



**Sapienza Università di Roma**

**XXX ciclo Dottorato di Ricerca in Biologia Ambientale ed Evoluzionistica  
(Curriculum Botanica)**

**Farmer crop variety mixtures to cope with disease epidemics in the common bean cropping  
system of the Ecuadorian highlands**



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**Dissertazione: Rome, 12 February of 2018**

## **Dedication**

To those who have been close to me during my education since long ago.

To the memory of my father Trinidad, my aunts Naty, María, Rebeca, my uncles Olmedo and Domingo, of whom I have very good memories, especially from my father and Naty.

An especial dedication to my mother, my cousin Jorge, aunt Mercedes, my wife Maria Luisa and specially to my daughters Maria Jose, Maria Teresa and Maria Paula, who were source of inspiration.

## **Acknowledgement**

To Massimo Reverbery and Fabio Attore from the Department of Environmental Biology and Evolution of Sapienza University for the guidance and friendship during the research. An especial acknowledgement to Devra Jarvis from Bioversity International for the opportune advice and encourage. To Paola De Santis, a key friend along this period with opportune help. To Lenin Ron, who was patient with data analysis.

To farmers from Cotacachi and Saraguro from whom I learned realities that are not available in the academy, but also pragmatism.

To Laura, Mayra's, Lorena, Italo, Andres, Cristian for the company and help during field work.

An especial acknowledgment to the National Institute of Agricultural Research (INIAP) for funding the Doctorate program.

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## **CHAPTER 1.**

### **1.1 INTRODUCTION**

#### **1.1.1 Common bean diversity and geographical areas**

Ecuador is part of the Andean center of diversity of wild and domesticated beans (Debouck et al., 1993; Freyre et al., 1996; Jarvis et al., 2016), and the cultivated common bean (*Phaseolus vulgaris* L.) grown in Ecuador belongs to the Nueva Granada and Peru races of the Andean gene pool (Singh et al., 1991). Phenotypically diverse common bean populations ranging from determinate (growth habit I and II), to indeterminate (growth habit III and IV) are grown in Ecuador; the former in the valleys and western foothills, and the later in the temperate highlands (Peralta et al., 2013). The determinate types called bush and the indeterminate types called climbing are grown in a wide range of agro-ecological conditions, which have generated a considerably diverse genetic base (Bonilla, 2010; Jacome, 2017; Torres, 2012).

In northern valleys, and in the central and northwest foothills, monoculture of bush improved varieties is an important agricultural activity of farmers (Peralta et al., 2013; Subia et al., 2007). Cultivation of bush type varieties is dynamic in the northern valleys and primarily depends on the Colombian market demanding the *Rojo Moteado* type, and at low Colombian demand, the local market favors varieties Panamito, Blanco de Leche and Calima Negro (Garver et al., 2008; Subia et al., 2007). In southern valleys on the other hand, common bean varieties are primarily traditional; however, improved varieties have been also released in this region (Jiménez et al., 1996).

The climbing types (volumbles in Spanish) are cultivated intercropped with maize in the temperate highland (2,000-3,000 masl) (Peralta et al., 2013). In this cropping system, climbing types have long been cultivated in a variety mixture approach, until *Canario* a local yellow large-seeded population, replaced variety mixtures in many common bean areas. However, in Cotacachi and Saraguro, northern and southern Ecuador respectively, variety mixtures are still an important cropping strategy (Chapter 2). A variety mixture is a phenotypically and genotypically diverse population that are planted, harvested, consumed and eventually commercialized together.

#### **1.1.2 Importance of common bean in Ecuador**

Bush and climbing are complementarily important common bean types in Ecuador. Both are produced for subsistence and commercial uses, providing the main plant protein of direct human consumption to the Ecuadorian population, and income to mostly small-scale farmers that grow common bean. Although in northern valleys, an important part of the bush common bean production is devoted for the Colombian and local demand, self-consumption, is also

important (Garver et al., 2008). On the other hand, bush types in southern Ecuador are grown for self-consumption and only for the local market.

The climbing common bean types grown intercropped with maize in a subsistence cropping system is key for food security (Photo 1). Production is primarily devoted to self-consumption, although part of the production is commercialized in the local market, which demands large seeds. *Canario* is the main climbing type cultivated along the highlands, and presently varieties Bombolin and a Cargamanto type are also grown for commercial purposes with wire fence supports mainly in central western Ecuador.



Photo 1. The climbing common bean type associated with maize.

Common bean is the main source of plant protein for direct human consumption in Ecuador (Peralta et al., 1997). Bush local varieties, and the climbing types Canario, Bombolin and Cargamanto provide plant protein to farmers along the highlands, and since these varieties are also commercialized in the local market (Peralta et al., 2013), they also provide plant protein at country wide. However, variety mixtures of bush and climbing types grown mostly for self-consumption primarily in Cotacachi and Saraguro are especially important in providing plant protein to native communities, who have less access to animal protein.

### 1.1.3 Common bean variety mixture intensification

Climbing type beans have likely been grown in mixtures (Photo 2) along the highlands probably since the domestication, and it is likely one of the oldest cropping systems in the Andes. However, at present variety mixtures are displaced to subsistence farming mainly in Cotacachi and Saraguro, inhabited by the local native communities Imbayas and Saraguros, respectively (Chapter 2). Variety mixture cultivation has been replaced in the rest of the maize/common bean areas by the local commercial type *Canario*.



Photo 2. Saraguro and Cotacachi variety mixtures. Upper center the *Canario* type

In Cotacachi, two types of variety mixtures, *Chacra* and *Allpa*, are intercropped with maize. The former is a late type belonging to growth habit IV, and the latter is an earlier type belonging to growth habits I, II, and III. The *Chacra* type is planted together with maize in the same planting site and climbs on the maize plant, whereas *Allpa* is planted between maize/*Chacra* plants (Photo 3), and only plants with growth habit III partially climb on the maize (Chapter 2). In Saraguro, *Chacra*, more often called *Mixturiado*, is the common bean type cultivated. It is also planted together and climbs on the maize plant as in Cotacachi. *Popayán* or *Toda Vida* (*Phaseolus coccineus*) is often part of the *Chacra* variety mixtures in some sites of Saraguro, complementing *Chacra* production as *Allpa* does in Cotacachi.

Two intensified approaches are clearly differentiated in Cotachi and Saraguro cropping system:

- 1) Species intensification: maize and beans
- 2) Bean type intensification: *Chacra* and *Allpa* (Cotacachi) and *Chacra* and *Popayán* (Saraguro)

This two-way intensification approach is primarily oriented to aim food security, ensuring availability of carbohydrates (maize) and protein (beans). In Cotacachi, the common bean intensification approach in addition ensures food provision along the year, the early maturing *Allpa* providing food early in the season and the late maturing *Chacra* providing food later in the season. In Saraguro, *Popayán* is considered more productive and more resistance to diseases than common bean (Chapter 2).



Photo 3. *Chacra* and *Allpa* intensification. *Chacra* climbs on maize plants and *Allpa* is planted in between.

Furthermore, intensification of *Chacra* and *Allpa* in Cotacachi, and *Chacra* and *Popayán* in Saraguro is characterized by cultivation of variety mixtures. Intraspecific diversity of these variety mixtures were found functional to buffer disease epidemics (Chapter 3), creating a high “mixture effect” (Espinoza and Ochoa, 2012), which is in agreement with benefits of variety mixtures (Di Falco and Chavas, 2007; Finckh, 2003; Jarvis et al., 2007; Thinlay et al., 2000; Thurston et al., 1999; Trutmann et al., 1996; Wolfe, 1985). Genetic vulnerability due to the risk of new biotypes of pest or pathogens entering within farmers’ fields (Brown, 2008), appears less likely to occur in Cotacachi and Saraguro due to diversity of resistance present in variety mixtures, which makes the common bean cropping system less vulnerable (Chapter 3).

Intensification is the strategy presently proposed to improve agricultural productivity to satisfy increasing food demand (Struik et al., 2014). Sustainable intensification, meaning “a process or system where agricultural yields are increased without adverse environmental impact and without the conversion of additional non-agricultural land” (Pretty and Bharucha, 2014) is still in debate. Commercial intensification are suggested to concentrate in three main modern actions: improving yield to approach yield potential, improving soil quality, and exploiting precision agriculture (Cassman, 1999). Intensification in traditional agriculture is primarily associated with crop management efficiency by mainly improving soil fertility, low tillage, crop rotations, and intercropping; strategies that are proposed mainly derived from experimentation (Devkota et al., 2016; Mungai et al., 2016; Nyagumbo et al., 2016; Pradhan et al., 2016; Wani et al., 2016).

Intensification in commercial agriculture appears achievable with modern technologies in construction, especially modern breeding and the efficient use of modern technology. Intensification in traditional agriculture appears a challenge and difficult to attain, and the strategies proposed appears not to satisfy globally marginalizing food inadequate households (Ritzema et al., 2017). Realistic intensification approaches for traditional agriculture appears to be still in construction, and therefore, it is time to learn from existence experiences. The common bean intensification approach implemented by Cotacachi farmers adapted to local agro-socio-economic conditions fits properly with sustainable intensification expectations. This low input approach has kept providing food for long time even under adverse climatic conditions in a rainfed agriculture. Traits and principles of this traditional intensification appears useful in planning intensification initiatives in other cropping systems (Chapter 4).

#### **1.1.4 Biotic constraints**

Main constraints of common bean in Ecuador are pest and diseases (Lepíz et al., 1995; Peralta et al., 2007). Common diseases of bush and climbing types are rust (*Uromyces appendiculatus*), anthracnose (*Colletotrichum lindemuthianum*), ascochyta leaf spot (AsLS) (*Ascochyta phaseolorum*), and powdery mildew (*Erysiphe polygoni*); whereas, angular leaf spot (ALS) (*Pseudocercospora griseola*) and spider mites (*Tetranychus urticae*) are reported only for bush beans in the valleys (Peralta et al., 2007). However, in this study, ALS and spider mites were recorded also for climbing types in the highlands (Chapter 4), likely because climatic conditions are becoming conducive for ALS and spider mites in the highlands. White fly (*Thiauleurodes vaporariorum*) is a very important constrain of bush types in the valleys and western foothills (Subia et al., 2007), and this insect has not yet reached the highlands.

### 1.1.5. Common bean rust

Rust is a very important constraint of common bean cultivation worldwide (Mmbaga et al., 1996b; Pastor-Corrales, 2002), and in Ecuador, it is the most important biotic constraint of bush and climbing common bean (INIAP, 2003; Lepíz et al., 1995; Ochoa et al., 1998; Peralta et al., 2007). Rust in bush types is controlled in Ecuador with up to four fungicide sprays per cycle (Subia et al., 2007), and on climbing types farmers mostly rely on interspecific diversity (intercrops) and on intraspecific diversity (variety mixtures) (Chapter 2).

*U. appendiculatus* is an obligate parasite that establish a biotrophic relationship with the bean plant (Mendgen and Hahn, 2002). The pathogen can infect cultivated and wild *P. vulgaris*, *P. coccineus*, *P. lunatus* and *P. augusti*; however, cultivated and wild types of *P. coccineus* and *P. lunatus* are more resistant than wild or cultivated *P. vulgaris* (Acevedo et al., 2013, 2005).

*U. appendiculatus* is a macrocyclic and autoecious pathogen, completing sexual and asexual stages in the bean plant (McMillan et al., 2003). However, the sexual stage in tropic and subtropics regions is less frequent than in temperate regions, which has contributed to the evolution of asexual populations (Taylor et al., 1999).

*U. appendiculatus* is a very diverse and virulent pathogen (Acevedo et al., 2013, 2005; Araya et al., 2004; Jochua et al., 2008; Mmbaga et al., 1996a; Ochoa et al., 2007), and even considered to carry excess virulences (Alexander et al., 1985). Pathogen diversity has been shown to be higher in the tropics than in temperate regions (Mmbaga et al., 1996a), and since the sexual stage is low frequent in the tropics, the high pathogen diversity appears to be associated primarily with mutation and the high selection pressure of resistance present in wild and cultivated *P. coccineus* and *P. lunatus* (Acevedo et al., 2013). In Ecuador, in addition of resistance of wild and cultivated *P. coccineus* and *P. lunatus*, resistance introduced through modern breeding in bush varieties appears also a selection pressure for pathogen evolution in the valleys, where modern bush resistant varieties are cultivated (Chapter 3).

In this study, common bean rust has been selected to evaluate the contribution of common bean variety mixtures in reducing disease epidemics. In a first step, rust epidemics of variety mixtures were evaluated in farmers' fields and the epidemics correlated with common bean mixture diversity. In a second step, resistance operating in genotypes components of variety mixtures were studied at seedling stage (greenhouse) and adult plant stage (field). In a third step, epidemics of rust were studied in the *Chacra/Allpa* mixture intensification approach in Cotacachi.

## 1.2 SCOPE OF THE STUDY

The maize/common bean cropping system has been for a long time an important component of highland agroecosystems, key for food security in Ecuador. In the highlands, farmers have adjusted to the cropping system a series of practices that have allowed common bean production to be sustainable. An important aspect of this cropping system is the high inter and intraspecific diversity. Although *P. vulgaris* (climbing) is the main bean intercropped with maize, *P. coccineus* is also part of the intercrop especially in Saraguro. Both, *P. vulgaris* and *P. coccineus* are in addition cultivated as variety mixtures of diverse genotypes.

The common bean crop management approach described is being practiced in the subsistence maize/common bean cropping system in Cotacachi and Saraguro. The aim of this cropping system is to ensure food security through reducing crop vulnerability to biotic and abiotic constraints and ensure food availability along the cropping season by growing diverse maturing types.

Despite the importance of the common bean cropping system in the highlands of Ecuador, little is known about traits that contribute to common bean sustainability. This study by linking participatory diagnosis with scientific analysis of the common bean variety mixture intensification aims to understand vulnerability, sustainability and resilience of the common bean cropping systems in the highlands of Ecuador.

In this study, farmer's knowledge of his agroecosystem was approached through participatory diagnosis, and the hypothesis stated was that the cropping system management resulted from the experience obtained by farmers throughout a close contact with field activities.

In a second step we approached the study of resistance that operates in variety mixtures. Hypothesis of this study was that the high phenotypic and genotypic diversity found in variety mixtures are also associated with high diversity of resistance. We studied richness of resistance at seedling stage, which is associated with major genes, and then evaluated the efficiency of this resistance in the field. Field studies also allowed establish contribution of partial resistance as a complement of major gene resistance in reducing disease epidemics in the field.

In a third step we assessed reaction to diseases and yield performance of the *Chacra/Allpa* intensified variety mixture approach in Cotacachi. Hypothesis of this study was that intensification does not increase disease epidemics due to an efficient resistance expression in the field, and the global yield (*Chacra + Allpa*) is improved, and therefore, the *Chacra/Allpa* intensification in addition of contributing to food security is a beneficial practice, creating a less vulnerable and resilient cropping system.



Results of this study guide a better visualization of the importance of common bean variety mixtures. It will allow to design strategies of diversity conservation, enhance productivity and eventually use the principles derived from this study in similar traditional cropping systems, and even in modern agro-ecosystems.

### **1.3 OBJECTIVES**

1. Study farmers' knowledge about diversity, pest and diseases, crop management, and the status and reaction to rust of common bean variety mixtures in the highlands of Ecuador.
2. Utilizing seedling and adult plant studies characterize the resistance to rust operating in Cotacachi and Saraguro variety mixtures.
3. Study the reaction to pest and diseases and yield performance of the *Chacra/Allpa* intensification approach in the highlands and valleys of Ecuador.

## CHAPTER 2

### Common bean variety mixture diversity: a farmers and scientific view of its contribution to the highlands agroecosystem sustainability in Ecuador.

#### Abstract

Common bean has been for long time the most cultivated legume for direct consumption in Ecuador. It is still cultivated mostly in traditional agroecosystems, in which farmers have implemented a series of management strategies to maintain crop sustainability. By linking participatory diagnosis, crop diversity assessment, and disease evaluation in the field, we established rationality of farmers' practices for the climbing common bean cropping system in the highlands of Ecuador. Climbing common bean is primarily grown intercropped with maize in a variety mixture strategy involving high genetic diversity. Two different types of variety mixtures are intercropped with maize in Cotacachi (north Ecuador): *Chacra*, a late maturing type of growth habit IV and *Allpa* an early maturing type of growth habit I, II, and III. In Saraguro (south Ecuador), only *Chacra* variety mixtures are cultivated; however, Popayán (*Phaseolus coccineus*) is often part of *Chacra* variety mixtures. Common bean cultivation in the intensified approach in Cotacachi and Saraguro aims food security by a better food provision of *Chacra* and *Allpa* types along the year, and by buffering biotic and abiotic constraints, which is achieved by planting genetically diverse variety mixtures. A negative association was observed between variety mixtures richness and evenness with rust epidemics. Farmers were knowledgeable about mixture diversity, mixture benefits, and pest and disease epidemics. They were able to name in an analytical way most components of variety mixtures, they perceived inter-specific diversity (intercrop) and intra-specific diversity (variety mixtures) reducing pest and diseases epidemics. Farmers also perceived evident differences among diseases, disease transmission, and disease resistance and its durability. However, as expected and reported frequently in literature, farmers associated disease origin with disease conducive conditions such as rain and clouds. Resistance and its durability are for farmers closely associated traits; resistant for farmers is absence of disease, which was easily detected in *Phaseolus coccineus*, which is a case of nonhost resistance or marginal host.

#### 2.1 Introduction

Common bean (*Phaseolus vulgaris* L) has long been an important crop along the highlands of Ecuador (Peralta et al., 1997). The cultivated common bean of Ecuador belongs to Nueva Granada and Perú races of the Andean gene pool (Singh et al., 1991). Presently, determinate types (growth habits I and II) and indeterminate types (growth habit III and IV) are grown in

Ecuador (Peralta et al., 2013). Molecular genetic studies of determinate and indeterminate types show a diverse genetic structure of common bean in Ecuador (Bonilla, 2010; Torres, 2012; Jacome, 2017).

Bush (determinate) types are primarily cultivated in the low warm valleys and eastern foothills on around 24,374 ha (Peralta et al., 2013). In the northern valleys, bush type cultivation is dynamic and depends on the Colombian market, which favors the *Rojo Moteado* varieties (Subia et al., 2007). At low Colombian demand varieties Panamito, Blanco de Leche and Calima Negro are grown for the local market (Garver et al., 2008). In southern Ecuador, on the other hand, modern and traditional varieties are cultivated only for the local market (Jiménez et al., 1996). In northern and southern valleys, and in the eastern foothills, production is also devoted for self-composition (Garver et al., 2008; Lepíz et al., 1995).

Climbing (indeterminate) types are grown mostly intercropped with maize (*Zea mays* L) in temperate regions (around 15 °C ) on 97,217 ha (Peralta et al., 2013). These common bean types have been adapted to grow in colder agro-ecological areas (15 °C) below the common bean temperature areas (17.5 - 25 °C) (Gepts, 1998). Climbing types are primarily cultivated in traditional agro-ecosystems for self-consumption and for the local market. These types of common bean are key sources of plant protein for farmers of the highland communities who have low access to animal protein, as well as main source of plant protein at country level (Peralta et al., 2013, 1997). In spite of the improved varieties released to grow intercropped with maize (INIAP, 2004, 1999, 1994, 1993), the cultivated climbing common bean varieties are still traditional.

To maintain sustainability of climbing common bean cultivation in their agroecosystems, farmers have developed a series of local management strategies: 1) the crop has been intercropped with maize in an ancient cropping system; 2) phenologically diverse common bean types have been adapted to this cropping system; and 3) cultivation of variety mixtures is the key practice in this cropping system.

Cultivation of diverse intraspecific diversity displayed in a **variety mixture** approach appears the key crop management aspect that maintains common bean sustainability in the highland of Ecuador. Although this diversity approach appears sustainable, cultivation as sole variety of the commercial *Canario* has displaced variety mixtures to the native communities of Imbayas and Saraguros in Cotacachi and Saraguro, respectively. *Canario* is a local yellow large-seeded type derived from variety mixtures. Other large-seeded varieties as Bombolin and Cargamanto are also becoming popular commercial climbing varieties.

