer (N-10%, P-26%, K-10%, S-4%, Ca-10%, Mg-4% and micronutrients-5%) was row applied at planting at a rate of 200 kg ha^{-1} at all the sites except Arusha.

The trial plots were rain fed but supplemental irrigation was done at the trial plots in Arusha, Homabay and Kitale to avert potential water stress due to low rains during the early stages of the trial. The plots were kept weed free biweekly using hand weeding using hoes commencing soon after emergence and with a break during flowering. The trials were constantly monitored and kept free of aphids and other insect pests with the application of insecticide (dimethoate 40% v/v).

Table 4.2. Twenty rust resistant/heat tolerant snap bean breeding lines targeted for selection under East African field environments.

Line name	Line code	Pedigree	Field entry no.	Seed plant^{-1}	Pod plant^{-1}	Seed wt, g plant^{-1}	Seeds Pod^{-1}	Single seed wt, g	Rust Gene Status
L1	10AHT2F5	(Amy x HT2) F3-4 P1	1	104	30	14.5	3.47	0.14	Heterozyg *Ur-4, Ur-11*
L2	10AHT31F5	(Amy x HT3) F3-4 P1	2	104	47	22.59	2.21	0.22	Homozyg *Ur-11*
L3	10AHT32F5	(Amy x HT3) F3-4 P3	3	118	29	15.74	4.07	0.13	Heterozyg *Ur-11*
L4	10AHT33F5	(Amy x HT3) F3-2 P4	4	170	47	24.87	3.62	0.15	Heterozyg *Ur-11*
L5	10BHT11F5	(Bronco x HT1) F3-1 P2	5	95	30	15.23	3.17	0.16	Homozyg *Ur-4, Ur-11*
L6	10BHT12F5	(Bronco x HT1) F3-4 P3	6	94	29	10.99	3.24	0.12	Homozyg *Ur-4, Ur-11*
L7	10BHT3F5	(Bronco x HT3) F3-1 P3	7	103	33	17.55	3.12	0.17	Homozyg *Ur-4, Ur-11*
L8	10BHS1F5	(Bronco x HS1) F3-2 P3	8	81	28	17.38	2.89	0.21	Homozyg *Ur-4, Ur-11*
L9	10JHT11F5	(PV712 x HT1) F3-2 P1	9	85	22	16.49	3.86	0.19	Homozyg *Ur-4, Ur-11*
L10	10JHT12F5	(PV712 x HT1) F3-2 P2	10	93	24	13.91	3.88	0.15	Homozyg *Ur-4, Ur-11*
L11	10JHT31F5	(PV712 x HT3) F3-3 P4	11	107	29	19.46	3.69	0.18	Homozyg *Ur-4, Ur-11*
L12	10JHT32F5	(PV712 x HT3) F3-4 P3	12	95	34	13.37	2.79	0.14?	Homozyg *Ur-4, Ur-11*
L13	10MHT11F5	(Masai x HT1) F3-2 P3	13	107	20	15.08	5.35	0.14	Homozyg *Ur-4, Ur-11*
L14	10MHT12F5	(Masai x HT1) F3-4 P3	14	164	38	24.09	4.32	0.15	Homozyg *Ur-4, Ur-11*
L15	10MHT2F5	(Masai x HT2) F3-1 P4	15	145	41	19.5	3.54	0.13	Homozyg *Ur-4, Ur-11*
L16	10MHS1F5	(Masai x HS1) F3-2 P4	16	106	32	15.37	3.31	0.15	Homozyg *Ur-4, Ur-11*
L17	10THT11F5	(Teresa x HT1) F3-1 P3	17	98	50	19.33	1.96	0.20	Homozyg *Ur-4, Ur-11*
L18	10THT12F5	(Teresa x HT1) F3-4 P3	18	102	20	14.88	5.10	0.15	Homozy *Ur-11*, heter *Ur-4*
L19	10THT3F5	(Teresa x HT3) F3-2 P1	19	90	33	13.65	2.73	0.15	Homozyg *Ur-4, Ur-11*
L20	10THS1F5	(Teresa x HS1) F3-4 P4	20	98	37	17.45	2.65	0.18	Homozyg *Ur-4, Ur-11*

4.2.4 Data collection and analysis

The snap bean entries and differential cultivars were monitored for symptoms of rust and other diseases. Dates on which first disease symptoms were observed at the sites were noted so as to account for possible site differences in rust severity and its effect on yield. The genotypes were scored for common bean rust at flowering (R6) and at pod filling (R8) developmental stages. Genotype reaction to rust (severity) was evaluated following the CIAT common bean evaluation scale (Schoonhoven and Pastor-Corrales, 1987). Quantitative information on genotypic reaction to rust and rust incidence was also obtained by counting the number of rust infected plants per plot, while rust severity was determined by the number of visible rust pustules formed per leaflet. The sets of rust incidence and severity data were used in a correlation plot to obtain a simultaneous quantification of genotype differences in reaction to rust.

Data on genotypic performance was obtained by recording the number of pods produced per plant. The total number of pods produced per plot was counted, while excluding plants at the end of the rows, and then divided by the total number of plants examined within the plot. The data was statistically analyzed using regression analysis of variance and the means separated using Tukeys HSD test procedure. The statistical analyses were done using JMP 7 software (SAS Institute Inc., Cary, NC, 1989-2008). Information on rust resistance status, pod yield, plant type and pod quality attributes were used to select lines to target for advancement. Selection for plant type and pod quality was visually done.

4.3 Results and Discussion

4.3.1 General observations

Information on snap bean genotype and differential cultivar reaction to rust and adaptation to different field environments was obtained from all the four sites planted.

Mean minimum and maximum temperatures at the sites during the field trial period were: Arusha (13.0º C and 24.3º C) Homabay (27.0º C and 33.5º C), Kakamega (15.5º C and 28.9º C) and Kitale (13.6º C and 25.7º C) (Figure 4.2). The lowest and the highest mean temperatures were recorded at Arusha and Homabay, respectively.

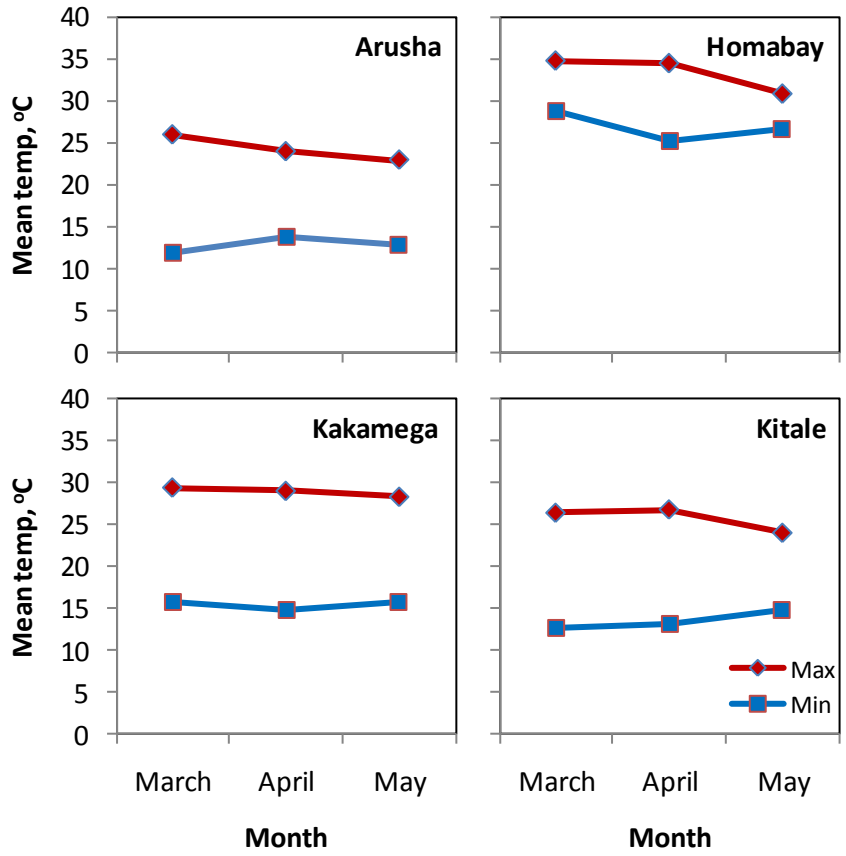

Figure 4.2. Mean monthly minimum and maximum temperatures during 2010 field trials at Arusha, Homabay, Kakamega and Kitale sites.