The main purpose of genetic mixtures (crop variety mixtures) cultivation is to slow down pest and pathogen spread (Wolfe, 1985). Several recent studies have shown that a diverse genetic basis of resistance is beneficial for the farmer because it allows a more stable management of pest and disease pressure than a monoculture (Di Falco and Chavas, 2007; Finckh, 2003; Jarvis et al., 2007; Thinlay et al., 2000; Thurston et al., 1999; Trutmann et al., 1993).

Cultivation of common bean variety mixtures in the highlands of Ecuador is likely oriented among other reasons to buffer epidemics of pest and diseases, which are the main constraints of common bean in Ecuador (Lepíz et al., 1995; Peralta et al., 2007). Rust (*Uromyces appendiculatus*), anthracnose (*Colletotrichum lindemuthianum*), and ascochyta leaf spot (AsLS)(*Ascochyta phaseolorum*) are the main diseases identified in bush and climbing common bean types, whereas angular leaf spot (ALS) (*Pseudocercospora griseola*) and spider mites (*Tetranychus urticae*) have been reported only in bush types in the valleys (Lepíz et al., 1995; Peralta et al., 2007).

Most pest and disease management strategies concentrate on reducing the current or coming season's crop loss. Few crop management programmes are oriented to providing options that could reduce the risk to future crop loss, i.e., reducing genetic vulnerability within the farmers' field. Vulnerability is intended here as the probability of crop loss due to a new biotype of pest or pathogen entering into the farmer's production (Brown, 2008), a phenomenon more likely to occur in an area consisting of one or few varieties that share a very similar resistance structure.

Despite the importance of the crop management strategies developed by farmers to maintain common bean productivity as part of maize/common bean cropping system in the highlands of Ecuador, little is known about features that sustain crop productivity. In this study, taking in consideration that farmers have been in contact and depended for food security on the common bean variety mixtures, we hypothesized that farmers have a deep understanding of the cropping system they maintain and depend on, which has allowed the conservation and a sustainable use of the common bean diversity in the highland of Ecuador.

**Main Objectives of this study were:**

1. To study the farmers' knowledge of variety mixtures in regards to local identity, crop management and reactions to pest and diseases, together with knowledge and management of pest and diseases.
2. Analyze common bean diversity at household and community of Cotacachi and Saraguro variety mixtures using richness, evenness and divergence.

3. To establish functionality of variety mixtures at household in regards to rust epidemics by associating rust severity with richness and evenness.

## **2.2 Materials and methodology**

### **2.2.1 Site description**

The study was undertaken in Cotacachi province of Imbabura and Saraguro province of Loja, northern and southern Ecuador, respectively. Cotacachi town is located at 0° 11' 37" N, 78° 13' 43" W and at 2,418 masl, and Saraguro town is located at 3° 37' 22" S, 79° 14' 18" W and 2,525 masl. Both regions share similar climatic and agro ecological conditions. Cotacachi has an average temperature of 15 °C with an annual average rainfall of 906 mm, and Saraguro has an average temperature of 14.1 °C with an annual average rainfall of 577 mm. In both regions, the maize/common bean cropping systems is the most important agricultural activity of two important ethnic groups, Imbayas and Saraguros in Cotacachi and Saraguro, respectively. In Cotacachi, Imbaya farmers are bilingual speaking the local language Quechua and Spanish; whereas in Saraguro, Saraguros speak primarily Spanish. In both regions, maize and common bean are mainly devoted to self-consumption, although part of the production is commercialized in the local market.

### **2.2.2 Materials and experimental design**

This study had four linked components: (1) participatory diagnosis through standardized focus group discussions; 2) household surveys to collect information from farmers on crop varietal diversity and disease management practices; (3) key farmers meetings to complement varietal diversity information; and 4) field assessment of rust at household.

### **2.2.3 Participatory diagnosis**

Participatory Diagnosis (PD) meetings using the methodology proposed by Barahona and Levy (2003) were carried out in each of the two regions encompassing Cumbas Conde and Morochos in Cotacachi; and Tenta, Cañicapa, Gañil and Selva Alegre in Saraguro. PD was conducted in 2008 following a globally agreed set of guidelines described in Jarvis and Campilan (2006).

First PD step was Focal Group Discussion (FGD) meetings conducted per region. Five FGDs comprising a separate group category (young women, old women, young men, old men, and leaders) were organized in Cotacachi and Saraguro. Young farmers were considered those under 25 years of age, old farmers those over the age of 25. Each FGD had a minimum of 10 and a maximum of 12 people participating. A standardized set of questions grouped under seven themes (Jarvis and Campilan, 2006) was used to ensure that all groups were asked the same set

of questions. In Cotacachi, a bilingual rapporteur speaking Quechua was included to have better communication, because some of the participants did not communicate well in Spanish.

Samples of common bean varieties (plants and seeds) and samples of diseases brought by farmers to FGDs were used as a basis for discussion among farmers and researchers. to understand farmers' knowledge of varietal diversity, the value – be it agronomic, adaptive, or quality or use traits for the different varieties, pest and disease symptoms, host–pest/pathogen differences in plant health, and host–pest/pathogen interactions. Plant materials were assigned by the farmers into groups of plants, which were determined to be the same variety. Importance was given to ensuring consistency of variety names and descriptions of varieties given by farmers (Sadiki et al., 2007).

In FGD meetings one individual farmer volunteer per variety led the documentation of describing the specific variety, with inputs from the other farmers. Documentation included recording the name or names given by the group to the variety, whether the variety was traditional or modern, and the morphological, agronomic, adaptive, and quality traits used by the group to describe the variety. Varieties that have been lost and seed flow were also analyzed during FGDs. This information was then organized by the researchers in a table of traits versus varieties. For variety mixtures, farmers were asked to sort out the phenotypes that compose the mixture and provided a name for each of them. The final step was to check this table with the farmers to ensure there was agreement across the groups.

To determine farmers' knowledge and perceptions of pests and diseases and host–pest/pathogen interactions, descriptions of the plant symptoms for the diseases and pests observed were gathered, including a list of the symptoms on the different plant parts (leaves, stem, fruit, root) at different growth stages. Pictures of other diseases, not brought by farmers were then shown and farmers were asked to identify and give any name they had for these diseases. Farmers then ranked the severity of damage from the different pests and diseases identified and drew what they believed was the source of the different pests and diseases in their systems. Farmers were then asked to identify varieties they brought, and then rank varieties according to their level of resistance to the complex of pest and diseases in their systems. Finally, farmers were asked to describe practices they use to select good planting materials and how they manage pests and diseases in their farms.

#### **2.2.4 Household surveys**

In 2009, households in each region were selected using a randomly stratified design (by region and village), to ensure geographic representation across the target villages within each agro-

ecological region, totaling 120 households (60 households for each region). The household survey (HHS) was designed to complement and quantify information collected in FGDs, and to link crop varietal diversity on farm to observations of damage by rust in the farmers' fields discussed below.

At household the area planted and cultivation time of each variety farmer grow were recorded. Then, belief statements (Heong and Escalada, 1999) were used to test the level of knowledge of variety traits: commercial value, yield potential, grain quality, and variety earliness. Belief statements assessed were: 1) strongly agree, 2) agree, 3) doubt, 4) disagree and 5) strongly disagree.

Farmers surveyed at HHS cultivated mostly variety mixtures and together with them phenotypes that were part of variety mixtures were identified. Farmers were asked to sort out from the variety mixture all phenotypes and name to each of them. The named phenotypes and a representative sample of variety mixtures were taken from households with two objectives: a) to further analyze identity of phenotypes at key farmers meetings, and b) to establish the phenotype frequency in the variety mixture to calculate diversity indexes (richness, evenness, and divergence). The status of variety mixtures was studied in 2009 and 2015 in Cotacachi and in 2015 in Saraguro.

The same belief statements used to test knowledge of variety traits were used to test farmers' knowledge and attitudes towards pest and disease origin, disease transmission, durability of disease resistance, and benefits of variety mixtures. Farmers' knowledge information of each of these topics assessed with belief statements were derived for FGDs farmers associated information.

Resistance to diseases of varieties farmers identified at FGDs was assessed by farmers using the scale: 1) low resistant, 2) intermediate, and 3) highly resistant. Durability of resistance of the same varieties was also assessed by farmers using the scale: 1) durable, 2) intermediate and 3) low durable.

#### **2.2.5 Key farmer meetings**

Key farmers meetings (KFM) were organized in Cotacachi and Saraguro with the most knowledgeable farmers in the community. Objectives of KFM were: a) establish identification criteria which farmers use to name phenotypes components of variety mixtures, and b) complement identification of phenotypes derived from HHS. During phenotypic identification, name duplications or same name for different varieties were resolved. In case of name duplications, the most common name was agreed with farmers to be assigned, and for the same



name of morphologically different phenotypes, a letter (A, B...) was added to the name. Key farmers work allowed a precise phenotypic identification, which was the key input for diversity index (richness and evenness) calculations.

#### **2.2.6 Disease assessment in surveyed farmers' fields**

Rust was the most prevalent disease in the 2015 crop season and the on-farm disease severity was estimated for the 60 surveyed households in Cotacachi and Saraguro. Disease severity (DS) was calculated based on observations from 30 plants sampled for each household, made at 10 different points across the farmers' fields and assessing three plants at each point—one in front, one on the left and the third on the right. Disease severity of the 30 plants per household was assessed using a scale of 1-9 levels proposed by CIAT (Van Schoonhoven and Pastor-Corrales, 1987).

#### **2.2.7 Data analysis**

Processed data of variety mixtures was analyzed using descriptive statistics to score scale responses, frequency distributions and mean comparisons. Total area planted to each variety, both traditional and commercial was calculated based on GPS measurements, farmers' diagrams and descriptions of their plots using the methods described in Jarvis and Campilan (2006).

Variety traits and disease resistance based on belief statements were analyzed using the Fisher's exact test. For this test, "strongly agree" and "agree" responses were considered positive answers, whereas "disagree" and "strongly disagree" were considered negative answers. This test finds the statistical association between the positive or negative responses of the variety used by the farmer and the trait analyzed. Perception of farmers of durability of resistance, disease origin, disease transmission, and benefits of variety mixtures were analyzed using the Chi-square test, by which belief statements were also classified as positive or negative. For this test the statistical significance was set at 0.05.

Frequency of phenotypes of variety mixtures were compared among sites and years in Cotacachi, and among sites in Saraguro using the Pearson correlation test. Standard diversity indices for phenotypes components of variety mixtures (Jarvis et al., 2008), including richness (number of phenotypes), and evenness estimated as a complement of D ( $1 - D$ ), where D is the Simpson measure of dominance were calculated and transformed logarithmically  $1/(1 - \ln)$  (Magurran AE, 2004; Jarvis et al., 2008). The average number of phenotypes per household and the mean household evenness was calculated for both Cotacachi and Saraguro regions. The total region richness was calculated by summing the number of distinct varieties found across villages in the community. Also community richness and evenness were transformed logarithmically.

Divergence (i.e., the partition of diversity between and within households) was calculated as the difference between community and household evenness divided by the community evenness.

The Standardized Disease Index (SDI) ( $\text{Average DS} \times \text{Percentage of plants effected (incidence)}/\text{total range of DS}$ ) was calculated for rust at household level and then correlated (Pearson correlation test) with richness and evenness of the community. Data of all studies were processed in the MS excel (Microsoft Office Professional Plus, 2013) computer program, and the statistical analysis were done using the R statistical program version 3.4.

## **2.3 Results**

### **2.3.1 Farmers' knowledge about variety mixtures**

#### **Cotacachi**

Variety mixtures that farmers brought to FGDs were classified into two categories: *Chacra* and *Allpa*. Old and young women brought statistically more phenotypes in their variety mixtures than old and young men. However, old men were more knowledgeable than the rest of groups identifying phenotypes absent in the variety mixtures and phenotypes lost at the community. Most phenotypes identified in variety mixtures including absent and lost were traditional, except Cargabello and TOA, which were the improved varieties grown in *Allpa* and *Chacra* variety mixtures, respectively (Table 1).

**Table 1. Bean phenotypes of *Chacra* and *Allpa* variety mixtures identified by farmers at Focal Group Discussions (FGDs) in Cotacachi.**

Phenotype name	Focal Group Discussions					Phenotype	Focal Group Discussions				
	Old women	Young women	Old men	Young men	Leaders		Old women	Young women	Old men	Young men	Leaders
<b><i>Chacra</i> present at FGDs</b>						<b><i>Chacra</i> absent at FGDs</b>					
Chakra <sup>1</sup>	+	+	-	-	+	Hantzy suku poroto	+	-	+	-	-
Canario	+	+	-	-	+	Bolivar	-	-	-	-	+
Raku canario	-	+	+	-	-	Café poroto	-	-	+	-	-
Blanco canario	+	-	-	-	-	Cascajo poroto	-	+	-	-	-
Hamtzy canario	-	-	+	-	-	Hamtzy lacre	-	-	+	-	-
Bolon	-	-	-	+	-	Inda poroto	-	-	-	+	-
Molon	-	+	-	-	+	Hamtzi azul poroto	+	-	-	-	-
Suku poroto <sup>1</sup>	+	-	-	-	-	Killu tabla poroto	-	-	+	-	-
Capulis pototo	+	+	-	-	-	Killu yana poroto	-	-	+	-	-
Killu poroto	-	+	+	-	-	Lacre poroto	-	-	+	-	-
Killu raku poroto	+	-	-	-	-	Palillo poroto	-	-	-	-	+
Conejo poroto	+	-	-	-	-	Puca capulis poroto	-	-	+	-	-
Puka poroto	-	+	-	-	-	Puka hantzy poroto	-	-	-	+	-
Puka lichi vaca	+	-	-	-	-	Tabla raku killu	-	-	+	-	-
Puka ishtaka	-	+	-	-	-	Toro poroto	-	+	-	-	-
Raku azul poroto	-	-	-	+	-	Verde poroto	-	-	+	-	-
Azul poroto	-	+	-	-	-	Yura molon	-	+	-	-	-
Yura poroto	+	-	-	-	-	Yura yana raya	-	-	+	-	-
Yura hamtzi poroto	-	-	+	-	-	Suku hamtzi	+	-	-	-	-
Yana poroto	+	-	-	-	-	<b><i>Allpa</i> absent at FGDs</b>					
Lichi vaca yana poroto	-	-	-	+	-	Hantzy yura allpa	-	-	+	-	-
Viruchuro poroto	+	-	-	-	-	Hantzy suku allpa	-	-	+	-	-
Toa <sup>2</sup>	-	-	-	-	+	<b>Total absent at FGDs<sup>7</sup></b>					
Popayán <sup>3</sup>	+	+	-	-	+	<b><i>Chacra</i> lost at the community</b>					
<b><i>Allpa</i> present at FGDs</b>						caspy raku poroto	-	-	-	+	-
Allpa <sup>4</sup>	+	-	+	+	+	Sarahua poroto	-	-	+	-	-
Matambre <sup>4</sup>	+	+	-	-	-	Hamtzi Vaca poroto	+	-	-	-	-
Raku allpa	-	-	+	-	-	Suku Lichi vaca poroto	-	-	+	-	-
Suku allpa	-	-	+	-	-	<b><i>Allpa</i> lost at the community</b>					
Cargabello <sup>5</sup>	+	+	-	-	+	Killu allpa	+	-	-	-	-
<b>Total brought at FGDs<sup>6</sup></b>	<b>15</b>	<b>12</b>	<b>7</b>	<b>4</b>	<b>7</b>	<b>Total lost at community</b>	<b>2</b>	<b>-</b>	<b>2</b>	<b>1</b>	<b>-</b>

<sup>1</sup>Chakra and poroto are generic names for *Chacra*. <sup>2</sup>Improved variety released in 1993. <sup>3</sup>*Phaseolus coccineus*.

<sup>4</sup>Synonyms and generic names. *Allpa* meaning soil (quechua) and matambre (Spanish) meaning killing hunger.

<sup>5</sup>Bush variety introduced from lower valleys.

<sup>6</sup>Women (young and old) brought to FGDs more phenotypes than men and leaders (Chi square; p-value = 0.008).

<sup>7</sup>Old man identified more absent phenotypes than the rest of groups (Chi square; p-value = 0.0522).

Phenotypes from Cotacachi sampled at HHSs were analyzed at KFM to establish the identification criteria, complement HHSs identity, and identify name synonymies or name duplications. Name synonymies were partly due to language synonymies and since, the Quechua name was used to name the phenotype at FGDs. Identification of phenotype components of the *Chacra* and *Allpa* variety mixtures was based mainly on seed traits: color, color patterns, size, and shape of the seed. If the genotype had a kidney shape (expected for common bean) and medium size, the phenotype name was only the main color accompanied often by the generic name, allpa or chacra. If more than one color was involved, the name was composed by both colors in order of predominance. If the second color had stripe patterns, the term listado

(meaning stripe) was part of the name. An important seed color trait used was a spot surrounding the hilum, which is called shimi (mouth). Point patterns called shiku were also part of the name. Notorious shape and seed size were also part of the name: bola meaning round, raku meaning big, hamtzi meaning thin and suni meaning long. Names of fruits and animals were also used to identify phenotypes resembling fruits and animals. This is the case of conejo (rabbit), vaca (cow), and capulis (local prunus). Vaca name had variation according to the color: yana leche vaca (white and black), puka leche vaca (white and red). Some well-known names were readily used: thus, veltran, suku, and cascajo. Modern varieties that were part of the variety mixture, maintained their commercial names. If *Chacra* and *Allpa* had the same phenotypic appearance, then farmers complemented the name with the word chakra or allpa. This is the case for instance of suku allpa and suku chakra, killu listado allpa and killu listado chakra, among others. In Cotacachi, name differences between FGDs and HHSs were due to name synonymies and the most common name was agreed to be used at KFMs. Using the same name for different phenotypes were only found in Cotacachi for crema chakra and yura chakra in *Chacra*, and for Cargabello in *Allpa*. *Chacra* phenotypes sampled at HHSs and identified at KFMs are shown in Table 2, and *Allpa* phenotypes sampled at HHSs and identified at KFMs are shown in Table 3.