Rainfall was adequate at the sites but was not well distributed throughout the period of the study (Figure 4.3). There was a dry spell in the third week of the trial at Homabay, Kakamega and Kitale, which affected plant growth. In Arusha there were

heavy rains during the third and fourth weeks of the trial, which caused water-logging, that negatively impacted plant growth and resulted in considerable plant mortality affecting overall stand establishment at the sites.

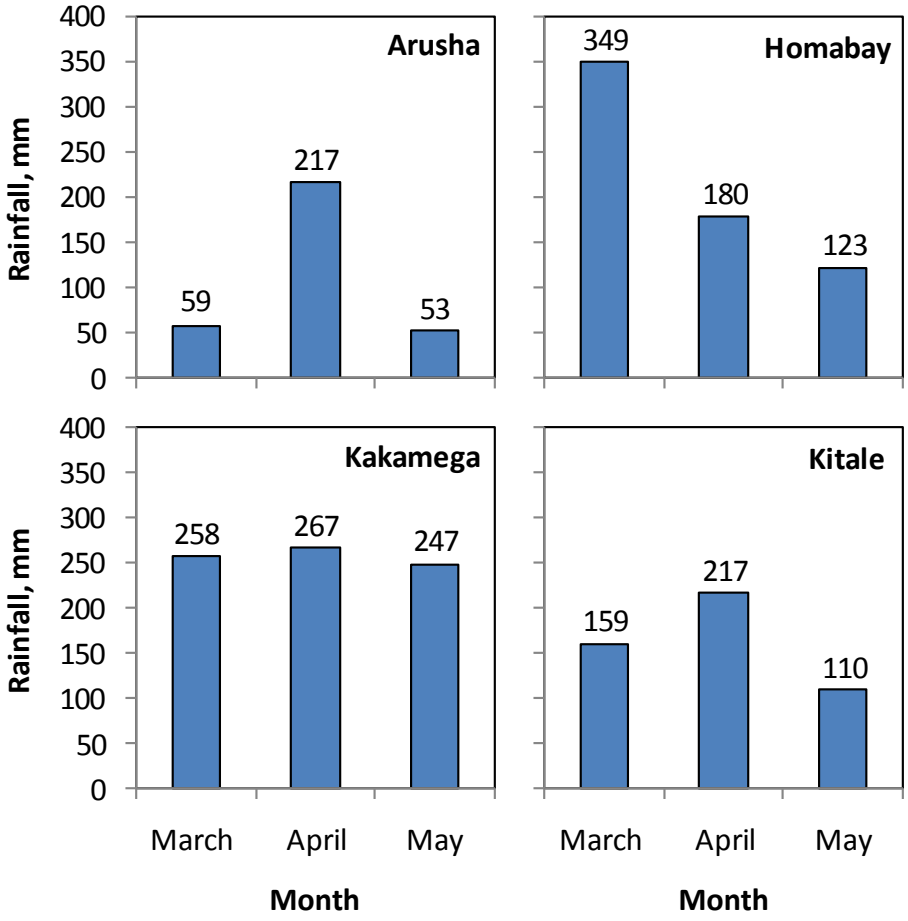

Figure 4.3. Mean monthly rainfall during 2010 field trials in Arusha, Homabay, Kakamega and Kitale sites at Arusha, Homabay, Kakamega and Kitale sites.

Stand establishment was highest in Kakamega followed by Kitale, Homabay and Arusha in descending order (Figure 4.4). Bean anthracnose and angular leaf spot

diseases were observed at varying levels at the four sites. There were high incidences of angular leaf spots at Arusha, moderate incidence of anthracnose in Kakamega and Kitale. Common bean rust was observed at all four sites.

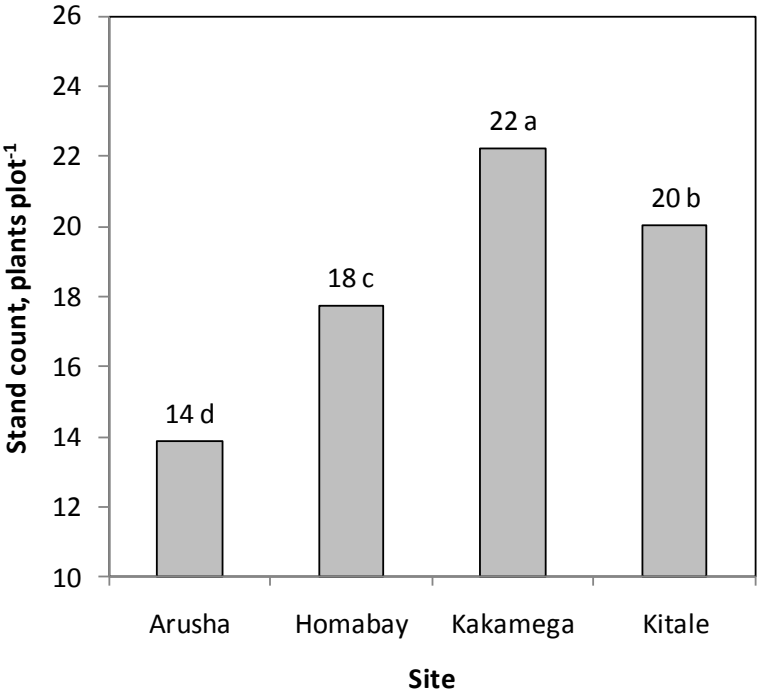

Figure 4.4. Mean stand count of 36 snap bean genotypes at Arusha, Homabay, Kakamega and Kitale during 2010 field trials. Maximum stand per plot would be 25. Means not followed by same letter are significantly different according to Tukeys HSD.

4.3.2 Reaction to common bean rust

The sites differed significantly in severity of the observed common bean rust (Figure 4.5). Rust Severity was highest in Kakamega followed by Kitale (though not significant difference) and then followed by Arusha and Homabay. Thus the bean rust was more severe at the higher altitude sites (> 1500 m). The high rust severity at the high altitude sites may be attributed to higher virulence diversity of the pathogen at

these sites as data on bean differential cultivar's reaction to rust at the sites attest (Table 4.3).

With the exception of PI 260418, most of the Andean differential cultivars were susceptible at three of four locations. On the other hand, with the exception GN 1140, most of the Middle American cultivars were resistant at three of four locations. These results suggest that the races of the rust pathogen prevalent in these four locations are Andean. The virulence spectrum of the races of the rust pathogen at high altitude sites (Kakamega and Kitale) was greater than at low altitude sites (Arusha and Homabay). Evidence of differences between high altitude and low altitude sites in virulence diversity of the bean rust was observed on the reaction of differential cultivars 'Aurora', from the Andean gene pool, and 'Redlands Pioneer' from the Middle American gene pool (Table 4.3). 'Aurora' (which has the *Ur-3* gene) and 'Redlands pioneer (*Ur-13*) were rust resistant at Arusha and Homabay – the low altitude sites but were susceptible at Kakamega and Kitale sites.