**Table 2. Frequency at community (%) of *Chacra* phenotypes from Cumbas Conde and Morochos (Cotacachi) obtained from 60 households in the cropping seasons of 2009 and 2015**

Phenotype <sup>1</sup>	Cumbas Conde <sup>2</sup>		Morochos <sup>3</sup>		Phenotype <sup>1</sup>	Cumbas Conde <sup>2</sup>		Morochos <sup>3</sup>	
	2009 <sup>4</sup>	2015 <sup>5</sup>	2009 <sup>4</sup>	2015 <sup>5</sup>		2009 <sup>4</sup>	2015 <sup>5</sup>	2009 <sup>4</sup>	2015 <sup>5</sup>
Hantzy canario	23.53	21.95	22.54	22.43	Canario alargado grande	0.43	1.51	0.00	0.05
Raku canario	17.61	19.10	10.89	21.48	Puka leche vaca	0.40	0.57	0.31	0.00
Toa	9.30	5.77	1.32	6.22	Lacre chakra	0.39	0.49	0.12	0.22
Crema chakra A	6.74	11.39	9.39	10.63	Morado hanptzi	0.32	1.69	1.45	0.28
Killu chakra	5.25	1.69	6.76	0.44	Bola yura chakra	0.32	1.42	0.31	0.00
Morado listado chakra	4.65	0.91	0.23	2.33	Suku listado leche vaca	0.31	1.07	0.00	0.00
Puka hantzi poroto chakra	2.96	1.40	15.68	0.84	Yana crema shiku popayan	0.27	0.11	0.63	0.00
Kishu listado chakra	2.91	1.09	0.90	1.11	Crema chakra C	0.23	0.41	0.00	0.00
Suku listado chakra	2.41	2.19	1.55	1.88	Yura chakra A	0.22	0.23	0.00	0.00
Puka bola poroto	1.98	1.79	0.88	4.23	Kishu vaca	0.22	0.84	0.00	0.12
Killu listado chakra	1.86	1.70	0.54	1.30	Yura yana shimi poroto	0.19	0.58	0.00	0.41
Canario cuadrado	1.60	0.46	2.30	0.12	Café chakra	0.18	0.14	0.19	0.28
Hanptzi yana morado	1.54	2.62	1.67	1.24	Yana listado	0.17	0.18	0.00	0.07
Capulis poroto	1.26	0.56	0.36	2.95	Bola soldado chakra	0.16	0.81	0.13	0.00
Killu puka listado	1.26	0.96	0.47	0.50	Yura chakra B	0.15	0.28	0.21	0.00
Yana leche vaca	1.23	1.06	0.51	0.00	Yura bola leche vaca	0.09	0.11	0.06	0.08
Café listado chakra	1.21	0.15	0.00	0.00	Hanptzi bola yana chakra	0.05	0.00	0.00	0.07
Suku chakra	1.20	1.49	2.60	1.37	Chakra listado bola crema	0.04	0.18	0.83	0.22
Torta	1.12	0.00	0.97	0.12	Cascao poroto	0.00	0.35	0.10	0.00
Crema listado chakra	0.99	0.08	0.04	0.06	Puka café poroto	0.00	0.67	0.00	0.00
Hanptzi puka chakra	0.90	0.67	0.66	0.40	Hermano capulis poroto	0.00	0.34	0.00	0.12
Yana bola chakra	0.78	1.42	0.23	0.18	Killu yana listado chakra	0.00	0.17	0.00	0.00
Yana chakra	0.74	0.50	1.21	0.29	Zuni yana chakra	0.00	0.13	0.00	0.00
Canario alargado	0.70	2.37	6.58	13.91	Café hanptzi poroto	0.00	0.14	0.00	0.00
Yura lacre shimi	0.55	1.43	0.71	0.00	Morado con crema	0.00	4.00	0.39	0.23
Bola puka listado chakra	0.54	0.44	4.20	0.15	Hanptzi yana poroto	0.00	0.00	0.00	0.23
Soladado chakra	0.52	0.25	0.28	0.00	Chakra poroto uva	0.00	0.00	1.29	0.11
Crema chakra B	0.52	0.14	0.51	3.33					

<sup>1</sup>Names derived from key farmers meetings

<sup>2</sup>Pearson coefficient of correlation among years in Cumbas Conde: 0.89\*\*\*

<sup>3</sup>Pearson coefficient of correlation among years in Morochos: 0.76\*\*\*

<sup>4</sup>Pearson coefficient of correlation among sites in 2009: 0.80\*\*\*

<sup>5</sup>Pearson coefficient of correlation among sites in 2015: 0.91\*\*\*

## Saraguro

The variety mixtures analyzed by farmers in Saraguro at FGDs are shown in Table 4. Farmers in Saraguro brought to FGDs the *Chacra* type, more often called *Mixturiado* (mixture made), and Popayán (*P. coccinesus*). Varieties brought to FGDs in Saraguro were entirely traditional. In Saraguro, among present and absent, nine variety mixtures were brought to FGDs. Farmers named the variety mixture with its generic name, chacra or mixturiado, adding the origin (lowland or highland), and often the size of the seed. Farmers in Saraguro were not knowledgeable about names of phenotypes present in variety mixtures and considered the variety mixture, a varietal unite. Farmers also brought a variety mixture of the commercial type *Canario*, called by them at FGDs Bola Amarillo. Curiously, poroto negro and huevo de chirote were absent at FGDs, but they were referred in KFM phenotypes components of variety mixtures. Only young and old women, and old men were available for FGDs in Saraguro.

**Table3. Frequency at community (%) of *Allpa* phenotypes from Cumbas Conde and Morochos (Cotacachi) obtained from 60 households in the cropping seasons of 2009 and 2015.**

Phenotypes <sup>1</sup>	Cumbas Conde <sup>2</sup>		Morochos <sup>3</sup>	
	2009 <sup>4</sup>	2015 <sup>5</sup>	2009 <sup>4</sup>	2015 <sup>5</sup>
Crema listado allpa	33.68	45.63	41.73	72.31
Puka listado allpa	16.94	3.3	6.97	2.78
Puka allpa	15.68	7.78	4.83	1.21
Hantzi crema listado allpa	8.95	0.00	20.00	4.97
Killu puka listado allpa	5.94	5.49	0.00	1.01
Cargabello B	4.25	0.43	11.32	0.42
Suku allpa	3.81	5.25	9.55	3.43
Cargabello A	3.76	17.49	3.41	3.20
Cargabello puka	2.91	0.38	0.00	0.35
Cargabello morado	1.76	4.22	0.58	5.96
Yura crema listado allpa	0.94	0.00	0.00	1.68
Yana listado allpa	0.67	1.69	1.61	0.85
Killu listado allpa	0.55	0.71	0.00	0.00
Hantzi crema listado allpa	0.16	0.00	0.00	0.17
Morado suku allpa	0.00	2.02	0.00	1.66
Crema allpa	0.00	5.56	0.00	0.00
Yana listado allpa	0.00	0.05	0.00	0.00

<sup>1</sup>Names derived from key farmers meetings

<sup>2</sup>Pearson coefficient of correlation among years in Cumbas Conde: 0.80\*\*

<sup>3</sup>Pearson coefficient of correlation among years in Morochos: 0.88\*\*

<sup>4</sup>Pearson coefficient of correlation among sites in 2009: 0.84\*\*

<sup>5</sup>Pearson coefficient of correlation among sites in 2015: 0.93\*\*

Key farmers of Saraguro, as in FGDs meetings, were low knowledgeable about phenotypic identity. Although they also used seed traits for naming the phenotypes, they were not as analytical as farmers in Cotacachi. They used mainly the color of the seed sometimes together with seed size (small or big). Stripes were also used as a trait and took the name rayadito (striped), and this trait was often used together with seed size. As in Cotacachi, names were associated with animal traits: huevo de chirote (egg of birth), corazón (heart), conejo (rabbit). Well-known phenotypes were also readily identified, as bolongo, chavelito, shiro, shano, and suku. Synonymies were less frequent in Saraguro and easily resolved at KFMs. The same name for different phenotypes was more often, and a letter (A, B...) was added to the name to differentiate phenotypes. *Chacra* phenotypes sampled at HHSs and identified at KFMs are shown in Table 5. Conejo and suku were common names for the same phenotypes in Cotacachi and Saraguro.

**Table 4. Bean phenotypes of *Chacra* and *Popayán* variety mixtures identified by farmers at Focal Group Discussions (FGDs) in Saraguro.**

Phenotype name	Young women	Old women	Old men		Young women	Old women	Old men
<b>Present at FGDs</b>				<b>Lost varieties</b>			
<i>Chacra</i> <sup>1</sup>	+	+	+	Achatado	-	-	+
<i>Misturiado</i> pequeño de Sierra <sup>1</sup>	-	-	+	Vaquitas	-	-	+
<i>Misturiado</i> de costa y sierra <sup>1</sup>	-	-	+	Chindo	+	-	-
Bola Amarillo <sup>2</sup>	+	+	+	Postrado	+	-	-
<i>Popayán</i> de costa y sierra <sup>3</sup>	-	-	+	Singa negra	-	+	-
<b>Total present at FGDs</b>	<b>2</b>	<b>2</b>	<b>5</b>	Bolson	-	+	-
<b>Absent at FGDs</b>				Calentura	-	+	-
Poroto negro	-	-	+	<b>Total lost at community</b>	<b>2</b>	<b>3</b>	<b>2</b>
Toda vida <sup>3</sup>	-	-	+	<sup>1</sup> <i>Chacra</i> and <i>Mixturiado</i> are generic and synonyms in Saraguro.			
Huevo de chirote	-	+	+	<sup>2</sup> Synonym of <i>Canario</i> (commercial variety).			
Mezcla ratoncito con rayado	-	-	+	<sup>3</sup> Synonyms belonging to <i>Phaseolus coccineus</i> .			
<b>Total absent at FGDs</b>	-	1	4				

### 2.3.2 Traits and management of variety mixtures

At HHSs, *Chacra* variety mixtures were cultivated in Cotacachi and Saraguro and *Allpa* variety mixtures were cultivated only in Cotacachi. *Canario* was considered by Cotacachi and Saraguro farmers a *Chacra* type; however, in this section *Canario* is considered a different type for the commercial status of *Canario* at the country level. *Popayán* was grown as part of *Chacra* variety mixtures in Cotacachi and Saraguro. In both regions, a variety mixture as described by farmers, is planted, harvested, cooked, and eventually commercialized as a varietal unite. High average of time span cultivation of all variety mixtures allows us to conclude that variety mixtures have been cultivated for a long time, except for *Popayán* in Saraguro that appears to have been recently introduced to cultivation (Table 6).

The perceptions of farmers about variety traits are shown in Table 6. *Canario* was the only variety positively associated with the commercial trait, *Chacra* and *Allpa* were not associated, and *Popayán* was inversely associated with this trait in both Cotacachi and Saraguro. Perception of yield, earliness, and grain quality was similar for all varieties in Cotacachi and Saraguro; except for *Allpa*, which were associated with earliness in Cotacachi. These results show that farmers considered all varieties yield similarly, all are late and have similar grain quality. Similarly, all common bean variety mixtures in both Cotacachi and Saraguro regions were perceived not associated with resistance to any disease and only *Canario* was inversely associated (susceptible) to AsLS (Table 6). *Popayán* belonging to *P. coccineus* on the other hand was highly associated with resistance to all diseases in both regions. Perception of durability of resistance was somehow different among regions. In Cotacachi farmers did not perceive durability of resistance in any variety mixture, whereas in Saraguro resistance of *Chacra* and *Canario* was considered of low durability and resistance of *Popayán* was considered durable.

**Table 5. Frequency at community (%) of *Chacra* phenotypes from four Saraguro sites obtained from 60 households in the cropping season of 2015.**

Phenotypes <sup>1</sup>	Saraguro sites <sup>2</sup>				Phenotypes <sup>1</sup>	Saraguro sites <sup>2</sup>			
	Tenta	Cañicapa	Gañil	Selva Alegre		Tenta	Cañicapa	Gañil	Selva Alegre
Canario A	13.21	14.27	23.81	21.74	Not defined B	0.56	0.40	0.41	0.39
Canario B	9.39	8.90	10.23	22.47	Pequeño negrito	0.22	1.25	0.00	0.27
Sangre cuy A	13.72	6.12	11.01	14.29	Morocho C	0.00	0.06	1.62	0.00
Pequeño alargado A	14.69	8.94	6.53	3.64	Shanito pequeño B	0.00	0.00	0.41	0.95
Canario C	4.53	7.13	7.42	7.66	Chavelito A	0.00	1.27	0.00	0.00
Rayado pequeño A	8.53	4.61	5.54	0.60	Shiro rayado pequeño	0.36	0.34	0.21	0.27
Canario E	0.33	5.31	0.95	8.40	Chacra manchas	1.12	0.00	0.00	0.00
Negro chacra B	2.83	3.92	2.27	1.04	Huevo de chirote B	0.00	0.77	0.21	0.00
Shanito pequeño A	2.14	1.35	1.47	4.76	Pequeño alargado B	0.35	0.00	0.00	0.48
Shanito A	0.74	4.99	2.86	0.00	Azulito	0.00	0.20	0.21	0.36
Corazón	2.95	3.18	0.85	1.58	Rayado pequeño C	0.33	0.24	0.21	0.00
Suco	2.71	1.83	1.99	1.33	Morocho A	0.40	0.00	0.18	0.00
Conejo	2.87	4.14	0.52	0.00	Negro chacra A	0.22	0.33	0.00	0.00
Canario D	2.80	1.30	1.45	1.30	Blanco largo	0.14	0.35	0.00	0.00
Shiro colorado	1.15	0.30	5.76	0.00	Shiro colorado grande	0.48	0.00	0.00	0.00
Rayado grande A	0.79	3.86	0.44	1.40	Café B	0.00	0.00	0.00	0.40
Not difined A	0.92	3.36	0.45	0.83	Rojito B	0.00	0.00	0.31	0.00
Shanito rayado	0.92	0.41	3.01	0.00	Sangre cuy C	0.08	0.00	0.24	0.00
Shiro pequeño A	0.38	0.89	2.47	0.39	Chavelito B	0.00	0.16	0.00	0.12
Sangre cuy B	0.58	0.46	2.36	0.39	Shano bola grande	0.00	0.00	0.00	0.24
Tipo TOA alargado	3.25	0.08	0.00	0.00	Bolongo negro	0.00	0.24	0.00	0.00
Vaca café	0.67	0.14	0.18	1.90	Rojito A	0.25	0.00	0.00	0.00
Chacra rayado	0.00	2.20	0.00	0.39	Rayado grande B	0.22	0.00	0.00	0.00
Bolongito	1.05	0.24	1.23	0.00	Plomito	0.22	0.00	0.00	0.00
Chacra morado	0.15	0.25	1.79	0.27	Pequeño alargado C	0.00	0.20	0.00	0.00
Rayado pequeño B	2.16	0.18	0.00	0.00	Shiro pequeño B	0.00	0.00	0.18	0.00
Shanito rayado pequeño	0.14	1.49	0.50	0.12	Bolongo	0.00	0.00	0.18	0.00
Café A	0.97	0.56	0.44	0.13	Shanito C	0.00	0.00	0.10	0.00
Shano azulito	0.00	0.92	0.00	1.16	Rayado pequeño D	0.08	0.05	0.00	0.00
Huevo de chirote A	0.33	0.96	0.00	0.73	Morocho B	0.07	0.00	0.00	0.00
Shanito B	0.00	1.80	0.00	0.00	Canario pequeño	0.00	0.05	0.00	0.00

<sup>1</sup>Names derived from key farmers meetings

<sup>2</sup>Pearson coefficient of correlation: Tenta with Cañicapa 0.83\*\*, Tenta with Gañil 0.82\*\*, Tenta with Selva Alegre: 0.73\*\*, Cañicapa with Gañil: 0.84\*\*, Cañicapa with Selva Alegre: 0.81\*\*, Gañil with Selva Alegre: 0.85\*\*.

Seed flow of variety mixtures is limited in Cotacachi and Saraguro, most farmers interviewed used their own seed, 60% in Cotacachi and 70% in Saraguro. In Cotacachi, seed interchange occurred among parents, relatives and neighbors (36%). Only 4% of farmers bought seeds from local markets, and the variety INIAP-412 TOA was obtained in the past from local official agriculture extension services. In Saraguro, seed interchange occurred among parents, neighbors and friends (25%) and, only 5% of farmers bought seeds from local markets.

Seed selection of variety mixtures was done at the store right after harvest in Cotacachi and Saraguro, and the main practice at store is selection of healthy seed. Phenotypic composition of variety mixtures did not appear relevant for most farmers, and frequency of phenotypes in the mixture appears entirely dependent of biotic and abiotic selection pressure in the previous season. Seed selection was not associated with either selecting plots or selecting plants in the field; except in Saraguro, where some farmers eventually select productive plants in the field.



**Table 6. Variety mixture cultivation time, probability of the Fisher's association test of farmers' perception of variety traits and disease resistance, and frequency (%) of farmers' perception of durability of disease resistance.**

Variety mixture <sup>1</sup>	Cultivation time <sup>2</sup>	Variety trait Fisher's probability <sup>3</sup>				Disease resistance Fisher's probability <sup>3</sup>			Durability of resistance (%) <sup>4</sup>	
		Commercial	Yield	Earliness	Quality test	Rust	P. mildew	AsLS	Durable	Non durable
Cotacachi										
Chacra	26	0.53 <sup>ns</sup>	1 <sup>ns</sup>	1 <sup>ns</sup>	1 <sup>ns</sup>	0.80 <sup>ns</sup>	0.08 <sup>ns</sup>	0.84 <sup>ns</sup>	0.52	0.48
Canario	29	0.00 <sup>***</sup>	1 <sup>ns</sup>	1 <sup>ns</sup>	1 <sup>ns</sup>	0.57 <sup>ns</sup>	0.22 <sup>ns</sup>	(0,00) <sup>***</sup>	0.42	0.58
Allpa	28	0.57 <sup>ns</sup>	1 <sup>ns</sup>	0.00 <sup>***</sup>	1 <sup>ns</sup>	0.81 <sup>ns</sup>	0.40 <sup>ns</sup>	0.18 <sup>ns</sup>	0.47	0.53
Popayán	17	(0.00) <sup>***</sup>	1 <sup>ns</sup>	1 <sup>ns</sup>	1 <sup>ns</sup>	0.00 <sup>***</sup>	0.01 <sup>**</sup>	0.00 <sup>***</sup>	0.50	0.50
Saraguro										
Chacra	25	0.07 <sup>ns</sup>	0.27 <sup>ns</sup>	1 <sup>ns</sup>	0.44 <sup>ns</sup>	0.75 <sup>ns</sup>	0.67 <sup>ns</sup>	0.47 <sup>ns</sup>	0.13	0.87 <sup>*</sup>
Canario	13	0.00 <sup>***</sup>	0.08 <sup>ns</sup>	1 <sup>ns</sup>	0.56 <sup>ns</sup>	0.60 <sup>ns</sup>	0.40 <sup>ns</sup>	0.44 <sup>ns</sup>	0.23	0.77 <sup>*</sup>
Popayán	7	0.33 <sup>ns</sup>	0.85 <sup>ns</sup>	1 <sup>ns</sup>	0.45 <sup>ns</sup>	0.00 <sup>***</sup>	-	0.00 <sup>***</sup>	1 <sup>***</sup>	0.00

<sup>1</sup>*Chacra*: mixtures of genotypes of growth habit IV. *Canario*: mixtures of yellow large-seeded genotypes of growth habit IV. *Allpa*: mixtures of genotypes of growth habit I, II and III. *Popayán*: mixtures of *Phaseolus coccineus* genotypes grown as part of *Chacra* variety mixtures.

<sup>2</sup>Average of time (years) of variety mixture cultivation in the community obtained from 60 households.

<sup>3</sup>The farmers' perception based on belief statements of the 60 households was subjected to the Fisher's exact association test: ns, not significantly associated; \*\* significantly associated at 0.01; \*\*\* significantly associated at 0.001. In parentheses, the farmers' perception was negatively associated with the trait.

<sup>4</sup>The farmers' perception based on belief statements was classified as resistance or susceptible and subjected to the Chi-square test: <sup>ns</sup> non-significant, \* significant at 0.05, \*\* significant at 0.01, \*\*\* significant at 0.001.

### 2.3.3 Status of variety mixtures

Cultivation of *Canario* variety mixtures as sole variety had low frequency, only one farmer from Cotacachi and three farmers from Saraguro cultivated *Canario* variety mixtures; however, all *Chacra* variety mixtures contained *Canario* phenotypes and therefore, *Canario* in this section is studied as a *Chacra* variety mixture. *Popayán* was rare in Cotacachi households and frequent in Saraguro households, and in both regions *Popayán* was part of *Chacra* variety mixtures. In Cotacachi and Saraguro, *Chacra* variety mixtures were cultivated by all farmers, while *Allpa* and *Popayán* were less frequent in Cotacachi and Saraguro respectively (Table 7). *Allpa* was similarly frequent in Cumbas Conde and Morochos, while *Popayán* was more frequent in Gañil than in the rest of sites of Saraguro (Table 7).

**Table 7. Frequency, richness and evenness at household (HH) and community (Comm) and divergence at community of phenotypes components of variety mixtures from Cotacachi and Saraguro in the cropping season of 2015.**

Variety mixture type	Frequency (%)		HH richness <sup>3</sup>		HH evenness <sup>4</sup>		Comm richness <sup>5</sup>	Comm evenness <sup>6</sup>	Divergence <sup>7</sup>
	HH <sup>2</sup>	Comm <sup>1</sup>	Mean	St dev.	Mean	St dev.			
<b>COTACACHI</b>									
<b>Cumbas</b>									
<i>Chacra</i>	77	100	12.40	5.57	0.75	0.15	51.00	0.90	0.16
<i>Allpa</i>	23	86	4.30	1.93	0.51	0.17	14.00	0.74	0.41
Global	...	...	15.57	7.67	0.78	0.14	65.00	0.94	0.17
<b>Morochos</b>									
<i>Chacra</i>	77	100	6.95	2.82	0.62	0.19	38.00	0.86	0.28
<i>Allpa</i>	23	90	2.37	2.19	0.17	0.27	14.00	0.47	0.69
Global	...	...	8.81	4.39	0.67	0.17	52.00	0.85	0.21
<b>Global Cotacachi</b>	...	...	12.17	7.10	0.72	0.17	72 <sup>6</sup>	0.91	0.21
<b>SARAGURO</b>									
<b>Tenta</b>									
<i>Chacra</i>	95	100	10.53	4.07	0.78	0.09	45.00	0.92	0.16
<i>Popayán</i>	5	0.4	2.00	0.89	0.34	0.29	7.00	0.80	0.58
Global	...	...	11.27	4.42	0.79	0.08	52.00	0.93	0.15
<b>Cañicapa</b>									
<i>Chacra</i>	93	100	15.09	7.88	0.83	0.08	51.00	0.94	0.12
<i>Popayán</i>	7	0.36	3.75	2.50	0.46	0.32	8.00	0.71	0.35
Global	...	...	18.10	6.98	0.85	0.08	59.00	0.91	0.07
<b>Gañil</b>									
<i>Chacra</i>	74	100	10.89	6.74	0.76	0.12	41.00	0.90	0.16
<i>Popayán</i>	26	89	4.00	2.14	0.51	0.33	11.00	0.76	0.37
Global	...	...	14.00	5.52	0.81	0.11	52.00	0.93	0.13
<b>Selva Alegre</b>									
<i>Chacra</i>	100	100	9.50	6.86	0.60	0.32	33.00	0.86	0.31
<b>Global Saraguro</b>	...	...	11.83	7.71	0.65	0.33	75 <sup>6</sup>	0.96	0.32

<sup>1</sup>Percentage of farmers cultivating each variety mixture type at the community.

<sup>2</sup>Average of each variety mixture type at household.

<sup>3</sup>Average of the number of phenotypes in the variety mixture of 60 households.

<sup>4</sup>Average of the complement of the Simpson measure of dominance (Magurran AE, 2004) of phenotypes in the variety mixtures of 60 households..

<sup>5</sup>Number of different phenotypes found in the 60 households.

<sup>6</sup>The complement of the Simpson measure of dominance (Magurran AE, 2004) of phenotypes of 60 households variety mixture

<sup>7</sup>Calculated as the difference between community and household evenness divided by the community evenness.

At household level, richness and evenness were similarly high in Cotacachi and Saraguro (Table 7). The overall average household richness reached 12.17 phenotypes in Cotacachi and 11.83 phenotypes in Saraguro, and the average household evenness (Simpson) reached 0.72 in Cotacachi and 0.65 in Saraguro. However, the average household richness and evenness of *Chacra*, the most important type, were very high in Cotacachi and Saraguro. The average richness and evenness of *Allpa* and *Popayán* were also important in Cotacachi and Saraguro, respectively. Richness and evenness varied among sites within regions, being higher in Cumbas Conde in Cotacachi, and in Cañicapa in Saraguro. Standard deviation of richness and evenness for *Chacra* in Cotacachi and Saraguro were relatively narrow, showing that richness and evenness of most household located around the average.

Richness of the community was also considerably high in Cotacachi and Saraguro (Table 7). Seventy-two different phenotypes including *Chacra* (55 phenotypes) and *Allpa* (17 phenotypes) were identified in Cotacachi and, 75 phenotypes including *Chacra* (62 phenotypes) and *Popayán* (13 phenotypes) were identified in Saraguro. Within Cotacachi and Saraguro sites, richness was also considerably high especially for *Chacra*.

Phenotype names derived from KFM in Cotacachi of the 55 *Chacra* phenotypes and 17 *Allpa* phenotypes sampled at HHSs at community are shown in Table 2 and Table 3, respectively. Likewise, names derived from KFM of the 62 *Chacra* phenotypes of Saraguro are shown in Table 5.

Community evenness was also high at Cotacachi and Saraguro, reaching 0.91 in Cotacachi and 0.96 in Saraguro, with high values for *Chacra* in both regions, but also with considerably high values for *Allpa* and *Popayán* in Cotacachi and Saraguro, respectively (Table 7). These results show that diversity of all types were evenly distributed in the community in both regions.

Divergence as a measure of the probability of any two randomly chosen households within the same community to grow different phenotypes was globally low in Cotacachi (0.21) and Saraguro (0.32), and especially low for *Chacra* in most sites in both regions. This low values appear to be associated with high richness, which reduces the probability that two randomly chosen phenotypes are different. On the other hand, divergence values of *Allpa* in Morochos (0.41) and *Popayán* in Tenta (0.58) were high due to low richness of these types at these sites.

Pearson correlation coefficients between richness with evenness were positive and significant at all sites of Cotacachi and Saraguro (Table 8), indicating that diversity indexes were closely associated in this study.

Variety mixtures in Cotacachi were planted in farms with an average area of 2,881 m<sup>2</sup>, with a minimum area of 221 m<sup>2</sup> and a maximum area of 10,687 m<sup>2</sup>. In Saraguro, the average farm area was 1,028 m<sup>2</sup>, with a minimum area of 180 m<sup>2</sup> and a maximum area of 4,200 m<sup>2</sup>. Pearson correlation coefficient between richness and evenness with area planted was negative and significant only in Cumbas Conde (Cotacachi) and Cañicapa (Saraguro) (Table 8).

**Table 8. Pearson coefficient of correlation among richness, evenness, area planted and the Standard Disease Index of rust for *Chacra* variety mixtures in Cotacachi and Saraguro**

	Cotacachi		Saraguro			
	Cumbas Conde	Morochos	Tenta	Cañicapa	Gañil	Selva Alegre
Richness x evenness	0.71**	0.74***	0.80***	0.85***	0.83***	0.8***
Richness x Area planted	-0.46*	0.08	0.19	-57*	0.23	-
Richness x SDI	-0.80**	-0.46*	-0.34	0.17	-0.15	0.59
Evenness x Area planted	-0.51**	0.12	0.05	-0.74*	0.23	-
Evenness x SDI	-0.59**	-0.04	-0.21	0.03	-0.29	0.2
Area planted x SDI	0.34	0.05	0.65*	0.67*	-0.17	-

\* significant at 0,05

\*\* significant at 0,01

\*\*\* significant at 0,001

In Cotacachi, phenotypes sampled at community at HHSs in 2009 and 2015 are shown for *Chacra* in Table 2 and for *Allpa* in Table 3. Within sites for *Chacra* and *Allpa*, frequency of phenotypes varied greatly in both years for *Chacra* ranging from less than 1 to 23.5% and for *Allpa* ranging from less than 1 to 72.3%. The most frequent *Chacra* phenotypes were canario bola pequeño, canario bola grande, Toa, crema chakra and killu chakra. The most frequent *Allpa* phenotypes were crema listado allpa, puka listado allpa, puka allpa and hantzi crema listado allpa. For both types, *Chacra* and *Allpa*, Pearson correlation coefficient of phenotypes among sites in both years was positive and significant showing that diversity of variety mixtures among Cumbas and Morochos was very similar. Similarly, the Pearson correlation coefficient of *Chacra* and *Allpa* phenotypes among years in both sites was positive and significant, showing that differences of phenotypes identified among 2009 and 2015 appeared primarily due to scape sampling of low frequent phenotypes.

In Saraguro, frequency of phenotypes of *Chacra* sampled at community at HHSs in 2015 is shown in Table 5. Frequency of *Chacra* phenotypes within sites varied from less than 1 to 23.8 %, and the most frequent *Chacra* phenotypes were pequeño largo, sangre de cuy, canario A and canario B. Person coefficient of correlation of *Chacra* phenotypes among all site combinations was positive and significant, showing that diversity of *Chacra* variety mixtures is similar among Tenta, Cañicapa, Gañil and Selva Alegre.

Frequency of phenotypes of *Popayán* sampled at community at HHSs in 2015 is shown in Table 9, and frequency of *Popayán* phenotypes varied from less than 1 to 37.2 %. Pearson coefficient of correlation was positive but not significant, showing that diversity of *Popayán* variety mixtures were different among Tenta, Cañicapa and Gañil.

**Table 9. Frequency at community (%) of Popayán phenotypes from three Saraguro sites in 2015.**

Phenotype	Tenta	Cañicapa	Gañil
T5	30.67	1.92	13.77
T13	20.00	0.00	0.00
T7	16.00	0.00	1.86
T2	14.66	45.45	0.57
T4	10.67	15.07	37.14
T3	4.00	21.63	23.74
T1	4.00	2.50	0.56
T9	0.00	0.64	15.39
T10	0.00	0.00	2.37
T11	0.00	0.00	2.37
T8	0.00	4.01	1.14
T6	0.00	0.00	1.09
T12	0.00	8.78	0.00

**Pearson coefficient of correlation:**

Tenta with Cañicapa: 0.14<sup>ns</sup>

Tenta with Gañil: 0.13<sup>ns</sup>

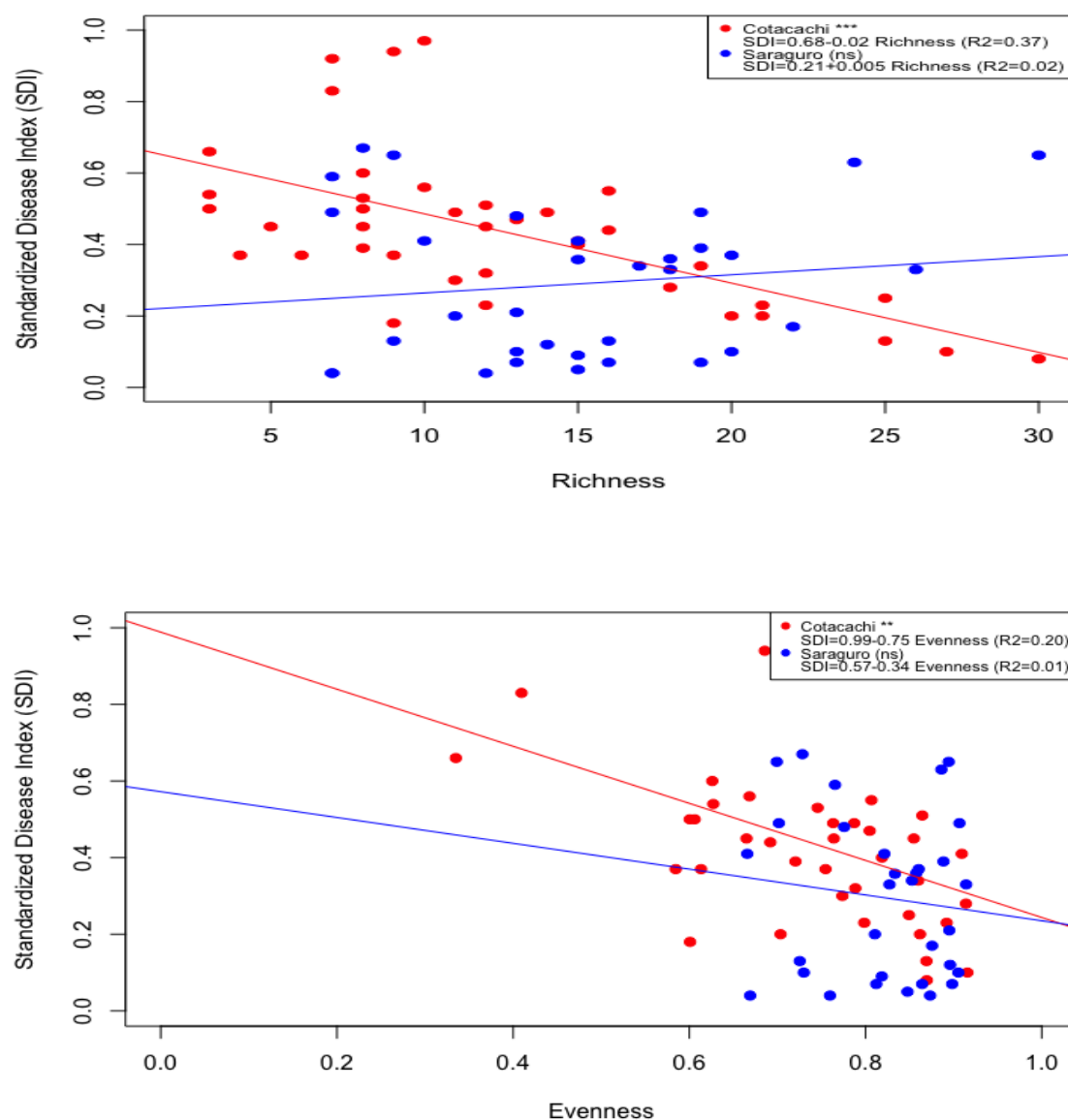
Cañicapa with Gañil: 0.20<sup>ns</sup>

#### **2.3.4 Diseases epidemics and farmers' knowledge**

Disease incidence of rust, measured by the Standardized Disease Index (SDI) for *Chacra* variety mixtures varied across sites and regions (Table 10). SDI was higher in Cotacachi (43.4) than in Saraguro (28.2); however, in Gañil (Saraguro) SDI was also high (37.7). Pearson correlation coefficient between richness and evenness with SDI was negative and significant in Cumbas Conde and Morochos (Cotacachi). However, although not significant, correlation was also negative in Saraguro (Table 8). This negative association of richness but especially evenness with the average SDI for Cotacachi and Saraguro is shown in Fig. 1. Correlation between the area planted with SDI was only positive and significant in Tenta and Cañicapa (Saraguro) (Table 8).

Farmers' names for diseases provided by farmers at Cotacachi and Saraguro are shown in Table 11. In both regions, farmers used to name diseases, symptom appearance and conducive conditions (rain, cloud). To name diseases in Cotacachi, young women used Quechua names, while old women and old men used Spanish names. Leaders on the other hand used Spanish

technical names. In Saraguro, farmers used Spanish and mostly technical names. This diversity of identification led to many synonymies (Table 11).



**Fig. 1. Association between Standardized Disease Index of rust in the field with richness and evenness of Cotacachi and Saraguro variety mixtures.**

In Cotacachi, farmers used the technical name lancha (blight) for all diseases, rust and powdery mildew were differentiated among each other, and anthracnose and AsLS were not differentiated among each other. In Saraguro, rust and powdery mildew using technical names were more clearly differentiated than in Cotacachi, and anthracnose and AsLS were not differentiated among each other (Table 11).

Farmers' perception about disease origin and disease transmission based on belief statements is shown in Table 12. In Cotacachi, farmers' perception was statistically in favor of disease origin related with rain and clouds, and disease transmission among neighboring plants and neighboring fields. Responses to diseases carried by animals were inconsistent. Although statistical differences were found among responses for the belief statements analyzed, doubt responses were frequent. In Saraguro, belief statements results were quite similar than in Cotacachi; farmers also agree with diseases originating in the rain, and unlike Cotacachi, they also believe that diseases are favored by pesticides. Farmers from Saraguro also associated disease transmission with neighboring plants and neighboring field, and doubt responses were less frequent in this region.

**Table 10. Richness, evenness and Standard Disease Index of rust of *Chacra* variety mixtures in Cotacachi and Saraguro in 2015.**

Variety mixtures in Cotacachi and Saraguro in 2019.				
Region/site	Richness <sup>1</sup>	Evenness <sup>2</sup>	Standardized Disease Index <sup>3</sup>	
			Mean	St dev.
<b>COTACACHI</b>				
Cumbas	12.35	0.75	37.6	21.86
Morochos	6.95	0.62	49.2	20.51
Average Cotacachi	9.65	0.72	43.4	21.19
<b>SARAGURO</b>				
Tenta	10.53	0.78	19.6	19.13
Cañicapa	15.09	0.83	39.0	21.54
Gañil	10.89	0.76	37.6	15.96
Selva Alegre	9.50	0.60	16.5	11.59
Average Saraguro	11.83	0.65	28.2	17.06

<sup>1</sup>Average of the number of phenotypes in the variety mixtures of 60 households.

<sup>2</sup>Average of the complement of the Simpson measure of dominance (Magurran AE, 2004) of phenotypes in the variety mixtures of 60 households.

<sup>3</sup>(Average DS × incidence)/total range of DS.

Farmers' perception about durability of disease resistance of traditional and modern varieties, and benefits of variety mixtures is shown in Table 13. Although resistance of local varieties was considered more durable than resistance of improved varieties, an important fraction of farmers gave doubt responses. Similarly, farmer's perception of variety mixtures being less affected by diseases were neutral in Cotacachi, and in favor of this belief statement in Saraguro. However, when resistance and durability of resistance is analyzed individually for each variety mixture and for each disease (Table 6), farmers from Cotacachi and Saraguro clearly discriminated resistance of *Popayán* from the relative susceptibility of common bean varieties. Perception of resistance durability was somehow different among regions. In Cotacachi, farmers did not perceive

durability of resistance, while in Saraguro resistance of *Chacra* and *Canario* were considered low durable while resistance of *Popayán* was considered durable.

In Cotacachi, benefits of yield and economical income of variety mixtures were not clearly perceived with high frequency of doubt responses; however, in Saraguro both benefits were clearly perceived with low percentage of doubt responses (Table 13).

**Table 11. Farmers' identity for main bean diseases found in Cotacachi and Saraguro**

Diseases	Farmers name at FGDs
<b>COTACACHI</b>	
<b>Anthraxnose</b> <i>Colletotrichum lindemuthianum</i>	Lancha, yana lancha, lluvia, gota a la vaina, microbio, tranquilador
<b>Ascochyta Leaf Spot</b> <i>Ascochyta phaseolorum</i>	Lancha, yana lancha, lluvia, pudrición, pica hoja
<b>Powdery mildew</b> <i>Erysiphe polygoni</i>	Lancha, yura lancha, lancha blanca, ucchufa, polvo blanco, mariposa
<b>Rust</b> <i>Uromyces appendiculatus</i>	Lancha, killu lancha, lancha amarilla, roya, puntos rojos, lancha harinosa, puca polvo
<b>SARAGURO</b>	
<b>Anthraxnose</b> <i>Colletotrichum lindemuthianum</i>	Lancha, lluvia, hongos, nube, pudrición, tizón temprano, apestado
<b>Ascochyta Leaf Spot</b> <i>Phoma exigua</i> var. <i>fobeata</i>	Lancha, lluvia, hongos, nube, pudrición, lancha negra, lancha agua, manchas negras,
<b>Powdery mildew</b> <i>Erysiphe polygoni</i>	Cenicilla, cenicilla blanca, lancha verano, oideo, polvillo, polvillo blanco
<b>Rust</b> <i>Uromyces appendiculatus</i>	Lancha amailla, roya

### 2.3.5 Disease control approaches

Main strategy used by farmers to manage pest and disease in Cotacachi and Saraguro is inter and intra specific diversity (Table 14). In Cotacachi, all FGDs considered interspecific diversity (intercrops) and intraspecific diversity (variety mixtures) strategies to cope with pest and diseases. In most FGD's (except young men and leaders) farmers agree that more diverse the interspecific diversity (quinoa, lupines, faba bean and peas) more the contribution to disease control. In common bean/maize intercropping, old women suggested *Allpa*, old men suggested *Canario* and young



**Table 12. Farmers' perception based on belief statements of origin and transmission of common bean diseases in Cotacachi and Saraguro**

Belief Statement <sup>1</sup>	Disease origin <sup>1</sup>					Disease transmission <sup>1</sup>					
	Rain		Clouds		Pesticides	Neighbour field		Neighbour plants		Animals carry diseases	
	Cotacachi										
	Cotacachi	Saraguro	Cotacachi	ns	Saraguro	Cotacachi	Saraguro	Cotacachi	Saraguro	Cotacachi	Saraguro
Strongly agree	29.51	30.34	9.84	11.48	20.88	9.84	32.26	32.79	69.89	9.84	52.69
Agree	39.34	20.22	32.79	16.39	27.47	22.95	37.63	32.79	13.98	13.11	25.81
Doubt	24.59	35.96	42.62	54.10	31.87	47.54	20.43	22.95	12.90	54.10	21.51
Disagree	6.56	8.99	8.20	16.39	15.38	14.75	6.45	8.20	3.23	18.03	-
Strongly disagree	-	4.49	6.56	1.64	4.40	4.92	3.23	3.28	-	4.92	-
Chi-square test	***	***	***	ns	**	*	***	***	***	ns	***

<sup>1</sup>For the chi-square test, the belief statements strongly agree and agree were classified as **positive** answers and the belief statements disagree and strongly disagree were classified as **negative** answers.