Even among Arusha and Homabay sites where there was no statistical difference in overall rust severity on the genotypes grown but which was on average were lower than the severity at Kakamega and Kitale (Figure 4.5), there were differences in virulence diversity of the bean rust as shown by the reaction of the Andean differential cultivar 'PC 50' which has *Ur-9* and was susceptible at all the sites except Arusha (Table 4.3). The virulence diversity of rust at the sites may be the result of differences in diversity of bean cultivars grown in the different regions and how this interacts with climatic conditions that favor the development of compatible isolates of the rust. Most common production in Eastern Africa is in the cool highland areas (Asfaw et al., 2009). As a result there is a higher diversity in the bean genotypes grown and consequently a wider spectrum of virulent isolates of common bean rust at the highlands compared to the low altitude areas.

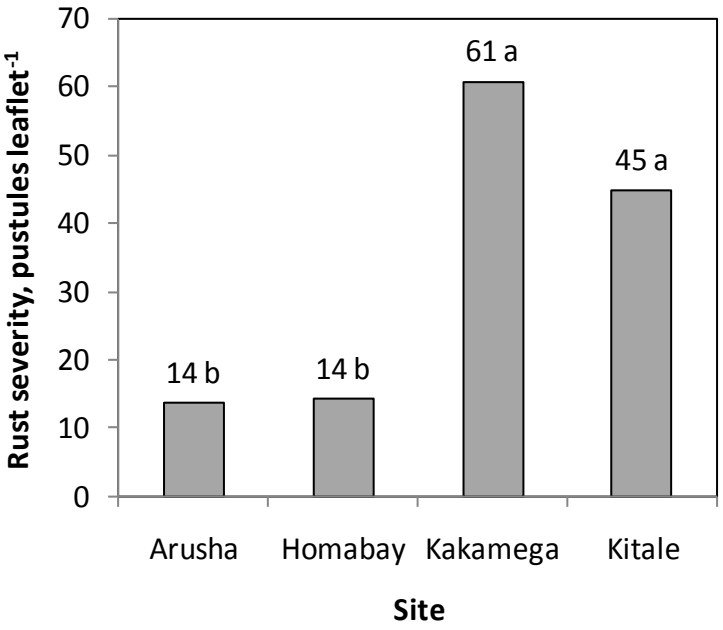

Figure 4.5. Mean rust severity on 36 snap bean genotypes at Arusha, Homabay, Kakamega and Kitale during 2010 field trials. Means not followed by same letter are significantly different according to Tukeys HSD.

Among the controls, 'HS1', 'HT1', 'HT2', 'PV698', 'PV712' and 'Teresa' were rust resistant at all the sites. However, 'Teresa' (which has *Ur-5*) was not completely resistant as a few rust pustules were observed on 20% of its plants at Kakamega (Tables 4.4 and 4.5) indicating the presence of a virulent isolate that overcomes the *Ur-5* gene at a low frequency. Palati which also has *Ur-5* gene was rust infected but at a low frequency in Homabay and Kakamega (Tables 4.4 and 4.5). The reaction of 'Palati' to rust at the two sites further supports the possible presence at these sites of a rust isolate that overcomes the *Ur-5* and is consistent with previous findings from this site (Chapter 3). The cultivars 'Amy', 'Barrier', 'Brio', 'Bronco', 'Hystyle' and 'Masai' were rust susceptible across the four sites. 'Bronco' had the most intense pustule formation on leaves (Figure 4.6).

Table 4.3. Characteristics of 12 bean differential cultivars and reaction to common bean rust at Arusha, Homabay, Kakamega, and Kitale field sites in East Africa and implied virulence diversity of the pathogen at contrasting field sites in the region.

Differential cultivar	Rust resistance gene[a]	Gene pool	Binary value	Reaction to rust by site[bc]			
				Arusha	Homabay	Kakamega	Kitale
Early Gallatin	*Ur-4*	Andean/Mid Amer	1	S	S	S	S
Redlands Pioneer	*Ur-13*	Andean	2	R	R	S	S
Montcalm	Unknown	Andean	4	S	S	S	S
P.C. 50	*Ur-9 and Ur-12*	Andean	8	R	S	S	S
Golden Gate Wax	*Ur-6*	Andean/Mid Amer	16	S	S	S	S
PI 260418	*Ur-P (Unknown)*	Andean	32	R	R	R	R
Great Northern 1140	*Ur-7*	Middle American	1	S	S	S	S
Aurora	*Ur-3*	Middle American	2	R	R	S	S
Mexico 309	*Ur-5*	Middle American	4	R	R	R	R
Mexico 235	*Ur-3+*	Middle American	8	R	R	R	R
CNC	Unknown	Middle American	16	R	R	R	R
PI 181996	*Ur-11*	Middle American	32	R	R	R	R
Rust race				**21-1**	**29-1**	**31-3**	**31-3**

[a] Differential cultivar with Unknown in the rust resistance gene column have unidentified rust gene.
[b] S and R denote susceptible and resistant reactions, respectively.
[c] Based on susceptible reaction of the differential cultivars, the virulence diversity of the common bean rust at the sites was in the order of Arusha<Homabay<Kakamega=Kitale.

Table 4.4. Incidence of common bean rust on 36 snap bean genotypes at Arusha, Homabay and Kakamega field sites in East Africa.

Genotype	Line No.	Rust incidence (rust infected plants), %				
		Arusha	Homabay	Kakamega	Kitale	All sites
L1	1	18 b	2 e	17 d-f	-	13 e-g
L2	2	0 b	0 e	25 d-f	-	8 fg
L3	3	30 b	39 a-e	45 b-e	-	38 de
L4	4	17 b	9 e	24 d-f	-	17 e-g
L5	5	0 b	0 e	0 f	-	0 g
L6	6	29 b	15 de	42 c-f	-	29 ef
L7	7	9 b	9 e	17 d-f	-	12 fg
L8	8	0 b	0 e	0 f	-	0 g
L9	9	0 b	0 e	0 f	-	0 g
L10	10	0 b	0 e	0 f	-	0 g
L11	11	0 b	0 e	0 f	-	0 g
L12	12	0 b	0 e	0 f	-	0 g
L13	13	0 b	0 e	0 f	-	0 g
L14	14	7 b	14 de	10 e-f	-	10 fg
L15	15	0 b	0 e	0 f	-	0 g
L16	16	0 b	0 e	0 f	-	0 g
L17	17	0 b	0 e	0 f	-	0 g
L18	18	0 b	0 e	0 f	-	0 g
L19	19	2 b	0 e	16 d-f	-	6 fg
L20	20	2 b	0 e	0 f	-	1 g
HT3	21	92 a	60 a-d	90 a	-	80 ab
HS1	22	0 b	0 e	0 f	-	0 g
HT1	23	0 b	0 e	0 f	-	0 g
HT2	24	0 b	0 e	0 f	-	0 g
Opus	25	0 b	9 e	53 a-d	-	21 e-g
Palati	26	10 b	11 de	34 d-f	-	18 e-g
Hystyle	27	69 a	22 c-e	92 a	-	61 b-d
Barrier	28	84 a	34 b-e	80 a-c	-	66 a-c
PV698	29	0 b	0 e	0 f	-	0 g
Amy	30	95 a	73 a-c	81 a-c	-	83 ab
Juliet	31	4 b	15 de	18 d-f	-	12 fg
Masai	32	32 b	42 a-e	87 ab	-	54 cd
Brio	33	73 a	80 ab	84 ab	-	79 ab
PV712	34	0 b	0 e	0 f	-	0 g
Teresa	35	0 b	0 e	4 e-f	-	1 g
Bronco	36	86 a	85 a	95 a	-	89 a

*Within a site/column, means followed by the same letter are not significantly different according to Tukeys HSD.