<sup>ns</sup>not significant; \* significant at 0.05; \*\* significant at 0.01; \*\*\* significant at 0.001.

**Table 13. Farmers' perception based on belief statements of durability of disease resistance and benefits of variety mixtures in Cotacachi and Saraguro**

Belief statement	Durability of disease resistance <sup>1</sup>				Variety mixture benefits <sup>1</sup>					
	Modern varieties		Local varieties		Disease incidence		Better yield		Economical income	
	Cotacachi	Saraguro	Cotacachi	Saraguro	Cotacachi	Saraguro	Cotacachi	Saraguro	Cotacachi	Saraguro
Strongly agree	1	5	5	26	10	34	5	41	4	55
Agree	0	0	24	37	21	28	15	31	17	21
Doubt	16	39	20	17	15	17	26	15	23	10
Disagree	5	16	9	8	13	3	12	2	16	2
Strongly disagree	13	20	2	1	2	3	2	3	1	4
<b>Chi-square test</b>	***	**	*	**	*	**	ns	**	ns	**

<sup>1</sup>For the chi-square test, the belief statements strongly agree and agree were classified as **positive** answers and the belief statements disagree and strongly disagree were classified as **negative** answers.

<sup>ns</sup>not significant; \* significant at 0.05; \*\* significant at 0.01; \*\*\* significant at 0.001.

women suggested Toa and *Allpa* as the main components of the strategy. In Saraguro, farmers also agree with interspecific diversity as the strategy to cope with pest and diseases.

Recommendations to reduce damage of pest and diseases are shown in Table 15. Additional recommendation to inter and intraspecific diversity of Cotacachi and Saraguro farmers were selection of healthy seeds and discarding damaged seeds. Spray ash to control blight was a recommendation of young and old men in Cotacachi and all farmers of Saraguro. Spray plant extracts with insecticide properties were recommended for insect control of young and old men in Cotacachi and old women in Saraguro. In Saraguro, crop rotation, organic fertilization and drainage were also recommended for pest and disease control. Neither in Cotacachi nor in Saraguro farmers used pesticides to control pest and diseases in common bean. A special recommendation of old men in Cotacachi was planting and weeding during the first quarter moon phase.

## 2.4 Discussion

### 2.4.1 Farmers' knowledge about variety mixtures

Farmers were in general knowledgeable about diversity they manage and maintain. Old and young women brought higher diversity to FGD, but also they were more knowledgeable because they stay at home, manage crops in the farm in an interactive way. On the other hand, old men were more knowledgeable about phenotypes absent at FGDs, because they are more socially active in the community. Knowledge of old farmers that is passed through generations as verified in this study specially for women is very important as source of varietal diversity information (Sadiki et al., 2007).

**Table 14. Focal Group Discussions information on the use of inter/intra specific diversity to manage pest and diseases *on farm* in Cotacachi and Saraguro.**

Iner/intra specific diversity <sup>1</sup>	Old women	Young women	Old men	Young men	Leaders
<b>Cotacachi</b>					
Intercrop maize + <i>Chacra</i> + other crops <sup>2</sup>	+	+	+	-	-
Inetrcrop maize + common bean	-	-	-	+	+
Intercrop maize + <i>Chacra</i> + <i>Allpa</i>	+	-	-	-	+
Intercrop maize + <i>Chacra</i> + <i>Canario</i>	-	-	+	-	-
Intercrop maize + Toa + <i>Allpa</i>	-	+	-	-	+
<b>Saraguro</b>	-	-	-	-	-
Intercrops	+	+	+	-	-

<sup>1</sup>In this topic, farmers used *Chacra*, *Allpa* and *Canario* as generic names for variety mixtures

<sup>2</sup>Often including quinoa, lupinus, faba bean and peas

In Cotacachi, farmers clearly differentiated varieties they maintain in such a way that they could discriminate and give a name to almost all phenotypes in variety mixtures. Farmers name

phenotypes in the mixtures irrespectively in Quechua or Spanish; however, they were more skillful with their first language (Quechua), and they used Spanish mostly to communicate with rapporteurs. At KFM, Quechua was agreed to be used to name phenotypes components of variety mixtures. Some phenotypes had well-known local names and names associated with appearance of animals and fruits were readily identified. For the remaining phenotypes, farmers used analytical seed traits: size, shape, and color patterns. Use of seed traits to name common bean varieties has been reported (Mar and Holly, 2000); however, the way farmers used to name their varieties in Cotacachi was similar to breeder methods to classify seed types (Voysets, 2000).

**Table 15. Recomendations for pest and disease control of Cotacachi and Saraguro farmers at Focal Group Discussions**

	Cotacachi farmers				Saraguro farmers		
	Old women	Young women	Old men	Young men	Old women	Young women	Old men
Discard broken and damaged seeds	-	-	+	+	+	+	+
Select big and healthy seeds	+	+	-	-	+	+	-
Spray ash for lancha (blight)	-	-	+	+	+	+	+
Spray extracts of repellent plants	-	-	+	+	+	-	-
Crop rotation	-	-	-	-	+	+	+
Organic fertilization	-	-	-	-	+	+	-
Drainage	-	-	-	-	+	+	-
Moon stage <sup>1</sup>	-	-	+	-	-	-	-

<sup>1</sup>Plant and weeding in the first quarter moon phase

In Saraguro, although farmers used similar criteria to name phenotypes in their variety mixtures, they were not as skillful as Cotacachi farmers were, and different phenotypes often received the same name, which appears because Saraguro farmers lost their first language (Quechua), and also lost variety names.

#### 2.4.2 Traits and management of variety mixtures

In Cotacachi, two common bean types are intercropped with maize: the indeterminate *Chacra* and the determinate *Allpa*. *Chacra* (Spanish) also called chakra (Quechua) is a late type of growth habit IV, while *Allpa* (Quechua) called also matambre is an early type of growth habit I, II and III. Names farmers provided to these common bean types are associated with plant phenology and the growth habit. Thus, the chacra (chakra) name is associated with the intercrop, chacra meaning maize crop. *Allpa* means soil, it is due to the prostrate habit of this type that produce closely and sometime taking contact with the soil, and matambre meaning “killing hanger” in regards to the early nature of *Allpa* providing food early in the season.

In Cotacachi, *Chacra* is the most important type grown by all farmers, *Allpa* is grown on the other hand complementarily to *Chacra* by an important fraction of farmers, and *Popayán* is part of *Chacra*. in the field. *Chacra* is planted together with maize in the same planting site and climbs

on the maize plant, and *Allpa* is planted between maize plants, and the *Allpa* of growth habit III partially climbs on the maize plant. The intensification approach described is so far primarily associated with food security ensuring food provision during the crop season, *Allpa* providing food early in the season and then *Chacra* providing food later in the season.

In Saraguro, *Chacra* more often called *Mixturiado* was the only common bean cultivated type. *Chacra* is also planted together with maize and climbs on the maize plant as in Cotacachi. *Popayán* as in Cotacachi is also part of the *Chacra* type; however, frequency of *Popayán* at household in Saraguro was higher than in Cotacachi, suggesting that *Popayán* complements *Chacra* production as *Allpa* does in Cotacachi. *Popayán* in Saraguro is considered by farmers more productive and more resistant to diseases than common bean.

*Chacra* and *Allpa* in Cotacachi and *Chacra* and *Popayán* in Saraguro are grown in a variety mixture approach. *Canario*, the large-seeded commercial type is highly frequent in *Chacra* variety mixtures in both, Cotacachi (Table 2) and Saraguro (Table 5). In a few cases, farmers grow only *Canario* and in these cases, *Canario* is also grown as variety mixture. These results could suggest that farmers have interest in the commercial trait of *Canario*; however, they also appreciate the high grain quality of *Canario*; and therefore, high frequency of this type in Cotacachi and Saraguro mixtures is not necessarily devoted to commerce. These results show that variety mixtures are the key cultivation strategy of common bean in Cotacachi and Saraguro.

Cultivation of *Chacra* variety mixtures in Cotacachi and Saraguro 700 km apart suggests that *Chacra* variety mixtures have been the main common bean practice in the temperate highlands of Ecuador before monoculture of *Canario*. The local knowledge that appears old and the genetically diverse traditional varieties found in Ecuador (Bonilla, 2010; Jacome, 2017; Torres, 2012), suggest that *Chacra* variety mixtures likely have been domesticated from the old local wild types found in the region (Debouck et al., 1993). This appears a domestication event different from Mesoamerica, since both domestications are considered to have occurred independently (Gepts, 1998)(Chacón S et al., 2005)

Since *Allpa* variety mixtures are less adapted to highland (Chapter 4), they appear to have been introduced from the valleys, as a strategy to improve food security, by intensifying common bean cultivation. In this intensification approach, *Chacra* and *Allpa* have co-adapted to each other creating a compensatory and synergic system (Chapter 4). In addition to productivity benefits, this intensification approach also provides food during the entire year, which is an additional contribution to food security. Due to a flexible balance among types under biotic and abiotic stresses, common bean intensification also contributes to agro ecosystem resilience. This

is also a low input system, neither synthetic fertilizer nor pesticides are used; furthermore, it has been in place for long time, showing that the cropping system is sustainable.

Seed flow is limited in Cotacachi and Saraguro, and due to low seed exchange among farmers (mostly among relatives), recombination among genotypes in the mixture appears the main genetic diversification phenomenon that explains high genetic diversity in variety mixtures (Jacome, 2017). New genetic diversity appears to be constantly exposing to the changing biotic and abiotic conditions, to which a plastic efficient response of variety mixtures is taking place. Efficient response to pest and diseases is on the other hand due to resistance operating in variety mixtures (Chapter 3), which in turn has an important mixture effect (Espinoza and Ochoa, 2012), that reduce the overall damage of the crop.

### **2.4.3 Status of variety mixtures**

In Cotacachi, phenotype components of variety mixtures are traditional populations, except for Cargabello and Toa introduced in *Allpa* and *Chacra* variety mixtures, respectively. Cargabello was released in 1987 as INIAP-404 (INIAP, 1987), and Toa was released in 1993 as INIAP-412 TOA (INIAP, 1993). INIAP Cargabello derived from a selection of a local *Rojo Moteado* type grown in the Ecuadorian valleys in the 1970s and 1980s, and whether Cargabello is traditional or improved is unknown. Cultivation (introduction) of Cargabello in variety mixtures in Cotacachi has likely been done before it was officially released. Additionally, more than one type of Cargabello was found in Cotacachi, likely derived from recombination with other *Allpa* genotypes; a phenomenon that appears common in variety mixtures in Cotacachi (Jacome, 2017). INIAP-412 TOA was on the other hand released to introduce the commercial *Rojo Moteado* type in the highlands to improve small-scale farmers income (INIAP, 1993). However, farmers did not consider this variety commercial, and they included it in the *Chacra* variety mixture to improve diversity. INIAP-412 TOA appears also to have been recombined with other *Chacra* genotypes in the mixture (Jacome, 2017).

In order to correct dysfunctional traits of traditional varieties such as late maturity, aggressiveness to maize plant, and susceptibility to diseases, genetic breeding for climbing types has also been taking place in Ecuador (INIAP, 1999). In addition of variety INIAP-412 TOA, varieties INIAP-416 Canario, INIAP-421 Bolivar and INIAP-426 Canario Siete Colinas have been released in Ecuador (INIAP, 2004, 1999, 1994). Whether these varieties are part of the *Chacra* mixtures such as INIAP-412 TOA is difficult to know due to phenotypic similarities with local *Canarios*; however, farmers do not refer to them as part of the variety mixture, as they did for INIAP-412 TOA.

Richness at household of *Chacra* variety mixtures was considerably high in all sites of Cotacachi and Saraguro, showing that farmers still maintain good levels of *Chacra* diversity in their farms. Lower richness at household of *Allpa* in Cotacachi and *Popayán* in Saraguro is likely because these types complement productivity of *Chacra* in both regions; however, some farmers also maintain high richness of these types in their farms.

Based on farmers' names, high phenotypic *Chacra* richness was identified in the community in Cotacachi and Saraguro. Fifty-five and 62 different phenotypes were identified in Cotacachi and Saraguro, respectively; however, this level of richness was an underestimation, since using SSR markers, single seed lines belonging to same phenotypes were genetically different (Torres, 2012). In this study, 154 genotypes were identified from the 55 phenotypes from Cotacachi, and 128 genotypes were identified from the 62 phenotypes from Saraguro.

High levels of diversity were also found for *Allpa* in Cotacachi and for *Popayán* in Saraguro, with 17 *Allpa* phenotypes and 13 *Popayán* phenotypes identified, respectively; values which are likely also underestimated, since at least for *Allpa*, genetic differences were also found among lines of same phenotypes (Jacome, 2017).

#### **2.4.4 Disease epidemics and farmers' knowledge**

Disease epidemics of rust in the field were closely associated with variety mixture composition. Negative association of richness but especially evenness with SDI (Fig 1) showed the importance of genetic diversity in variety mixtures stability, which was in turn associated with richness of resistance in variety mixtures, which was considerably high in Cotacachi and Saraguro (Chapter 3).

Farmers were able to differentiate rust and powdery mildew from each other, and from anthracnose and AsLS; however, farmers were not able to differentiate among anthracnose and AsLS neither in Cotacachi nor in Saraguro, which appears due to the necrotrophic nature of these diseases that produce similar symptoms. Farmers also had difficulties perceiving pathogen biology associated aspects, as disease origin, which was primarily associated with conducive climatic conditions, as rain and clouds. However, they had evidences from the daily experience in the field related with disease conducive conditions. Farmers difficulties in differentiating diseases caused by neurotropic pathogen and association of diseases with climatic conditions (mainly rain) is a common fact for common bean diseases as in Central Africa (Trutmann et al., 1996).

In Cotacachi and Saraguro only *Popayán* was considered resistant to all diseases, and since *Popayán* belongs to another species (*P. coccineus*), farmers' perception of resistance is

associated with absence of disease, since *Popayán* is either nonhost or marginal host (Niks and Marcel, 2009). The same conclusion can be applied for durability of resistance; Saraguro farmers perceived durable resistance only in *Popayán*, because in the past they have not experienced disease in *Popayán*; for the same reason, because common bean diseases do not affect *Popayán*. Therefore, scientist knowledge has to be adjusted to farmers understanding of their agro-ecosystem for successful participatory initiatives.

Farmers in the field as expected perceived what is evident and objective for their reality, as rust and powdery mildew differences, disease transmission, high levels of resistance, durability of resistance; but not what is not evident, such as disease origin or seed transmission. However, they take disease control measures as healthy seed selection and eventually spray fungicides, not for climbing common bean but for other crops (Saraguro).

#### **2.4.5 Disease control approaches**

Inter and intraspecific diversity were the main approaches farmers mentioned as useful strategies to manage pest and diseases in their fields. Interspecific diversity such as intercrops appears to be the main strategy for the evident barrier effect that is more clearly perceived by farmers and proved to be effective (Finck and Wolfe, 2006). Variety mixtures were also mentioned as the strategy to cope with pest and diseases, and they included all types *Chacra*, *Allpa* and *Canario*, suggesting that all contain resistance, and in answers were explicit intensification of *Chacra* and *Allpa* in Cotacachi. Benefits of mixtures was experimentally demonstrated by the high mixture effect to rust (Espinoza and Ochoa, 2012), and complementarily, many sources of resistance has been identified for rust in genotypes components of variety mixtures (Chapter 3).

Healthy seed selection and discarding-damaged seed are key practices to reduce damage of seed transmitted diseases as anthracnose and AsLS. This strategy, together with interspecific and intraspecific diversity are the base of disease control of common bean in the highlands of Ecuador. Strategies are similar in Central African highlands for common bean traditional cultivation, where sanitation and resistance from mixture varieties are also key strategies for disease control (Trutmann et al., 1993).

A special recommendation of old men from Cotacachi was planting and weeding during the first quarter moon phase. Using moon phases for implementing crop practices that improve crop productivity is a common and widely accepted practice in traditional farming country wide in Ecuador.

## 2.5 Conclusions

- Farmers are well acquainted with the cropping systems they manage, especially about the diversity they maintain. Their knowledge is associated with morphological traits, phenology, varietal adaptation and varietal management. This knowledge is derived from a close contact with diversity and farmers' custom, where local language is a key tool.
- Although farmers, as expected, had difficulties in perceiving pest and disease biology, they can discriminate diseases and their epidemics. Based on this, they have adapted intercrops and variety mixtures as main strategies to control pest and diseases. These practices have been efficiently operating for quite a long time, and although advantages are not evident for the intrinsic benefits of intercrops and variety mixtures, farmers maintain these practices due to the general perception of benefits.
- Farmers have their own understanding about phenomena operating in their farms, which is consistent with their agroecosystem and socioeconomic realities. For instance, grain quality of *Canario* and *Chacra* is similar for subsistence farmers but different for commercial farmers.
- Disease resistance in view of the farmers is perceived as the complete absence of disease; a perception associated with either nonhost resistance or marginal host, and in addition durability of resistance for farmers is associated with the real durable nature of nonhost resistance or the marginal host nature.
- *Chacra* variety mixtures intercropped with maize have long been adapted to the temperate highlands of Ecuador, and then *Allpa* appears to have been introduced from the valleys and co-adapted with *Chacra* improving food security in the highlands of Cotacachi. In Saraguro, *Popayán* appears also to have been introduced into *Chacra* variety mixtures with similar purposes.
- Seed flow is very limited in Cotacachi and Saraguro, and therefore, recombination of genotypes within variety mixtures appears to be the main genetic diversification phenomenon that explains the high genetic diversity found in variety mixtures.
- *Chacra*, *Allpa* and *Popayán* variety mixtures are genetically diverse populations composed of traditional genotypes and, the modern varieties introduced in the mixture have improved genetic diversity; and in addition, high evenness at household and community improves functionality of diversity.



- Disease epidemics of rust in the field was closely associated with variety mixture composition. Thus, richness and evenness were inversely associated with rust epidemics in the field, which is in turn associated with richness of resistance in the mixtures.

## Chapter 3

### Resistance to *Uromyces appendiculatus* in common bean variety mixtures in the highland of Ecuador

#### Abstract

Common bean in Ecuador is cultivated in a centre of crop diversity in an ancient agroecosystem, with high intraspecific diversity and traditional crop management strategies. In these conditions, the crop has adapted to important diseases such as rust (*Uromyces appendiculatus*), one of the most important constraints of common bean in Ecuador. To adapt the crop to biotic constraints, farmers have adopted variety mixtures as the key crop management strategy. In this study, we characterized resistance to rust operating in variety mixtures, for which 67 Cotacachi and 65 Saraguro lines were derived from variety mixtures. These lines were studied at the seedling stage with representative Ecuadorian isolates of *U. appendiculatus*, and were complementarily evaluated in field experiments in Cotacachi, Saraguro, and Gualaceo. At seedling stage, 31 different resistance genes were identified in Cotacachi and 38 were identified in Saraguro. The pathogen population has already adapted to these resistance factors and therefore they are not useful for conventional plant breeding. However, efficiency of resistance of these resistance factors varied significantly, suggesting that some of them are durable. In addition, resistance genes identified at the seedling stage significantly explained resistance in the field. Partial resistance (PR) also complementarily explained resistance in the field, especially for Cotacachi lines. Both, resistance genes and PR dynamically operating in a close relationship with *U. appendiculatus* evolution has created a high mixture effect that has reduced common bean vulnerability in the highlands of Ecuador.