Table 4.5. Severity of common bean rust on 36 snap bean genotypes at Arusha, Homabay, Kakamega and Kitale field sites in East Africa.

Genotype	Line No.	Rust severity, pustules leaflet^{-1}				
		Arusha	Homabay	Kakamega	Kitale	All sites
L1	1	10 b	3 b	54 de	300	92 bc
L2	2	0 b	0 b	82 c-e	18	25 c-e
L3	3	10 b	23 b	43 e	184	65 b-e
L4	4	14 b	6 b	75 de	172	67 b-e
L5	5	0 b	0 b	0 e	0	0 e
L6	6	18 b	7 b	71 de	43	35 c-e
L7	7	9 b	7 b	35 e	75	31 c-e
L8	8	0 b	0 b	0 e	0	0 e
L9	9	0 b	0 b	0 e	0	0 e
L10	10	0 b	0 b	0 e	0	0 e
L11	11	0 b	0 b	0 e	0	0 e
L12	12	0 b	0 b	0 e	0	0 e
L13	13	0 b	0 b	0 e	0	0 e
L14	14	4 b	17 b	71 de	1	23 c-e
L15	15	0 b	0 b	0 e	0	0 e
L16	16	0 b	0 b	0 e	0	0 e
L17	17	0 b	0 b	0 e	0	0 e
L18	18	0 b	0 b	0 e	0	0 e
L19	19	5 b	0 b	39 e	47	23 c-e
L20	20	1 b	0 b	0 e	0	0 e
HT3	21	39 b	46 ab	135 b-e	30	63 b-e
HS1	22	0 b	0 b	0 e	0	0 e
HT1	23	0 b	0 b	0 e	0	0 e
HT2	24	0 b	0 b	0 e	0	0 e
Opus	25	0 b	9 b	62 de	100	43 b-e
Palati	26	0 b	8 b	18 e	0	7 e
Hystyle	27	26 b	37 ab	220 a-c	82	91 bc
Barrier	28	39 b	38 ab	270 ab	43	97 bc
PV698	29	0 b	0 b	0 e	0	0 e
Amy	30	26 b	46 ab	185 b-d	72	82 b-d
Juliet	31	2 b	3 b	36 e	1	11 de
Masai	32	60 b	11 b	233 ab	76	95 bc
Brio	33	32 b	105 ab	267 ab	67	117 b
PV712	34	0 b	0 b	0 e	0	0 e
Teresa	35	0 b	0 b	6 e	0	2 e
Bronco	36	198 a	147 a	283 a	300	232 a

*Site means followed by the same letter are not significantly different according to Tukeys HSD.

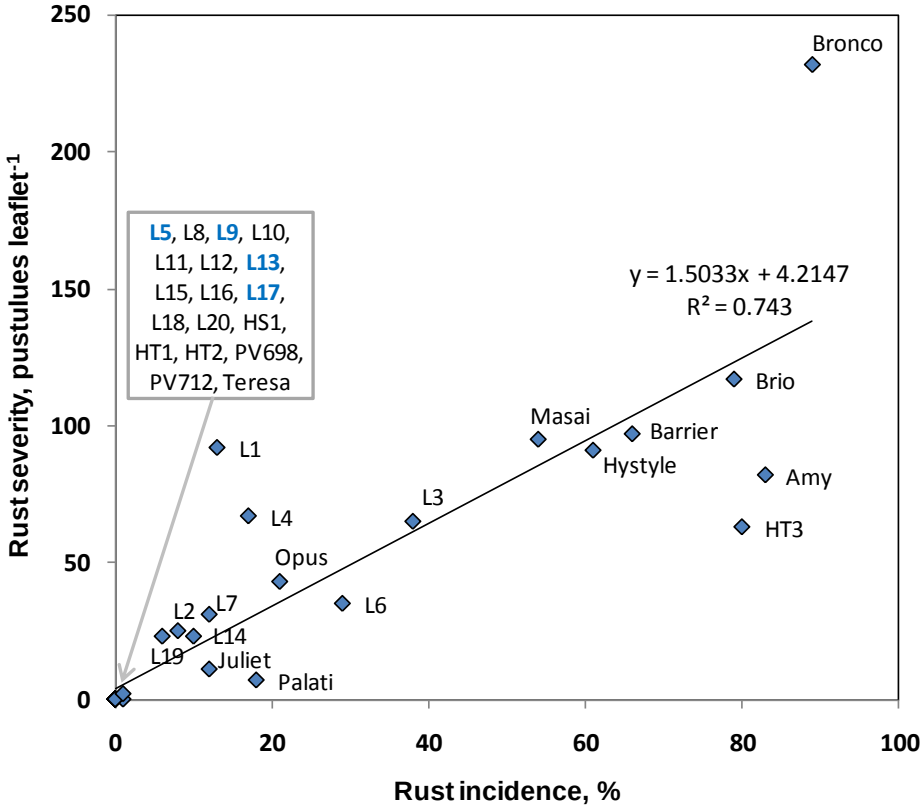

Figure 4.6. Rust incidence and severity on 20 snap bean breeding lines (L1-L20) and 16 control genotypes evaluated in 2010. The data points are genotype means over four field sites: Arusha, Homabay, Kakamega and Kitale.

Among the 20 snap bean breeding lines under evaluation, the rust resistant lines in Arusha, Homabay, Kakamega and Kitale were: 'L5', 'L8', 'L9', 'L10', 'L11', 'L12', 'L13', 'L15', 'L16', 'L17', 'L18', and 'L20' (Tables 4.4 and 4.5; Figure 4.6). Line 2 (L2) was rust free in Arusha and Homabay but was highly susceptible in Kakamega and Kitale. The other breeding lines that were heterozygous for the *Ur-11* gene included: 'L1', 'L3', 'L4', 'L6', 'L7', 'L14' and 'L19'. All the breeding lines (lines 1-4) previously selected from populations derived from 'HT1' crossed with

'Amy' were heterozygous for *Ur-11*. All four breeding lines (lines 9-12) descending from the cross between 'HT1' and 'PV712' were fixed for the *Ur-11* gene just as the two parents. The rust resistant breeding lines 'L5', 'L8', 'L9', 'L10', 'L11', 'L12', 'L13', 'L15', 'L16', 'L17', 'L18', and 'L20' were therefore subjected to further selection based on yield, pod quality and other phenotype qualities such as strong plant type.

4.3.3 Yield

The four sites significantly differed in the average pod yield of the 36 snap bean genotypes grown (Figure 4.7). The highest yield was from Homabay followed by Kakamega and Kitale (which were not significantly different from each other) and Arusha which had the lowest yields (Figure 4.7). Low soil fertility, lack of fertilizer nutrient inputs coupled with water logged soils at the Arusha site adversely affected bean growth and yield. Yield was high in Homabay because of fertile soils in addition to nutrient inputs at planting.

Genotypic differences in stand establishment did not significantly affect pod yield per plant across the four sites (Figure 4.8). For the top five genotypes the highest yield was from 'L17' followed by 'L19', 'Masai', 'L9' and 'PV698' across the four sites (Table 4.6). Effect of site temperature differences on yield was noticeable on 'Masai'. 'Masai' had lower yield in Homabay (which was the hottest of the sites) compared to its yield at the cool high altitude site in Kitale.

From the 12 snap bean breeding lines ('L5', 'L8', 'L9', 'L10', 'L11', 'L12', 'L13', 'L15', 'L16', 'L17', 'L18', and 'L20') which were confirmed to be rust resistant at the four field sites, the best yielding at each of the sites and across the sites were selected. Lines 'L5', 'L9', 'L13' and 'L17' were selected as the most promising lines to target for advancement on the basis of their high yields (equal to or more than

the grand mean at each of the sites), high pod quality and strong, upright bush plant phenotype in addition to being rust resistant (Figure 4.9). Across the four sites the five selected rust resistant lines had pod yields which were equal to or higher than those of their parent cultivars which are currently grown in or targeted to East Africa (Table 4.7).

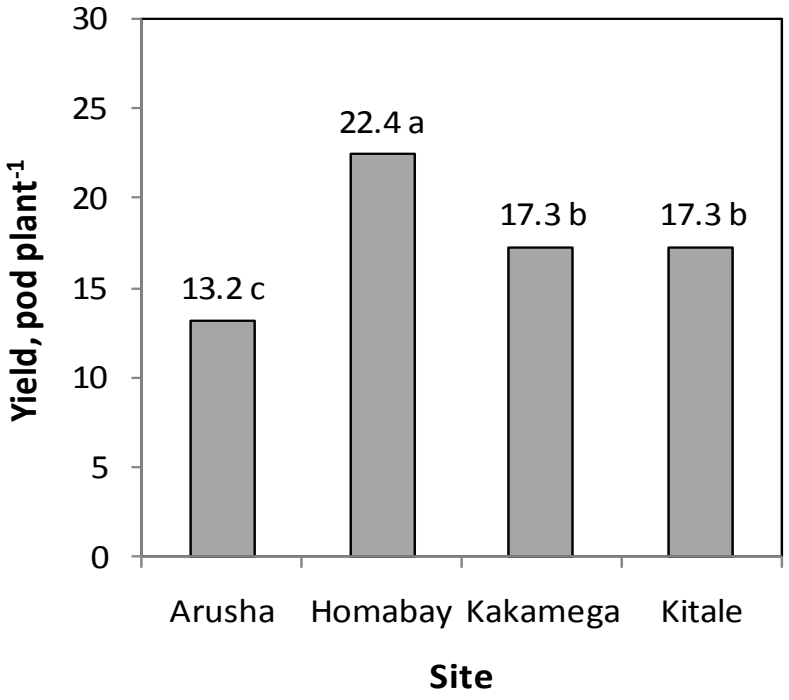

Figure 4.7. Mean pod yield of 36 snap bean genotypes at Arusha, Homabay, Kakamega and Kitale sites during 2010 field trials. Means not followed by same letter are significantly different according to Tukeys HSD**.**

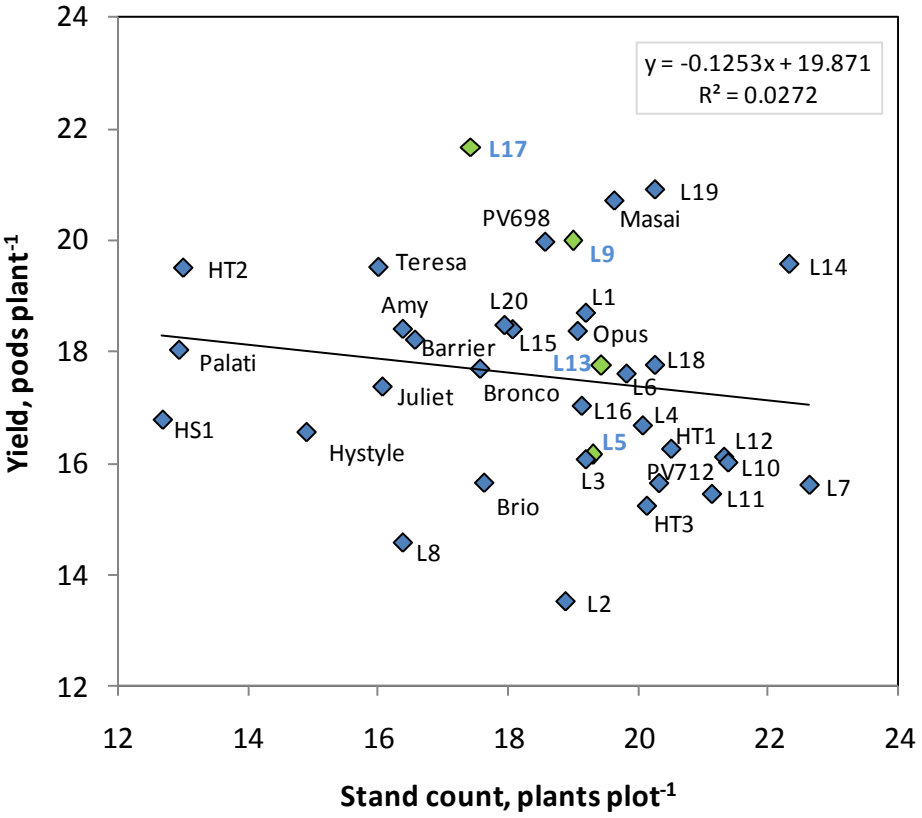

Figure 4.8. Effect of stand establishment on pod yield of 36 snap bean genotypes across Arusha, Homabay, Kakamega and Kitale field sites in East Africa during 2010 wet season.

Table 4.6. Mean pod yield of 36 snap bean genotypes at four field sites in East Africa.

Genotype	Line No.	Yield, pods plant^{-1}				
		Arusha	**Homabay**	**Kakamega**	**Kitale**	**All sites**
L1	1	15.0 a	26.8 ab	16.2 a	16.8 a	18.7 a-c
L2	2	10.5 a	12.7 b	15.3 a	15.7 b	13.5 c
L3	3	8.7 a	21.1 ab	18.6 a	15.9 b	16.1 a-c
L4	4	12.5 a	20.8 ab	16.8 a	16.7 ab	16.7 a-c
L5	5	9.3 a	20.7 ab	17.7 a	17.0 ab	16.2 a-c
L6	6	9.7 a	24.3 ab	19.5 a	17.0 ab	17.6 a-c
L7	7	9.9 a	20.0 ab	19.3 a	13.3 b	15.6 a-c
L8	8	7.0 a	18.9 ab	15.1 a	17.3 ab	14.6 bc
L9	9	14.2 a	28.7 a	15.6 a	21.4 ab	20.0 a-c
L10	10	11.0 a	20.1 ab	19.4 a	14.1 b	16.1 a-c
L11	11	9.4 a	21.0 ab	16.0 a	15.5 b	15.5 a-c
L12	12	11.8 a	19.6 ab	15.8 a	16.9 ab	16.0 a-c
L13	13	12.2 a	24.5 ab	17.7 a	16.6 ab	17.7 a-c
L14	14	19.9 a	21.1 ab	19.3 a	18.0 ab	19.6 a-c
L15	15	15.7 a	22.7 ab	15.6 a	19.6 ab	18.4 a-c
L16	16	12.2 a	23.9 ab	16.6 a	15.4 b	17.0 ac
L17	17	22.2 a	22.5 ab	20.2 a	21.8 ab	21.7 a
L18	18	14.2 a	21.1 ab	17.6 a	18.2 ab	17.8 a-c
L19	19	21.4 a	27.7 a	17.1 a	17.4 ab	20.9 ab
L20	20	15.9 a	25.1 ab	15.5 a	17.5 ab	18.5 a-c
HT3	21	8.4 a	20.0 ab	19.1 a	13.6 b	15.2 a-c
HS1	22	6.2 a	24.4 ab	17.7 a	18.9 ab	16.8 a-c
HT1	23	9.6 a	19.9 ab	17.5 a	18.1 ab	16.3 a-c
HT2	24	16.8 a	27.2 a	17.2 a	16.9 ab	19.5 a-c
OPUS	25	12.3 a	25.7 ab	21.2 a	14.3 b	18.4 a-c
Palati	26	15.0 a	24.5 ab	19.5 a	13.2 b	18.0 a-c
Hystyle	27	11.7 a	22.2 ab	19.5 a	12.8 b	16.6 a-c
Barrier	28	8.2 a	30.6 a	23.1 a	11.1 b	18.2 a-c
PV698	29	16.1 a	26.0 ab	14.3 a	23.5 ab	20.0 a-c
Amy	30	19.0 a	19.4 ab	13.5 a	21.8 ab	18.4 a-c
Juliet	31	13.9 a	23.6 ab	13.5 a	18.5 ab	17.4 a-c
Masai	32	20.0 a	19.0 ab	15.5 a	28.3 a	20.7 ab
Brio	33	8.6 a	21.5 ab	16.6 a	15.9 b	15.7 a-c
PV712	34	13.6 a	18.2 ab	15.2 a	15.7 b	15.7 a-c
Teresa	35	18.8 a	23.5 ab	14.7 a	21.1 ab	19.5 a-c
Bronco	36	14.1 a	19.6 ab	20.2 a	16.9 ab	17.7 a-c
Mean		**13.2**	**26.8**	**17.3**	**17.3**	**17.6**