#### 3.1 Introduction

Common bean (*Phaseolus vulgaris* L.) has been an important legume food crop in Ecuador for a long time (Peralta et al., 1997). The cultivated common bean belongs to Nueva Granada and Peru races (Singh et al., 1991), and a wide genetic structure of varieties ranging from bush (determinate) to climbing (indeterminate) are cultivated (Bonilla, 2010; Jacome, 2017; Torres, 2012). In addition to the diverse cultivated types, an important wild type intermediate between Mesoamerican and Andean wild types have been reported in the region (Debouck et al., 1993; Freyre et al., 1996).

Bush types are adapted to the warm lower valleys, whereas climbing types are adapted to temperate regions. Traditional varieties of both types have been cultivated along the highlands of Ecuador, until cultivation of *Rojo Moteado* (bush) and *Canario* (climbing) types were

prioritized for commercial purposes. Thus, *Rojo Moteado* improved varieties are grown in northern valleys and northwestern foothills (Subia et al., 2007), and *Canario* is grown along the temperate highlands. However, high diversity of local bush varieties are still cultivated in southern valleys (Jiménez et al., 1996), and high diversity of climbing varieties are primarily grown in Cotacachi and Saraguro, in northern and southern Ecuador, respectively (Chapter 2).

Climbing types are especially important because they are the main source of plant protein for communities of the Andean highlands, but also because *Canario* is commercialized in the local market. Climbing types are intercropped with maize in an ancient cropping system, where common bean cultivated as variety mixtures has been a key practice for food security (Chapter 2). A variety mixture is composed of diverse genotypes that are planted, harvested, cooked, and eventually commercialized as a varietal unite. Cultivation of *Canario*, a big-seeded type derived from variety mixtures has displaced variety mixture cultivation to mainly subsistence farming. Communities of Imbayas in Cotacachi and Saraguros in Saraguro in northern and southern Ecuador, respectively, are the main farmers that cultivate variety mixtures in Ecuador.

An important ecological service of variety mixtures is reducing disease epidemics, which is epidemiologically called “mixture effect” (Murray and Milgroom, 2012), and was shown to occur for Cotacachi variety mixtures (Espinoza and Ochoa, 2012). According to Wolfe (1985), the main purpose of genetic mixtures (crop variety mixtures) for pest and disease management is to slow down pest and pathogen spread. It is also clear that genetic mixtures allow a more stable management of pest and disease pressure than a monoculture (Di Falco and Chavas, 2007; Finckh, 2003; Jarvis et al., 2007; Thinlay et al., 2000; Thurston et al., 1999; Trutmann et al., 1993). In addition, most pest and disease management strategies concentrate on reducing the current or coming season’s crop loss to pest and diseases. Few crop management programs are oriented to providing options that could reduce the risk to future crop loss, i.e., reducing genetic vulnerability within the farmers’ fields. Vulnerability is intended here as the probability of crop loss due to a new biotype of pest or pathogen entering into the production system (Brown, 2008), a phenomenon more likely to occur in an area consisting of one or few varieties that share a very similar resistance structure.

Despite the recognized importance of variety mixtures, genetic improvement of common bean continues to favor monoculture. Common bean breeding in Ecuador has been mostly concentrated on bush types and many new varieties have been released in northern valleys (Peralta et al., 2009). Genetic improvement for climbing types is less active, although four commercial varieties have been released so far (INIAP, 1994, 2004, 1999, 1994, 1993). INIAP-TOA released in 1993 has been incorporated in the variety mixtures in Cotacachi (Chapter 2),

and whether the rest of varieties also have been incorporated in variety mixtures is unknown. The large-seeded Bombolin and Cargamanto, likely improved lines (not released), are grown for commercial purposes in the province of Bolivar.

Rust (*Uromyces appendiculatus*) is a global constraint of common bean cultivation (Mmbaga et al., 1996b; Pastor-Corrales, 2002), and in Ecuador is the most important biotic constraint of common bean (INIAP, 2003; Lepíz et al., 1995; Ochoa et al., 1998; Peralta et al., 2007). Rust in bush types is controlled in Ecuador with up to four fungicide sprays per cycle (Subia et al., 2007); and on climbing types farmers mostly rely on interspecific diversity (intercrops) and on intraspecific diversity (variety mixtures) (Chapter 2).

Virulence studies conducted worldwide and in Ecuador showed that *U. appendiculatus* is a very virulent pathogen (Jochua et al., 2008; Mmbaga et al., 1996b; Ochoa et al., 2007). Genetic improvement in Ecuador to develop rust resistance varieties in bush types has been very active, and most varieties released carry high levels of resistance (Peralta et al., 2009). Sources of resistance to rust for bush variety improvement have been foreign, mainly from CIAT gemplasm. Therefore, new selection pressure has been introduced to pathogen evolution in lower valleys, where modern varieties have been adopted by farmers. Due to the wind-borne nature of the pathogen, the new potentially virulent pathogen races are a threat not only for bush types in the valleys, but also for climbing types in the highlands. Therefore, resistance to rust in climbing types, from which farmers rely, is facing a new scenario, that deserve attention.

The mixture effect to rust observed for Cotacachi mixtures (Espinoza and Ochoa, 2012) is likely stable in time and space, since the cropping system has been operating with the pathogen for a long time. However, it is important to assess the degree of crop (variety mixtures) vulnerability to new races entering in the cropping systems (Brown, 2008), aspects of which are mainly approached in this study.

**Main objective of this study were:**

- a) Evaluate virulence diversity of *U. appendiculatus* in the main common bean cultivation areas of Ecuador.
- b) Using a representative *U. appendiculatus* population, study at seedling stage the resistance operating in genotypes components of variety mixtures,
- c) Assess by in farm and on experimental tests the resistance to *U. appendiculatus* in genotype components of variety mixtures.

## 3.2 Methods

### 3.2.1 Pathogen diversity

Eighty-five isolates of *U. appendiculatus* were collected in the main climbing and bush common bean areas of Ecuador. Initially, 10 samples from the northern highlands, northern valleys, southern highlands, and southern valleys were surveyed. From these surveys, low virulent races were identified in the southern highland and southern valleys, and highly virulent races were identified in northern highlands and northern valleys. To sample a more representative rust population in the northern region, additional surveys were organized to the northern highland and northern valleys. A total of 29 samples from the northern highlands, 35 samples from northern valleys, 10 samples from southern highlands, and 11 samples from southern valleys were studied. A single uredinium (pustule) isolate was derived from each of the samples surveyed. A diluted suspension of urediniospores were inoculated onto the susceptible variety Red Small Garden, and an isolated uredinium was allowed to grow by removing leaf tissue with other uredinium before sporulation. After sporulation, urediniospores from the isolated uredinium were inoculated on Red Small Garden for urediniospore multiplication.

Virulence studies were conducted at the Santa Catalina Experimental Station (INIAP), near Quito-Ecuador, following the methodology used by Jochua et al (2008). The standard differential varieties proposed by Steadman et al (2002) were used in this study. Differential varieties and the binary number used for race identification is shown in Table 1. Three seedlings of each differential variety were inoculated with single pustule isolates using a suspension of 2.5 mg of urediniospores in 30 ml of Tween 20 solution (40 µl of Tween 20 per 1 000 ml of distilled water). Seedlings were grown in a greenhouse at 25 °C and inoculated 8 days after planting at the first true leaf stage (V2) (Fernández et al., 1986). The seedlings were then transferred for 16 hours to a growth chamber for incubation at 20 °C and 100 % relative humidity. After incubation, seedlings were transferred to a growth chamber at 25 °C. Reaction Types (RT) of rust were assessed at 14 days after inoculation, and 18 days after inoculation. RTs were assessed using the 1-6 scale proposed by Stavely et al (1983)(Table 2). RT ranging from 1 to 3 was classified as resistant (incompatibility) and RT ranging from 3, 4 to 6 were classified as susceptible (compatibility). Isolates producing susceptible RT were considered to carry virulence to the resistance gene(s) carried by that specific differential. Race identification was based on the binary notation system proposed by Steadman et al (2002). In this system, authors has assigned a binary number to each differential within the Andean and Middle American gene pools (Table 1). Race identity results from the sum of binary numbers that belong to the differentials susceptible in each gene pool, and numbers are separated by a hyphen.

Table 1. Differential varieties, gene pool, resistance genes, and the binary numbers used to identify *U. appendiculatus* races

Differential number	Cultivar or line <sup>1</sup>	Gene pool	Resistance gene	Binary number <sup>1,2</sup>
1	Early Gallatin	Andean	Ur-4	1
2	Redlands Pioneer	Andean	Ur-13	2
3	Montcalm	Andean	.....	4
4	PC 50	Andean	Ur-9, Ur12	8
5	Golden Gate Wax	Andean	Ur-6	16
6	PI 260418	Andean		32
7	GN 1140	Meddle American	Ur-7	1
8	Aurora	Meddle American	Ur-3	2
9	Mex. 309	Meddle American	Ur-5	4
10	Mex. 235	Meddle American	Ur-3+	8
11	CNC	Meddle American		16
12	PI 181996	Meddle American	Ur-11	32

<sup>1</sup>Differential cultivars and binary numbers adopted in the Third International Bean Rust Workshop in South Africa in 2002 (Steadman *et al.* 2002).

<sup>2</sup>Race identity results from the sum of the binary numbers belonging to the susceptible differentials in each gene pool. Numbers of race identity are separated by a hyphen.

CNC=Compuesto Negro Chimaltenango.

Frequency of virulence (compatibility) to each differential in each region and at the country level was tabulated. RT values were converted to quantitative disease scores (QDS) as proposed by Mmbaga et al (1996a), which is a quantitative value that quantitatively corresponds to the infection type (Table 2).. Using QDS, the mean disease score (MDS), was calculated to establish the degree of virulence of isolates. ANOVA in which isolates and differentials were nested in regions was performed for MDS. ANOVA was performed using the AMMI function from Agricolae package with the R software version 3.4.

### 3.2.2 Resistant studies

#### 3.2.2.1 Experimental material

In the 2009 cropping season, household surveys were conducted in common bean areas of Cotacachi and Saraguro. In each region, 60 households were selected using a randomly stratified design (by region and village), to ensure geographic representation across the target villages within each agro-ecological region, totaling 120 households (60 households for each region). A representative seed sample of each household was analyzed together with farmers. Phenotypically similar seeds were grouped apart in different classes according to the farmer's criteria, which was primarily size, shape and color of the seed. Farmers were then asked to name each of the phenotypes sampled. Identity of phenotypes obtained at household surveys were

further analyzed with key farmers to resolve synonymies and same names for different phenotypes (Chapter 2).

**Table 2. Scale used at seedling stage to evaluate *Uromyces appendiculatus* reaction types and the quantitative disease scores (taken from Jochua, C. *et al.* 2008 )**

Reaction type <sup>1</sup>	Description	Quantitative disease score <sup>2</sup>	Rust reaction
1	Inmune, no visible symptoms	1.1	Resistant
2	Necrotic spots without sporulation	2.1	Resistant
2,3	Reaction 2 with few type 3	2.4	Resistant
3,2	Reaction 3 with few type 2	2.7	Resistant
3	Uredinia < 0.3 mm in diameter	3.1	Resistant
3,4	Reaction 3 with few type 4	3.4	Susceptible
4,3	Reaction 4 with few type 3	3.7	Susceptible
4	Uredinia 0.3 to 0.49 mm in diameter	4.1	Susceptible
4,5	Reaction 4 with few type 5	4.4	Susceptible
5,4	Reaction 5 with few type 4	4.7	Susceptible
5	Uredinia 0.5 to 0.8 mm in diameter	5.1	Susceptible
5,6	Reaction 5 with few type 6	5.4	Susceptible
6,5	Reaction 6 with few type 5	5.7	Susceptible
6	Uredinia 0.8 to 1.2 mm in diameter	6.1	Susceptible

<sup>1</sup>Evaluation at seedling stage in the greenhouse. Scale proposed by Stavelly et al (1983).

<sup>2</sup>Conversion from reaction types for statistical analysis (Mmbaga et al. 1996)

In the crop season of 2010, single seed lines were derived from each phenotype in Cumbas Conde and Cañicapa for Cotacachi and Saraguro phenotypes, respectively. Each phenotype was planted in a 4 m row in which two seeds of each phenotype were planted together with two seeds of maize every 0.8 m. Maize varieties Killu Sara and Zhima were planted to support common bean plants in Cotacachi and Saraguro, respectively. At growth plant stage V3 (Fernández et al., 1986) only one common bean plant per planting site was allowed to grow, plants were harvested individually and then at least one single seed line was derived from each phenotype. The number of lines derived per phenotype depended on the phenotypic variation among plants. All selected single seed lines were subjected to molecular genetic analysis using 10 SSR polymorphic markers (Gaitán-Solís et al., 2002). For this study, a representative collection representing the phenotypic variation identified by key farmers as well representing the genetic variation in each region was selected.

### 3.2.2.2 Seedling resistance studies

Sixty seven lines from Cotacachi and 65 lines from Saraguro were inoculated with 25 isolates from different geographic origins (Table 5). Isolates collectively carried all virulences identified in Ecuador, and represented the races of northern and southern Ecuador.

**Table 3.** Resistance identity (RI), the relative efficiency of resistance (RER), and the mean disease score (MDS) of common bean lines derived from Cotacachi variety mixtures to 25 *Uromyces appendiculatus* isolates collected in four common bean regions of Ecuador.

Line identity <sup>1</sup>	Farmers' identity <sup>2</sup>	RI <sup>3</sup>	RER <sup>4</sup>	MDS <sup>5</sup>	Line identity <sup>1</sup>	Farmers' identity <sup>2</sup>	RF <sup>3</sup>	RER <sup>4</sup>	MDS <sup>5</sup>
CF33P1	Yura lacre shimi 2	1	76	2.62	CF93P1	Crema listado allpa 5	19	20	3.79
CF16P3	TOA	2	68	3.02	CF56P4	Suku chagra poroto 1	20	12	4.33
CF113P1	Hanptzi crema listado allpa	3	40	3.44	CF94P1	Killu puka listado 2	20	12	4.38
CF17P1	Crema listado chagra 1	4	36	3.84	CF95P1	Crema chagra A2	21	12	4.61
CF15P3	Killu listado chagra 2	5	36	3.82	CF72P2	Kaka conejo	22	8	4.38
CF106P1	Hanptzi yana morado 3	6	24	4.06	CF111P1	Killu listado chagra 4	23	8	4.82
CF21P2	Crema chagra hanptzi	7	16	4.46	CF38P1	Zuni yana chagra	23	8	4.70
CF59P1	Rosado puka poroto	8	52	3.15	CF103P1	Carario grande	24	16	4.19
CF63P2	Morado chagra largo	8	52	3.20	CF97P1	Yana listado chagra	25	8	4.74
CF88P1	Hanptzi yana morado 2	8	52	3.05	CF5P1	Puka café poroto	25	8	4.04
CF60P4	Lacre chagra	9	36	3.91	CF1P1	Crema listado allpa 1	26	28	4.05
CF85P1	Crema chagra A1	9	36	4.12	CF100P1	Yana chagra	26	28	4.08
CF25P1	Yura bola leche vaca	10	28	3.68	CF19P1	Hermano capulis poroto	26	28	3.68
CF69P1	Café hanptzi poroto	11	28	4.26	CF52P1	Suku chagra poroto 1	27	20	4.23
CF15P1	Killu listado chagra 1	12	24	4.23	CF52P2	Suku chagra poroto 2	27	20	3.95
CF43P1	Killu puka listado 1	12	24	4.21	CF12P2	Bola lacre chagra poroto	27	20	4.00
CF54P4	Suku listado chagra 1	13	24	3.62	CF99P1	Chagra poroto uva	27	20	4.41
CF51P2	Morado listado chagra	13	24	4.39	CF55P1	Killu listado chagra 3	28	16	4.00
CF91P1	Crema listado allpa 4	14	24	4.57	CF49P1	Killu yana listado chagra 1	29	12	4.67
CF12P5	Crema listado allpa 2	15	20	4.26	CF29P3	Hanptzi yana morado 1	30	8	4.52
CF89P1	Crema listado chagra 2	15	20	4.41	CF64P1	Crema listado allpa 3	30	8	4.42
CF35P1	Suku listado leche vaca 1	16	20	4.50	CF13P2	Chagra listado bola crema	30	8	4.34
CF86P1	Morado zuku allpa	16	20	4.07	CF105P1	Suku listado leche vaca 2	31	8	4.52
CF28P2	Yura lacre shimi 1	17	20	4.32	CF55P3	Killu yana listado chagra 2	31	8	4.98
CF56P1	Suku listado chagra 2	18	20	4.23	CF61P1	Canario pequeño	(-)	0	4.93

<sup>1</sup>Genetically different single seed lines using SSR markers (Torres, 2012).

<sup>2</sup>Farmers' names established at key farmers meeting (second chapter).

<sup>3</sup>Lines differing among each other in at least one resistance reaction type according to Table 2 was considered to carry a different resistance gene.

<sup>4</sup>Percentage (%) of the 25 *U. appendiculatus* isolates to which a line is resistant according to resistance reaction types of Table 2.

<sup>5</sup>Mean of the quantitative disease scores (QDS) of the 25 *U. appendiculatus* isolates.

Pearson coefficient of correlation between RER and MDS: -0.89\*\*\*

Seedling management, inoculation, and evaluation were done using the methodology used for pathogen diversity studies. Resistance RTs were also discerned using the criteria proposed by Stevely et al 1983 (Table 2). Using resistance RT information, resistance genes were identified in lines studied. A resistance gene was discerned if a line differed from another in at least one resistance RT. In this study, RTs were also converted to QDS (Mmbaga et al., 1996b), and MDS was also calculated, to measure the degree of resistance of Cotacachi and Saraguro lines. Complementarily to MDS, the relative efficiency of resistance (RER), tabulated as the percentage of isolates to which the line is resistant was also included as a resistance parameter.

For statistical analysis, lines from Cotacachi and Saraguro were grouped in lines origin and isolates were grouped in four regions: northern highland, northern valley, southern highland and southern valley isolates. ANOVA was performed for MDS, in which lines were nested in lines origin, and isolates were nested in isolates region. ANOVA was performed using the AMMI function from Agricolae package. Pearson correlation coefficient between RER and MDS were



also calculated. ANOVA and the Pearson correlation test were conducted utilizing R software version 3.4.

Table 4. Resistance identity (RI), the relative efficiency of resistance (RER), and the mean disease score (MDS) of common bean lines derived from Saraguro variety mixtures to 25 *Uromyces appendiculatus* isolates collected in four common bean regions of Ecuador.