*Within a site/column, means followed by the same letter are not significantly different according to Tukeys HSD.

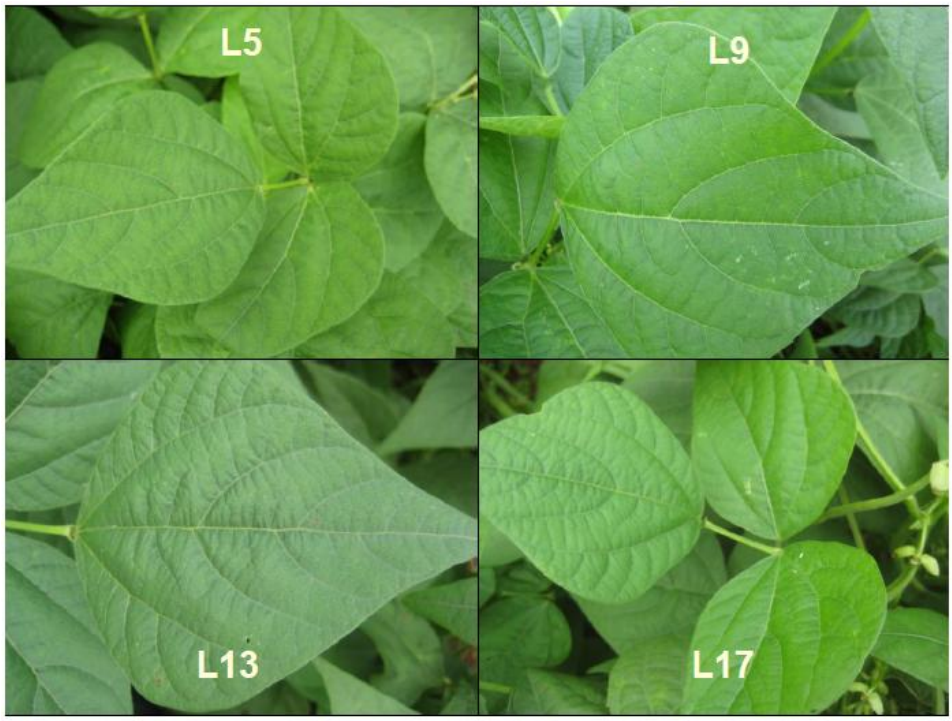

Figure 4.9. Rust resistant and heat tolerant snap bean breeding lines selected for eastern Africa.

Line 'L17' had consistently high pod load in Arusha, where it was the highest yielding, in Kakamega and Kitale where it was among the top three highest yielders and in Homabay where it had a mean yield equal to the overall site mean yield (Table 4.6 and Figure 4.10). The line also has high quality pods – straight, fleshy, small sieve sized pods. It has an upright bush plant growth habit with small to medium sized leaves that makes its canopy relatively open. Since 'L17' is a selection descending from 'HT1' and 'Teresa' (Table 4.2), it is also fixed or segregating for the *Ur-5* rust gene which as results from the present field studies (Chapter 4) have shown, is the next most important rust resistance gene (after *Ur-11*) for the East Africa region.

Further refinement of this line to ensure it is fixed for *Ur-5* and *Ur-11* would lead to a cultivar with a broad and durable rust resistance.

Table 4.7. Contrasts of pod yield of four field selected snap bean breeding lines and selected snap bean cultivars grown in the East Africa region.

Genotype*	Yield, pods plant^{-1}	Contrasts of yield of selected lines, (*P* value)			
		L5	L9	L13	L17
L5	16.2				
L9	20.0	0.024			
L13	17.7	0.346	0.186		
L17	21.7	0.001	0.320	0.020	
HT1	16.3	0.947	0.028	0.381	0.001
Amy	18.4	0.183	0.351	0.695	0.054
Juliet	17.4	0.469	0.123	0.827	0.011
Masai	20.7	0.007	0.670	0.081	0.569
PV712	15.7	0.295	0.010	0.216	<0.001
Teresa	19.5	0.047	0.781	0.295	0.294
Bronco	17.7	0.361	0.176	0.976	0.019

*Within a column contrast *P* values ≤0.05 (in shaded cells) indicate significant yield differences between corresponding breeding line selections and other genotypes used as controls.

Line 'L13' is a rust selection derived from a cross of 'HT1' and 'Masai' (Table 4.2). It has straight fleshy pods which are longer than those of 'Masai' and an upright growth habit. The line has good pod set and had yield equivalent to the mean site yields for Arusha, Kakamega and Kitale (Figure 4.10). Its pod yield at Homabay was higher than that of the grand mean for the site and also higher than the mean pod yields of both its parents – 'Masai' and 'HT1'. The performance of L13 yield wise at the hot Homabay site relative to its two parents shows that, in addition to being rust resistant (as opposed to its rust susceptible 'Masai' parent), this selection had a good