Line identity <sup>1</sup>	Farmers' identity <sup>2</sup>	RI <sup>3</sup>	RER <sup>4</sup>	MDS <sup>5</sup>	Line identity <sup>1</sup>	Farmers' identity <sup>2</sup>	RF <sup>3</sup>	RER <sup>4</sup>	MDS <sup>5</sup>
SF17P1	Pequeño negrito	1	92	2.29	SF74P1	Plomito 1	23	20	4.14
SF82P1	Blanco largo 1	2	84	2.41	SF82P2	Canario C1	24	12	4.15
SF108P1	Blanco largo 2	2	84	2.46	SF106P1	Shanito B	25	12	4.14
SF67P3	Chavelito A1	3	72	2.86	SF22P1	Blanco molongo rayado	26	12	4.12
SF70P1	Chavelito A2	3	72	2.87	SF103P3	Chacra morado 4	27	12	4.24
SF59P1	Chavelito B	4	56	3.30	SF65P2A	Pequeño alargado B	28	28	4.06
SF33P1	Chacra rayado 1	4	56	3.33	SF55P1	Canario pequeño	28	24	4.12
SF92P1	Chacra rayado 2	4	56	3.37	SF36P2	Suco 2	29	20	4.16
SF65P2B	Rayado grande B1	5	56	3.42	SF29P2	Suco 1	29	20	3.91
SF85P2	Canario B	6	52	3.55	SF97P1	Pequeño negrito	30	16	4.14
SF110P1	Conejo	12	48	3.16	SF48P1	Shanito pequeño A2	30	16	3.86
SF54P1	Rayado pequeño A	13	48	3.43	Shanito	Shanito rayado 1	30	16	4.30
SF34P1B	Shanito A	14	44	3.49	SF102P3	Shanito rayado pequeño 3	31	16	4.45
SF44P3	Rayado pequeño B	16	44	3.36	SF32P1	Shanito rayado pequeño 1	31	16	3.93
SF7P2	Sangre de cuy A	7	40	3.62	SF90P1	Bolongo 3	32	16	4.05
SF42P2A	Chacra morado 1	8	40	3.68	SF21P1	Shanito rayado 2	32	16	3.87
SF86P1	Chacra morado 3	15	40	3.63	SF9P1	Canario A	33	16	4.09
SF50P1	Rayado grande A2	17	40	3.82	SF81P1	Bolongo 2	34	16	4.20
SF19P2	Shanito pequeño B	18	40	3.40	SF115P1	Rayado grande B2	35	16	4.43
SF119P1	Plomito 2	9	36	3.85	SF118P1	Negro chacra 2	36	16	3.98
SF47P1	Chacra morado 2	10	36	3.53	SF103P1	Pequeño alargado C	36	12	4.66
SF28P1	Bolongo 1	11	16	4.27	SF23P1	Shanito pequeño A1	37	8	4.68
SF116P1	Rayado pequeño C	19	36	3.56	SF60P1	Shanito rayado pequeño 2	37	8	4.58
SF15P1	Negro chacra 1	20	36	3.89	SF77P1	Pequeño alargado A	38	8	4.72
SF31P1	Rayado grande A1	20	32	3.82	SF35P3	Shanito C1	38	8	4.60
SF114P1	Canario C3	21	32	3.92	SF98P1	Shanito C2	38	4	4.60
SF86P2	Canario C2	22	24	3.84	SF69P2	Canario	(-)	0	4.82

<sup>1</sup>Genetically different single seed lines using SSR markers (Torres, 2012).

<sup>2</sup>Farmers' names established in key farmers meeting (second chapter).

<sup>3</sup>Lines differing among each other in at least one resistance reaction type according to Table 2 was considered to carry a different resistance gene.

<sup>4</sup>Percentage (%) of the 25 *U. appendiculatus* isolates to which a line is resistant according to resistance reaction types of Table 2.

<sup>5</sup>Mean of the quantitative disease scores (QDS) of the 25 *U. appendiculatus* isolates.

Pearson coefficient of correlation between RER and MDS: -0.95\*\*\*

### 3.2.2.3 Field resistance studies

Forty-one lines from Cotacachi (Table 6) and 38 lines from Saraguro (Table 7) were evaluated to rust on farm and experimental station conditions. As in seedling studies, lines were selected to include most farmers' phenotypes and represented the genetic diversity of Cotacachi and Saraguro. Most lines evaluated in this study were also evaluated at the seedling stage. Three on-farm experiments were carried out in each Cotacachi and Saraguro regions, and three on-experimental station experiments were carried out in Gualaceo-Azuay. In Cotacachi, experiments were carried out in Cumbas Conde in 2013, 2014, and 2015. In Saraguro, experiments were carried out in Cañicapa in 2013 and 2014, and in Ensilada in 2013. In Gualaceo, experiments were carried out in the Austro Experimental Station (INIAP) in 2013, 2014 and 2016.

In Cotacachi and Saraguro, lines were evaluated in one row plots of 5 m long spaced by 0.8 m. In Gualaceo, lines were planted in three row plots, 5 m long, with a row spacing of 0.8 m. Two seeds of common bean and two seeds of maize were planted together in the same planting site, and planting sites were separated each other in the row by 0.8 m. In Cotacachi, the local variety

Killu Sara, and in Saraguro and Bullcay, the local variety Zhima were planted to support common bean plants.

**Table 5. Race identity, frequency of virulence, and mean disease scores (MDS) of 25 representative *Uromyces appendiculatus* isolates from four common bean regions of Ecuador evaluated on the 12 rust differential varieties, 67 lines from Cotacachi, and 65 lines from Saraguro.**

No	Site	Region	Race <sup>1</sup>	Frequency of virulence (%) <sup>2</sup>			Mean Disease Scores <sup>3</sup>		
				Differential varieties	Cotacachi lines	Saraguro lines	Differential varieties	Cotacachi lines	Saraguro lines
1	Gapsol-Chunchi	Southern highlands	37-0	25.0	20	38.9	2.23	2.54	3.00
2	Bola Oro-Chillanes	Southern highlands	37-0	25.0	48	57.4	2.23	3.20	3.52
3	Cañicapa 3-Saraguro	Southern highlands	45-0	33.3	40	42.6	2.59	3.30	3.19
4	Bulcay 1-Gualaceo	Southern valleys	45-0	33.3	46	64.8	2.92	3.37	3.52
5	Bulcay 2-Gualaceo	Southern valleys	44-0	25.0	44	70.4	2.44	3.31	3.77
6	Papaya 1-Saraguro	Southern valleys	44-0	25.0	62	42.6	2.80	4.03	3.45
7	Papaya 2-Saraguro	Southern valleys	62-0	41.7	54	48.1	2.83	3.70	3.57
8	Urcuqui 2-Ibarra	Northern highlands	4-0	8.3	76	44.4	2.38	4.21	3.71
9	Cumbas Conde 5-Cotacachi	Northern highlands	12-0	16.7	76	68.5	2.07	4.56	3.76
10	Cumbas Conde 12-Cotacachi	Northern highlands	13-0	25.0	88	42.6	2.64	4.64	3.43
11	Cumbas Conde 6-Cotacachi	Northern highlands	44-0	25.0	72	70.4	2.31	3.91	4.07
12	Cumbas 9-Cotacachi	Northern highlands	44-0	25.0	92	77.8	2.17	4.63	4.10
13	Morales Chupa-Cotacachi	Northern highlands	45-1	41.7	84	66.7	3.21	4.61	3.95
14	Morochos 3-Cotacachi	Northern highlands	45-1	41.7	84	68.5	2.82	4.62	3.93
15	Tumbaco 1-Quito	Northern valleys	47-0	41.7	88	70.4	2.69	4.25	3.62
16	Tumbaco 4-Quito	Northern valleys	45-1	41.7	74	75.9	3.12	3.95	3.78
17	Chalguayacu 1-Pimampiro	Northern valleys	45-0	33.3	94	68.5	3.21	4.84	4.05
18	Chalguayacu 2-Pimampiro	Northern valleys	45-1	41.7	90	72.2	2.77	4.67	4.05
19	Intag 6-Intag	Northern valleys	6-63	66.7	96	77.8	3.87	4.41	4.05
20	Chalguayacu 3-Pimampiro	Northern valleys	30-55	75.0	96	85.2	4.03	4.31	3.96
21	Intag 3-Intag	Northern valleys	62-62	83.3	92	77.8	3.98	3.92	3.66
22	Intag 1-Intag	Northern valleys	31-55	83.3	96	94.4	3.69	4.27	3.91
23	Yachay 1-Urcuqui	Northern valleys	30-63	83.3	100	94.4	4.17	4.97	5.18
24	Chaguayacu 5-Pimampiro	Northern valleys	62-63	91.7	96	90.7	4.53	4.43	4.32
25	Yachay 2-Urcuqui	Northern valleys	31-63	91.7	96	92.6	4.09	4.42	4.40

<sup>1</sup>Race identity: sum of the binary numbers belonging to the susceptible differentials in each Andean and Middle American gene pool, according to Table 1.

<sup>2</sup>Frequency of isolates producing susceptible reaction types according to scale of Table 2

Pearson coefficient of correlation: Differentials x Cotacachi lines 0.58\*\*, Differentials x Saraguro lines 0.77\*\*\*, Cotacachi x Saraguro lines 0.70\*\*\*.

<sup>3</sup>Mean of the quantitative disease scores (QDS) according to scale of Table 2

Pearson coefficient of correlation: Differentials x Cotacachi lines 0.38\*, Differentials x Saraguro lines 0.59\*\*, Cotacachi x Saraguro lines 0.70\*\*\*.

In all studies, lines were exposed to the local population of common bean rust. To insure a uniform rust epidemic, only in Bulcay (experimental station), a row of the susceptible local variety Canario was planted transversal to the experimental plots. Rust epidemic development was monitored during the crop season, and the evaluation initiated when the epidemic was uniform. Evaluations were performed every 2 to 3 weeks in four plants (replications) randomly selected along the row.

Disease severity (DS) was evaluated using the Cobb modified scale proposed by James (1972), and adjusted to common bean rust by Staveland (1983). This scale varied from 0-100%, 0% meaning absence of disease symptoms and 100% complete coverage of foliar area. Due to overlapping with other pest and diseases the most accurate DS evaluation was selected for statistical analysis.

For statistical analysis, Cotacachi, Saraguro, and Gualaceo regions were treated as experimental regions, each experiment within regions was treated as an experimental site, and lines from Cotacachi and Saraguro were treated as experimental lines. DS data was submitted to a double nested ANOVA with data transformed using Arcsine. In the ANOVA, replications and experimental lines were nested within experimental sites, and experimental sites were nested

within experimental regions. Person coefficient of correlation was calculated for DS among experimental regions, and for DS between experimental regions with RER and MDS.

**Table 6. Disease Severity in the field of lines derived from Cotacachi variety mixtures in three experimental regions.**

Line		Experimental regions <sup>3</sup>			Line		Experimental regions <sup>3</sup>		
identity <sup>1</sup>	Farmer identity <sup>2</sup>	Cotacachi	Saraguro	Bulcay	identity <sup>1</sup>	Farmer identity <sup>2</sup>	Cotacachi	Saraguro	Bulcay
CF7P1	Yana chacra	0.3	0.3	0.5	CF73P1	Hanptzi yana morado	11.7	19.2	36.9
CF100P1	Yana chagra	0.7	0.5	2.8	CF61P1	Canario pequeño	15.2	13.2	3.5
CF105P1	Suku kistado leche vaca	1.0	0.7	5.5	CF56P1	Suku listado chagra	16.7	26.7	16.9
CF86F1	Morado zuku allpa	1.0	4.9	22.9	CF108P1	Not defined	16.8	30.0	11.3
CF15P3	Killu yana listado chacra	1.1	0.5	11.8	CF58P1	Canario grande	17.0	29.6	43.8
CF59P1	Rosado puka poroto	1.3	0.3	1.0	CF97P1	Yana listado chagra	19.6	29.6	17.5
CF16P3	TOA	1.7	0.9	1.4	CF104 P3	Puka café poroto	20.0	41.7	31.7
CF63P2	Morado chagra largo	2.3	0.3	0.8	CF109P1	Suku listado chagra	23.8	10.7	21.2
CF1P1	Crema listado allpa	3.0	1.7	1.6	CF93P1	Crema listado allpa	23.8	30.4	29.5
CF19P1	Hermano capulis poroto	3.0	0.3	1.5	CF5P1	Puka café poroto	24.9	37.9	14.1
CF88P1	Hanptzi yana morado	3.0	1.1	16.9	CF55P1	Killu listado chagra	26.7	17.2	4.8
CF51P2	Morado listado chagra	3.0	0.7	12.0	CF15P2	Killu puka listado	26.7	24.2	39.5
CF69P1	Café hanptzi poroto	3.2	0.5	10.8	CF17P1	Crema oscuro listado chagra	27.5	13.8	26.7
CF25P1	Yura bola leche vaca	3.8	0.3	1.3	CF89P1	Crema listado chagra	27.9	42.1	30.4
CF35P1	Suku listado leche vaca	3.9	0.3	8.0	CF64P1	crema listado allpa	29.2	17.5	25.3
CF15P1	Killu listado chagra	4.0	0.3	1.6	CF24P2	Suku listado chagra	35.9	41.3	31.2
CF54P1	Suku listado chagra	5.3	28.3	21.3	CF29P3	Hnptzi yana morado	36.7	27.9	34.9
CF111P1	Killu listado chagra	6.0	21.1	20.3	CF56P4	Suku chagra poroto	40.0	50.4	51.3
CF52P2	Suku chagra poroto	6.1	0.3	12.0	CF28P2	Yura lacre shimi	50.4	30.8	36.7
CF91P1	Crema listado allpa	6.2	0.6	12.6	CF13P2	Chagra listado bola crema	54.6	47.9	36.6
CF85P1	Crema chagra A	7.8	0.3	1.1					

<sup>1</sup>Genetically different single seed lines using SSR markers (Torres, 2012).

<sup>2</sup>Farmers' name established at key farmers meetings (second chapter).

<sup>3</sup>DS evaluated using the Cobb modified scale (Stavely, 1983). Average from three experiments in each region  
Pearson coefficient of correlation among regions: Cot x Sar 0.81\*\*\*, Cot x Bul 0.73 \*\*\*, Sar x Bul 0.78\*\*\*.

### 3.3 Results

#### 3.3.1 Pathogen diversity studies

Using the binary notation proposed by Steadman et al (2002), 35 races out of the 85 isolates sampled were identified, with a rate of 1 race per 2.4 isolates. Southern races were of low virulent in the highlands for climbing types as well as in the valleys for bush types, and race 4-0 is the lowest virulent and might be the ancestor of pathogen evolution in the south. In the north, races from the highlands and valleys were very different, highland races of climbing types were virulent on the Andean differentials and on the differential CN 1140 of meddle America, and valley races of bush types were virulent all Andean and Meddle American differentials. Races 4-0 and 8-0 are the lowest virulent and appears to be the ancestors of pathogen evolution in the north, and races 45-0, 45-1, and 63-1 were common among highlands and valleys.

Frequency of virulence to each differential in the four regions studied is shown in Table 8. Virulence to the Andean differentials Montcalm, PC 50, PI 260418 and Early Gallatin was very frequent, whereas virulence to Redland Pioneer and Golden Gate Wax was infrequent in all regions, except in the Northern valleys where virulence to all differentials was very frequent. Virulence to the Meddle American differential GN 1140 was very frequent in Northern highlands and Northern valleys. Virulence to the rest of Meddle American differentials were very frequent (over 50%) only in Northern valleys, except for Mex. 235, to which virulence was low frequent.

**Table 7. Disease Severity in the field of lines derived from Saraguro variety mixtures in three experimental regions.**

Line identity <sup>1</sup>	Farmer identity <sup>2</sup>	Experimental regions <sup>3</sup>			Line identity <sup>1</sup>	Farmer identity <sup>2</sup>	Experimental regions <sup>3</sup>		
		Cotacachi	Saraguro	Bulcay			Cotacachi	Saraguro	Bulcay
SF15P1	Negro chacra	1.0	19.0	0.5	SF67P3	Chavelito A	20.0	39.6	30.8
SF42P2	Chacra morado	1.0	11.2	12.9	SCF68P1	Canario C	20.0	20.5	26.6
SF119P1	Plomito	1.0	25.8	27.1	SF87P1	Pequeño alargado B	20.0	31.7	38.8
SF14P3	Not defined	1.0	3.3	1.0	SF28P1	Bolongo	20.0	37.5	40.0
SF50P1	Rayado grande A	2.5	0.3	0.7	SF103P1	Pequeño alargado A	20.5	16.2	22.8
SF82P1	Blanco largo	2.5	0.3	0.5	SF90P1	Bolongo	22.5	25.0	8.5
SF74P1	Plomito	3.0	28.0	37.9	SF81P1	Bolongo	22.5	34.5	10.0
SF30P1	Shanito pequeño B	3.0	27.0	31.1	SF31P1	Rayado grande A	22.5	16.7	4.9
SF22P1	Not defined	5.5	17.4	4.8	SF64P1	Canario C	22.5	22.8	25.9
SF47P1	Chacra morado	5.5	33.3	37.9	SF112P3	Rojito B	22.5	25.8	36.1
SF92P1	Rayado grande B	5.5	24.6	36.3	SF35P3	Chacra rayado	25.3	34.2	7.6
SF2P1	Shanito B	6.5	33.3	26.7	SF118P1	Negro chacra	25.5	17.0	6.3
SF19P2	shanito pequeño A	7.5	32.5	21.4	SF100P1	Chacra morado	27.5	40.3	46.7
SF23P1	Shanito pequeño A	15.0	38.3	39.3	SF36P2	Suco	28.8	41.7	33.8
SF32P1	Rojito A	17.5	23.3	27.8	SF9P1	Canario C	31.3	27.5	34.2
SF98P1	Shanito A	17.5	30.0	29.4	SF106P1	Shanito A	32.5	24.7	21.3
SF7P2	Sangre de cuy A	17.5	35.7	38.5	SF97P1	Pequeño negro	32.5	30.8	33.3
SF58P1	Canario A	17.5	34.6	32.7	SF115P1	Rayado grande A	32.5	15.1	39.0
SF33P1	Chagra rayado	18.8	25.0	26.7	SF75P1	Bolongo	40.0	48.3	34.6

<sup>1</sup>Genetically different single seed lines using SSR markers (Torres, 2012).

<sup>2</sup>Farmers' name established in key farmers meetings (second chapter).

<sup>3</sup>DS assessed using the Cobb modified scale (Stavely, 1983). Average from three experiments in each region. Pearson coefficient of correlation among regions: Cot x Sar 0.45\*, Cot x Bul 0.33 ns, Sar x Bul 0.67\*\*\*.

Virulence to all differentials from the Andes and Meddle America were collectively found in Ecuador (Table 8). Northern highland races carried virulence to all Andean differentials, and they also carried virulence to the Meddle American differential GN 1140. Northern valley races had on the other hand virulence to all Andean and Meddle American differentials, aspect for which northern highlands and northern valley races were very different, only having in common races 45-0, 45-1 and 63-1. In the southern highlands on the other hand, races were only virulent to the Andean differentials, except Redlands Pioneer and Golden Gate Wax. In southern valleys, races were also low virulent, although collectively carried virulence to all Andean differentials. ANOVA for MDS for the Andean and Meddle American differential sets (Table 9) shows statistical differences among differentials, among regions and the interaction regions x differentials, which confirms the great diversity of races and race differences among regions.

Three main different virulence regions were identified in this study: 1) the northern highland region, which has virulence to all Andean differentials, and also to the GN 1140 Meddle American differential; 2) the northern valleys region, which has virulence to all Andean and Meddle American differentials; and 3) the southern region (highland and valleys), which has virulence to the Andean differentials. Representative isolates (races) from these regions are shown in Table 5.

**Table 8. Frequency of virulence to Andean and Mesoamerican differential varieties developed by 85 *Uromyces appendiculatus* isolates collected in northern and southern Ecuador.**

Differential number	Cultivar or line <sup>1</sup>	Gene pool	Frequency of virulence (%) <sup>2</sup>			
			Northern highlands	Northern valleys	Southern highlands	Southern valleys
1	Early Gallatin	Andean	64	74	60	73
2	Redlands Pioneer	Andean	11	66	0	9
3	Montcalm	Andean	93	100	100	100
4	PC 50	Andean	86	94	50	82
5	Golden Gate Wax	Andean	11	66	0	9
6	PI 260418	Andean	54	66	60	100
7	GN 1140	Meddle American	50	77	0	0
8	Aurora	Meddle American	0	51	0	0
9	Mex. 309	Meddle American	0	66	0	0
10	Mex. 235	Meddle American	0	26	0	0
11	CNC	Meddle American	0	57	0	0
12	PI 181996	Meddle American	0	40	0	0

<sup>1</sup>Differential cultivars adopted in the Third International Bean Rust Workshop in South Africa in 2002 (Steadman et al.2002).

<sup>2</sup>Frequency of isolates producing susceptible reaction types according to the scale of Table 2.

### 3.3.2 Resistance studies

#### 3.3.2.1 Seedling resistance studies

Using the 25 representative rust isolates from Ecuador (Table 5), resistance of the 67 lines from Cotacachi and 65 lines from Saraguro were studied. On the basis of resistance RT differing in at least one isolate, 31 different resistance genes were identified in the lines from Cotacachi (Table 3) and 38 resistance genes were identified in the lines from Saraguro (Table 4). Seventeen lines from Cotacachi and 11 lines from Saraguro (not shown in Tables) were susceptible to all isolates. Additionally, the resistance TR pattern of the 25 isolates with Cotacachi and Saraguro lines were different, implying that resistance genes identified in Cotacachi and Saraguro lines were different.