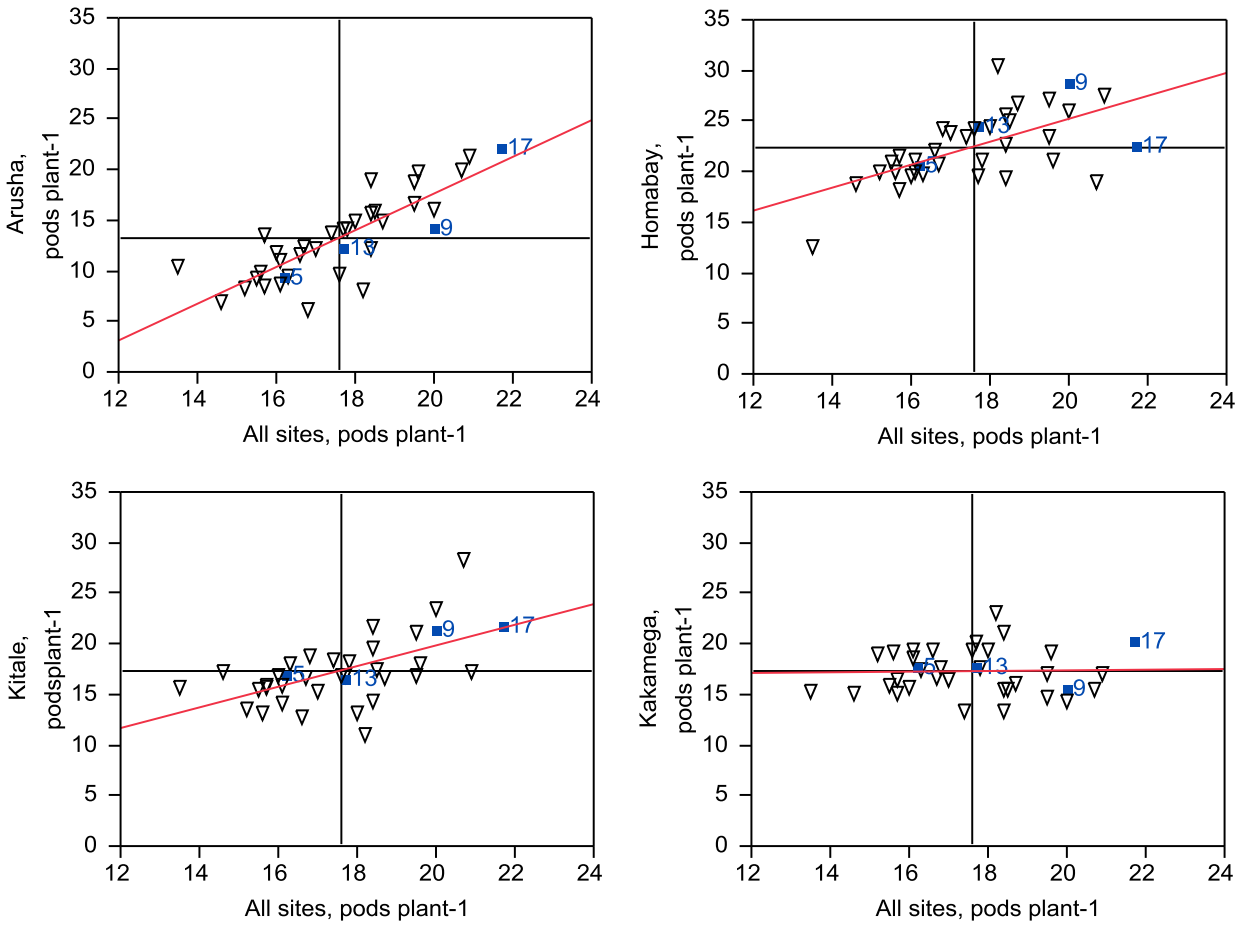

Figure 4.10. Correlations of mean yields of 36 snap bean genotypes in Arusha, Homabay, Kakamega and Kitale with yield across the four sites combined.

level of heat tolerance derived from its 'HT1' parent and higher pod set derived from 'Masai'. The high pod set trait in 'Masai' is verifiable at the cooler higher altitude sites such as Kitale (Table 4.6). Thus this line has better adaptation to and production potential in the warmer low altitude site at Homabay.

Line 'L9' is a rust resistant selection derived from crossing 'HT1' with 'PV712' (Table 2). It has an upright bushy plant type with straight, fleshy small sieve pods. Its mean pod yield across the four sites combined was significantly higher than means of each of its parents – 'HT1' and 'PV712'. It was the fourth best performing line across the four field sites for the trial (Table 4.6; Figure 4.8 and Figure 4.10). The line 'L9' was ranked best among the 12 rust resistant breeding lines at the Homabay site and the second best performing line among the 36 snap bean genotypes tested at the Homabay site. The high yield of 'L9' at Homabay which was the lowest altitude and hottest of the four sites shows that it has some good level of heat tolerance (derived from its 'HT1' parent) and that it was more adapted to this low altitude site than its parents and the other genotypes tested.

Line 'L5' is a rust resistant selection derived from 'HT1' and 'Bronco' (Table 4.2). It has an upright, bushy plant type with straight fleshy medium sieve pods. The mean pod yield for 'L5' across the four sites was lower than the overall mean for all the genotypes tested but was equal to the averages for the Kakamega and Kitale sites (Figure 4.10). Its yield across the four sites was not significantly different from that of its two parents – 'HT1' and 'Bronco'. This selection was made on the basis of its potential utility for advancement into a rust resistant, heat tolerant snap bean cultivar with medium sieve size pods targeted for consumption in the domestic markets in East Africa. Also being a larger pod type, 'L5' has the potential to be utilized as a dual purpose snap bean which can be consumed fresh or grown to maturity and consumed as a dry bean.

CHAPTER FIVE

COMBINING COMMON BEAN RUST RESISTANCE AND HEAT TOLERANCE IN SNAP BEANS FOR EASTERN AFRICA: CONCLUSIONS AND FUTURE WORK

This study focused on concurrent introgression of rust resistance and heat tolerance traits into snap beans. These two traits are of great importance in the tropics and subtropics where both common bean rust and high ambient temperatures limit snap bean production. Rust resistant snap bean breeding lines that were also heat tolerant were selected from populations segregating for the two traits. Resistance of the breeding lines to natural rust infestation and ability to yield well under high temperature field environments was tested and confirmed at contrasting field sites in East Africa and Puerto Rico. Snap bean cultivars (including those currently grown in East Africa) which were used as controls during the field trials, were either rust susceptible, heat sensitive or both, underscoring the importance of combining the two traits into genetic backgrounds of commercial cultivars.

Breeding lines developed here were utilized to make crosses with snap bean cultivars currently grown in East Africa to develop new cultivars that combine rust resistance, heat tolerance and important pod quality attributes including pod size. From this set of crosses, 20 candidate snap bean breeding lines were selected from greenhouse screens for heat tolerance and rust resistance (involving *Ur-4* and *Ur-11* gene combination). The 20 breeding lines were grown at contrasting field sites in East Africa from where they were selected for: resistance to local races of the rust, ability to yield well at both warm and cool sites, and desirable pod quality attributes: small sieve size, straight and fleshy pods and general adaptation to field site conditions. The top four breeding lines (‘L5’, ‘L9’, ‘L13’ and ‘L17’) that ranked high for the selection

attributes were picked and will be further field tested and refined before registration and subsequently released as cultivars targeted to East Africa and other regions. However to maximize the potential benefits from these improved snap beans in East Africa, future research building on the outcome of the current study should focus on specific areas some of which are suggested below:

1) During this study, selected snap bean breeding lines were field tested together with 12 commercial cultivars adapted or targeted to different regions including East Africa. When genotype reaction to natural rust infection at the different field sites was matched against their rust resistance gene status (that is the *Ur-* genes that they had), the *Ur-11* gene was established to be the most effective gene that conferred resistance against races of the rust found in the East African region. Genotypes that had *Ur-11* were free of rust symptoms unlike those that had no rust resistance genes or had only the *Ur-4* gene. The *Ur-5* was the next most effective against majority of the races of rust in the region since *Ur-5* containing genotypes were either rust free at some of the sites or had minimal rust infection other sites. The rust pathogen is highly variable and has potential to evolve new races which are able to overcome effective resistance genes such as the *Ur-11*.

   To minimize the possibility of new races developing and thus ensure a more durable and broad resistance against the rust, concurrent deployment of other effective resistance genes such as the *Ur-5* into *Ur-11* genotypes is needed. The present study has developed materials from which snap beans with rust resistance based on the combination of *Ur-4*, *Ur-5* into *Ur-11* genes. Selections having the three genes could be made from the breeding line ‘L17’ which is one the current promising selections targeted for further advancement. It was developed from a cross between ‘Teresa’ (which has *Ur*-5) and a heat tolerant breeding line with rust resistance based on *Ur-4* andUr-*11* gene combination. The addition of the *Ur-5*

gene to *Ur-4* and *Ur-11* will help in protecting cultivars from rust races overcoming one of the *Ur-4* or *Ur-11* genes, increasing the durability and effectiveness of the gene combination.

2) Snap bean genotypes that had *Ur-4* and *Ur-11* genes were identified by inoculating plants with rust races 108 and 67, respectively. This approach is laborious and time consuming and requires specialized greenhouse and incubation facilities for accurate screening of genotypes. Use of molecular markers linked to the rust resistance genes of interest has potential to increase the efficiency by which rust resistance genes are identified and deployed into bean genotypes. Molecular markers have been developed for various *Ur* genes including *Ur-4, Ur-5* and *Ur-11*. The *Ur-5* gene can be detected effectively as a co-dominant SCAR marker reproducible across snap bean cultivars tested. However, the currently published markers for the *Ur-11* gene lack reproducibility across different common bean genetic backgrounds. Broad utility of the *Ur-11* markers is essential to facilitate transfer of the rust gene into bean genotypes from Andean and Middle American gene pools. Future work would need to focus on validating the utility of published *Ur-11* markers across the gene pools of the common bean and to additionally develop a more robust marker that distinguishes the presence of *Ur-11* in bean genotypes drawn from different gene pools.
3) The current study focused on snap bean improvement for increased productivity in the East African region; however, it is important to incorporate additional traits into the target genotypes to introduce resistance to Bean Common Mosaic Virus (BCMV) and Bean Common mosaic Necrosis Virus (BCMNV). BCMV has a worldwide distribution because of its high rates (average 35%) of transmission via seeds produced by plants systematically infected prior to bloom (Schwartz et al., 2005). BCMNV is also seed borne in common bean and is commonly found in

eastern Africa where it has been associated with wild legume reservoirs. Aphids are the most important means of secondary spread of the viruses during growing seasons. Combined transmission through infected seed and aphids cause significant yield and quality losses in snap beans.

Introgression of *I* and *bc-3* genes confers resistance against all known strains of the BCMV and BCMNV. The dominant *I* gene inhibits all non-necrotic strains of the virus. However, the *I* gene can be activated by necrosis-inducing strains of BCMV and BCMNV, which can lead to complete failure of cultivars that have only the *I* gene, unless protected by other genes such as *bc-3,* hence the need to combine these genes. In the context of the current study it would be imperative to ascertain the *I* and *bc-3* gene status of the selected snap bean breeding lines that are targeted for further advancement and subsequent release as cultivars. This is necessary because the rust resistance source parents from which the current line selections have been derived had *I* and *bc-3* genes. Thus from the subsequent crosses and gene segregations, there is the likelihood that some of the selected lines may contain only the *I* gene which may adversely affect their performance once deployed to East African field environments in the presence of necrosis-inducing strains of BCMV and BCMNV. The current snap bean line selections for advancement should be further genetically improved by introgression of the *I* and *bc-3* gene combination which can be achieved with available molecular markers, or through inoculations with the NL-3 strain of BCMNV.

4) Anthracnose (caused by the fungus *Colletotrichum lindemuthianum*) is one of the most important diseases of beans in the world and yield losses can be up to 100% when contaminated seed are planted and prolonged conditions favorable to disease development occur during the crop cycle. In snap beans, anthracnose reduces pod

quality further reducing the yield biomass which can be marketed. It is an important disease of dry beans and snap beans in East Africa and most cultivars are susceptible. This disease is largely transmitted by seed, or through continuous planting in the same soil, and is a problem associated more with dry beans, as the seed production is undertaken in East African regions with contaminated soils. During the current study, anthracnose was observed at a majority of the field sites in which the field trials were conducted. Future work with the selected snap bean breeding lines could focus on improving them for resistance to anthracnose.

5) The present work focused on genetic improvement of snap beans for the tropics for tolerance to heat stress. Production environments where heat stress is prevalent are also often characterized by soil moisture deficits (drought stress) that adversely affect plant growth and yield. The drought stress which may be intermittent or terminal is especially of significance to rain-fed production environments where majority of farmlands in sub-Saharan Africa and many other tropical areas are situated. Snap beans grown in such regions are both heat stressed and water stressed resulting in reduced yield and quality.

   Breeding for drought tolerance in common bean for the warm tropics has focused on developing improved sources without regard to other varietal traits and market classes. There is presently scarce documentation on snap bean improvement for tolerance to drought stress and especially those targeted to East African production environments. Future work on the rust resistant heat tolerant snap beans could focus on genetic improvement for tolerance to drought stress to alleviate the risk that this poses to production under rain-fed systems in East Africa.

6) A major challenge to crop production in East Africa and sub-Saharan Africa as a whole is negative balances in soil nutrient stocks in majority of farms in the

region. Coupled with high intensity in crop production practices, the low fertility of majority of soils in the region has resulted in high nutrient input-dependent production systems. This significantly increases crop production costs in addition to reducing environmental quality. Sustainable crop production practices in such regions need to incorporate growing of nutrient efficient cultivars – those that have low internal and external nutrient requirements. Genetic diversity with respect nutrient requirements has been documented in the common bean and dry bean cultivars with ability to grow in soils low in nutrients such as P have been developed. There is presently no documentation of snap beans with ability to yield well under low fertility soils. Future work with the new snap bean lines would also need to focus on genetic improvement for higher nutrient use efficiency.

7) While most snap bean production in the East African region has been largely targeted to export markets in Europe, demand in the domestic markets has been rapidly increasing. The demand for snap beans in the domestic markets has so far been served by produce with similar quality attributes such as small-pod types that are targeted to the export markets. To nurture a robust and sustainable domestic market for snap beans, there is need to diversify the utility and quality of snap beans grown in the region in addition to improving for disease resistance and tolerance to abiotic stresses. The diversification on quality attributes should focus on aspects such as developing cultivars that have large sieve size pods to cater for consumers with preference for large sieve pods. In case not all the pods are harvested fresh, snap bean cultivars with large sieve sized pods also have potential to be utilized as large seeded dry beans since most dry beans grown and consumed in the East Africa region are the large seeded types. The snap bean line L5 which is one of lines selected from materials developed during the current study has

medium sieve sized pods and will need to be advanced and eventually released as a type that may have this dual use.

There is also need to focus on improving the nutritional quality of the snap beans in areas such as higher micronutrient content. Most of the work on increasing micronutrient content of the common bean has so far focused on dry beans and not snap beans. Genetic improvement of snap beans for higher micronutrient content has potential to make a significant contribution to human nutrition and health.

8) The current study focused on rust resistance in snap beans. However, bean rust affects many cultivars in the dry bean market class. Many dry bean cultivars currently grown in the East African region are rust susceptible. For example during the field trials carried out in East Africa as part of the current study, four of the five popular dry bean cultivars that were grown as guard rows manifested severe rust symptoms underscoring their susceptibility. The success of the *Ur-4* and *Ur-11* rust resistance gene combination that has been demonstrated in the current snap bean lines therefore needs to be transferred into popular dry bean cultivars grown in the East African region so as to minimize yield losses currently attributed to the bean rust.

   Effort has also been put into development of dry bean cultivars that are higher in nutritional quality attributes such as high content of micronutrients including iron and zinc. These high quality dry beans have been released in various regions including sub-Saharan Africa to contribute to the fight against malnutrition. However, the success of these new dry bean cultivars will only be realized if they have rust resistance among the genetic attributes that will enhance their performance in the rust prone production environments in the region. The *Ur-4* and *Ur-11* rust resistance gene combination that was demonstrated as effective in

the current study needs to be transferred to these novel high nutritional quality dry bean cultivars.

The research presented in this thesis has demonstrated the effectiveness of targeted gene combinations in controlling bean rust in East Africa that is applicable to all tropical and sub-tropical regions of the world. Combining these genes in a heat tolerant background will enable the expanded production of snap beans throughout these regions, enabling more efficient and environmentally friendly production of the crop in more marginal lower altitude regions. Combining these traits with others discussed will create new market opportunities for small-holder growers in these regions, enabling expanded production of a higher-value horticultural crop.

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