In Cotacachi, resistance genes 8, 9, 10, 11, 12, 14, 15, 19, 22, 24, 25, 26, 29 and 30 were identified in at least two lines (Table 3). Only the resistance gene 26 was carried by lines with the same phenotypic identity, and the rest of resistance genes were carried by lines with different phenotypic identities. In Saraguro, resistance genes 2, 3, 4, 20, 28, 29, 30, 31, 32, 36, 37 and 38 were identified in at least two lines (Table 4), from which resistance genes 2, 3, 4, 29 and 31 were found in lines with the same phenotypic identity, while resistance genes 20, 28, 30, 32, 36, 37 and 38 were carried by lines with different phenotypic identity.

In this study, Pearson correlation coefficient between RER with MDS was negative and statistically significant for Cotacachi (-0,89 \*\*\*) and Saraguro (-0.95\*\*\*) lines, showing that

expression of both, RER and MDS are closely associated and quantify similarly degree of resistance in the lines studied, and both indices are valid for the analysis. None of Cotacachi lines were resistant to all isolates, and RER values varied from 76 to 0%, while MDS values varied from 2.62 to 4.98 (Table 3). Similarly, none of the Saraguro lines were resistant to all isolates, and RER values varied from 92 to 0 %, and MDS values varied from 2.3 to 4.8 (Table 4).

**Tabl 9. ANOVA of the mean disease scores (MDS) of 85 *Uromyces appendiculatus* isolates collected in four common bean regions of Ecuador.**

	Df	Sum Sq	Mean Sq	F value	P > F <sup>1</sup>
<b>Andean origin differentials</b>					
Regions <sup>1</sup>	3	52.89	17.63	8.6294	0.000 ***
Isolates	81	165.48	2.043	2.0932	0.000 ***
Differentials	5	349.91	69.982	71.7022	0.000 ***
Regions x Differentials	15	60.82	4.055	4.1547	0.000 ***
Error	405	395.28	0.976		
<b>Meddle American origin differentials</b>					
Regions	3	263.21	87.736	21.3939	0.000 ***
Isolates	81	332.18	4.101	6.3585	0.000 ***
Differentials	5	102.94	20.587	31.9202	0.000 ***
Regions x Differentials	15	40.94	2.729	4.2319	0.000 ***
Error	405	261.21	0.645		

<sup>1</sup>\*\*\* = significant at  $P \leq 0.001$

<sup>2</sup>Northern highlands, northern valleys, southern highlands and southern valleys

ANOVA for MDS (Table 10) shows statistical differences for origin of lines, lines, region of isolates, and the interaction origin of lines x origin of isolates, which shows the high diversity of resistance of lines evaluated, and high virulence of the pathogen. Interactions between origin of lines x origin of isolates is due to differential response of resistance of lines grouped within origins with isolates grouped also within origins.

Cotacachi lines had slightly higher RERs than Saraguro lines to southern highlands and southern valley isolates, while Saraguro lines had higher RERs than Cotacachi lines to northern highland, and northern valley isolates (Table 11), which shows resistance specificity, being Cotacachi lines more resistant to southern isolates and Saraguro lines more resistant to northern isolates. An important epidemiological aspect to consider in addition to RER, is the diverse interactions observed between resistance genes and isolates for Cotacachi lines, as well as for Saraguro lines (not shown in the tables).

In regards to virulence diversity of the 25 isolates, all produced different virulence patterns for Cotacachi and Saraguro resistance genes, except isolates 15 and 16 from Morales Chupa and

Morochos that showed similar virulence patterns to all lines from Cotacachi and Saraguro (not shown in Tables). Therefore, isolates that belonged to the same race, as 1 and 2 (race 37-0), 3 and 4 (race 45-0), 5 and 6 (race 44-0), and 11 and 12 (race 44-0) (Table 5) using resistance of the standard differential varieties were in fact different when using resistance of Cotacachi and Saraguro lines.

**Table 10. ANOVA of mean disease scores (MDS) of Cotacachi and Saraguro lines assessed to 25 *Uromyces appendiculatus* isolates from four common bean regions**

Sources	Df	MS	F value	P>F <sup>1</sup>
Lines origin (Cot and Sar)	1	40.318	5.6746	0.019*
Lines	101	7.105	5.5218	0.000***
Isolates region <sup>2</sup>	2	182.095	141.5177	0.000***
Lines origin x isolates region	2	23.821	18.5125	0.000***
Error	2445	1.287		

<sup>1</sup> \* = significant at  $P \leq 0.05$ , \*\*\* = significant at  $P \leq 0.001$ .

<sup>2</sup> Northern highlands, northern valleys, southern highlands and southern valleys.

### 3.3.2.2 Field resistance studies

ANOVA for DS of field studies is shown in Table 12. Statistical differences were not found among experimental regions, showing that the average resistance of all lines from Cotacachi and Saraguro were similar in the three experimental regions: Cotacachi, Saraguro, and Bulcay. Statistical differences were on the other hand found for origin of the lines (Cotacachi and Saraguro), and for the interaction of experimental regions x origin of the lines. In addition, the average DS of Cotacachi lines was lower than the average DS of Saraguro lines, especially in Saraguro and Bulcay, where differences were significant (Table 13).

**Table 11. Relative efficiency of resistance (RER) (%) of Cotacachi and Saraguro lines to 25 *Uromyces appendiculatus* isolates collected in four regions of Ecuador**

Lines from	Origin of <i>U. appendiculatus</i> isolates			
	Northern highlands	Northern valleys	Southern highlands	Southern valleys
Cotacachi <sup>1</sup>	18.3	7.4	64	48.5
Saraguro <sup>2</sup>	37.3	18.2	53.7	43.5

PER: percentage of isolates to which the line was resistant according to Table 2.

<sup>1</sup> Average of 50 lines.

<sup>2</sup> Average of 54 lines.

DS values in the three experimental regions for Cotacachi lines is shown in Table 6. Lines CF7P1 and CF100P1 were highly resistant in the three experimental regions. Lines CF105P1 and CF86P1 were also highly resistant in Cotacachi, an important fraction of lines were highly resistant in Saraguro and another important fraction of lines were highly resistant in Bulcay. Most lines

scored from very low DS values to significantly high DS values in the susceptible lines CF29P3, CF56P4, F28P2 and CF13P2.

**Table 12. ANOVA of rust DS of Cotacachi and Saraguro lines evaluated in the field in three experimental regions (Cotacachi, Saraguro and Bulcay)**

	Df	MS	F value	P>F <sup>1</sup>
<b>Origin of lines nested in experimental regions</b>				
Experimental regions (Cot, Sar and Bul) <sup>2</sup>	2	0.559	2.429	0.106 ns
Error A	29	0.2301		
Origin of lines (Cotacachi and Saraguro)	1	7.647	119.785	0.000***
Experimental regions x origin of lines	2	0.56	8.764	0.000 ***
Error B	2729	0.064		
<b>Cotacachi lines nested in experimental regions</b>				
Experimental regions (Cot, Sar and Bul) <sup>2</sup>	2	0.594	7.31	0.002**
Lines	40	1.71	54.25	0.000***
Lines x experimental regions	80	0.12	3.82	0.000***
Error	1352	0.031		
<b>Saraguro lines nested in experimental regions</b>				
Experimental regions (Cot, Sar and Bul) <sup>2</sup>	2	2.01	4.71	0.018*
Lines	38	0.81	22.88	0.000***
Lines x experimental regions	76	0.16	4.65	0.000***
Error	1113	0.035		

<sup>1</sup> ns = not significant, \* = significant at  $P \leq 0.05$ , \*\* significant at  $P \leq 0.01$ , \*\*\* = significant at  $P \leq 0.001$

<sup>2</sup>Data obtained from three experiments

DS values in the three experimental regions for Saraguro lines is shown in Table 7. None of Saraguro lines were highly resistant in all regions. Lines CF15P1, CF42P2, CF119P1 and 14P3 were highly resistant in Cotacachi, lines CF50P1 and CF82P1 were highly resistant in Saraguro, and lines CF15P1, CF82P1, CF14P3 and CF50P1 were highly resistant in Bulcay. Most lines scored moderate to high DS values in the three regions.

**Table 13. Average of Disease Severity (DS) of Cotacachi and Saraguro lines evaluated in three experimental regions**

Origin of lines	Disease Severity at field <sup>1</sup>			Global average
	Cotacachi	Saraguro	Gualaceo	
Cotacachi <sup>2</sup>	14.9	15.8 a	17.3 a	16.1 a
Saraguro <sup>3</sup>	16.8	26.1 b	24.6 b	23.4 b

<sup>1</sup>Average from three experiments in each region.

<sup>2</sup>Forty one lines were included in the test.

<sup>3</sup>Thirty eight lines were included in the test.

Pearson correlation coefficients of DS (field) with RER and MDS (greenhouse) for Cotacachi lines were significant, with intermediate values for all regions evaluated, except for Cotacachi with MDS (Table 14), showing that field resistance is explained to a certain extent by the contribution of resistance genes identified at seedling stage. On the other hand, for Saraguro lines, Pearson coefficient of correlation was only significant between DS (field) in Cotacachi with RER (greenhouse) (Table 14), and therefore resistance genes of Saraguro lines are only contributing



with field resistance in Cotacachi, and the pathogen population of Saraguro and Bulcay have already developed virulence to most resistance factors of Saraguro lines.

**Table 14. Pearson coefficient of correlation between disease severity (DS) in the field in three regions with the relative efficiency of resistance (RER) and mean disease scores (MDS) at the green house for Cotacachi and Saraguro lines.**

DS at	Cotacachi lines <sup>1</sup>		DS at	Saraguro lines <sup>2</sup>	
	RER	MDS		RER	MDS
Cotacachi	-0.45*	0.33 ns	Cotacachi	-0.51*	0.41 ns
Saraguro	-0.61***	0.47*	Saraguro	-0.32 ns	0.29 ns
Bulcay	-0.52***	0.46*	Bulcay	-0.28 ns	0.27 ns

<sup>1</sup>Forty four lines were included in the test.

<sup>2</sup>Thirty three lines were included in the test.

Pearson correlation coefficients of DS among experimental regions for Cotacachi lines were positive and significant for all correlations (Table 6), showing that expression of resistance in Cotacachi lines in the field had similar patterns in the three regions. The correlation coefficient of DS among experimental regions for Saraguro lines was on the other hand positive and significant between Saraguro and Cotacachi, and between Bulcay and Saraguro regions (Table 7), showing that among these regions, pattern of resistance reaction was similar. Correlation values were in general higher for Cotacachi lines than for Saraguro lines.

### 3.4 Discussion

#### 3.4.1 Pathogen diversity studies

Utilizing differential varieties proposed by Steadman et al (2002), the population of *U. appendiculatus* was considerably diverse, with 35 races identified with a rate of 1 race per 2.4 isolates. However, the pathogen population was even more diverse when isolates were further discerned with Cotacachi and Saraguro lines. Similar diverse pathogen population was observed in Ecuador using differential cultivars proposed by Stavely et al (1983), and also isolates were further discerned using local bush varieties (Ochoa et al., 2007). Similarly high *U. appendiculatus* diversity has been reported worldwide in countries where common bean rust is an important disease (Alexander et al., 1985; Araya et al., 2004; Arunga et al., 2012; Jochua et al., 2008; Mmbaga et al., 1996b; Nyang et al., 2016; Stavely, 1984; Stavely et al., 1989).

Since Ecuador is part of the Andean gene pool, *U. appendiculatus* races are expected to carry virulence primarily to the Andean differential cultivars, since Andean genes are the main selection pressure for the pathogen to develop virulence, which is the case of southern highlands and southern valleys isolates, which carry virulence only to genes carried by Andean

differential varieties. Similar results were found by Sandlin et al. (1999), who found that Andean isolates were virulent on Andean land races and non on Middle American land races. However, in this study, virulence to Meddle American differential cultivars was found in northern valleys, which appears associated with introduction to cultivation varieties carrying Meddle American resistance genes, likely in the modern varieties resistance to *U. appendiculatus* released for the lower valleys (Peralta et al., 2009). Most modern improved varieties of Ecuador derive from international genetic material mostly from CIAT, which likely carry Meddle American resistance genes. Since modern resistant varieties have been recently released with different degrees of adoption, evolution to *U. appendiculatus* to adapt to these new sources of resistance has been quite fast.

The Ecuadorian population of *U. appendiculatus* in addition of carrying virulence to the resistance in all Andean and Meddle American differentials, has also developed virulence to the 31 resistance genes identified in Cotacachi lines and the 38 resistance genes identified in Saraguro lines, none of the lines has a relative efficiency of resistance (RER) of 100% (Table 3 and 4). Northern highland and northern valley races are highly virulent to the resistance genes in the Cotacachi and Saraguro lines, showing that *U. appendiculatus* evolution is more active in northern Ecuador, especially in the northern valleys (Table 5).

Results of this study also show that the standard differential varieties used in this study (Steadman et al., 2002) did not discern virulence in Ecuador well, neither did the previous standard differentials varieties (Stavely et al., 1983). However, although local varieties used in this study can help better discerning *U. appendiculatus* diversity in the highlands, diversity in the valleys is likely different, and can be discerned with a similar study including traditional and modern bush varieties.

Difficulties in characterizing pathogen variability of *U. appendiculatus* using the old differential varieties (Stavely et al., 1983), was already pointed out by Mmbaga et al (1996a), and an agreement was made to use the standardized differential set proposed by Steadman et al (2002) and used in this study. The later standard differential set helped partially identify virulence variation among regions, since similar differences in virulence patterns were also observed with Cotacachi and Saraguro lines, which was shown by the significant correlation coefficient for MDSs between differential varieties with Cotacachi and Saraguro lines (Table 5). However, the correlation coefficient of MDSs between Cotacachi with Saraguro lines was much higher, showing that local varieties associate better with pathogen evolution, and additional varieties appear necessary to better characterize variability of *U. appendiculatus* in Ecuador.

An important epidemiological aspect that is also evident in this study is the presence of highly virulent isolates in northern Ecuador. In northern valleys, most races were extremely complex with virulence to most differential cultivars but also to most Cotacachi and Saraguro resistance genes. Since complex races were frequently sampled in northern valleys, virulence development by the pathogen does not appear to have a cost in pathogen fitness. These results support (Parlevliet, 1981) disagreement on the "stabilizing selection" theory proposed by Van Der Plank (1968). Stabilizing selection apparently does not appear to operate in *U. appendiculatus*, and for most *Ur* genes, the pathogen appears easily develop virulence, as for Middle American genes in the valleys.

Another important epidemiological aspect is that since *U. appendiculatus* is an airborne pathogen, valley races appears to be a potential threat for common bean cultivation in the northern highlands. However, valley races have not been detected in the highlands, probably due to restricted climatic adaptation or to less efficient competition of valley races with highlands races.

### **3.4.2 Resistance diversity studies**

#### **3.4.2.1 Seedling resistance studies**

Resistance genes identified in Cotacachi and Saraguro lines resulted in RT 1, 2 and 3 (Table 2), which is governed by major genes (de Souza et al., 2011; Haley et al., 1993; Park et al., 2007; Thibivilliers et al., 2009). Therefore, resistance genes identified in this study have a similar function as the genes of differential varieties discerning pathogen diversity and will be useful for characterizing pathogen variability in Ecuador.

Exposure of resistance genes to the pathogen for a long time in traditional common bean cultivation has acted as selection pressure, to which the pathogen has evolved. In this evolution process, races of the pathogen from these regions are expected to be differentially grouped, as northern highland and northern valleys races for Middle American virulence. This expectation was partially observed in this study; northern isolates were more virulent to Cotacachi resistance factors and southern isolates were more virulent to Saraguro resistance factors (Table 11). However, southern isolates have virulence to an important fraction of northern resistance genes (Cotacachi) (Table 5), and northern isolates have virulence to most southern resistance genes (Saraguro)(Table 5). Therefore, southern and northern isolates have many virulence in common, suggesting that resistance factors were integrated in the past in both regions or had a common origin, which is supported by the fact that variety mixtures have been cultivated since long ago along the highlands of Ecuador (Chapter 2).

In this study, some resistance genes of Cotacachi and Saraguro lines were identified in more than one line, in most cases belonging to different phenotypic identities. Since all lines studied from Cotacachi and Saraguro were genetically different, resistance factors appear to be recombined in the genetically diverse common bean populations, since recombination appears a common phenomenon in variety mixtures (Jacome, 2017).

From the strict breeding point of view, major gene resistance is a low promising strategy for genetic improvement of common bean in Ecuador. In this study, we were unable to identify an efficient source of resistance to all races studied, despite the diverse germplasm evaluated. However, high RER values of some Cotacachi and Saraguro lines, carrying resistance genes that have been exposed to the pathogen for long time suggests the presence of potential sources of durable resistance in these lines, as proposed by Mmbaga et al (1996a) and also admitted by Parlevliet (1981) for some resistance genes. Therefore, movement of lines with high RERs among regions appears to be a strategy to improve control of common bean rust for a more sustainable common bean cultivation in Ecuador.

Resistance genes identified in this study are not useful for classical breeding programs oriented to monoculture. However, they have been collectively useful in traditional cultivation of variety mixtures, reducing rust epidemics by protecting complementarily to the avirulent fraction of the pathogen population, which has resulted in a high mixture effect (Espinoza and Ochoa, 2012).

#### **3.4.2.2 Field resistance studies**

Field resistance of Cotacachi and Saraguro lines assessed under diverse epidemiological conditions integrated a wide range of line/pathogen interactions, which together with greenhouse information allowed a better understanding of resistance in Cotacachi and Saraguro lines.

DS values of Cotacachi lines correlating with RER and MDS with intermediate values in the three experimental regions (Table 14) showed that differences of DS in the field is explained to certain extent by major resistance genes conferring high levels of resistance. In addition, positive and significant correlation of DS of Cotacachi lines among the three experimental regions (Table 6) showed that resistance of Cotacachi lines followed a similar pattern in the three regions, suggesting that part of resistance is not race specific; therefore, partial resistance (PR) also operates in Cotacachi lines, which explains the high level of resistance in the field. PR has been reported for common bean rust (Alexander et al., 1985; Habtu and Zadoks, 1995; Statler and Mcvey, 1987), and in Ecuador, effective levels of PR were also reported in bush types (Ochoa et al., 2007).

DS of Saraguro lines correlated only with RER in Cotacachi (Table 14), showed that resistance genes of Saraguro lines are only efficient in Cotacachi, which is understandable because Bulcay and Saraguro regions are close to each other with similar pathogen populations, virulent to resistance genes of Saraguro lines. Similarly, coefficient of correlation of DS among regions although positive and significant between Saraguro and Cotacachi regions and especially between Bulcay and Saraguro (Table 7), values were lower than in Cotacachi, showing also lower levels of PR in Saraguro lines. However, the average DS of Saraguro lines in the three sites (Table 13) is still lower compared with susceptible lines from Cotacachi and Saraguro. PR of Saraguro lines is therefore likely adequate for the lower virulence of the Saraguro rust population.

Resistance genes conferring high levels of major resistance and PR conferring quantitative resistance appear to be operating together in the common bean cropping systems of Cotacachi and Saraguro. This cropping system appears less vulnerable, as proposed in the vertical and horizontal resistance model designed by Parlevliet and Zadoks (1977), which is less vulnerable to pathogen mutation due to the high richness of resistance, aspect that it is also pointed out in the model.

Integration of resistance genes of any nature in variety mixtures appears dynamic in a close relationship with pathogen evolution; thus, in northern Ecuador, where pathogen evolution has been more active, selection of more efficient sources of resistance (PR) has buffered the effect of pathogen evolution. On the other hand, resistance of Saraguro lines has been less threatened by pathogen evolution and although resistance is also rich, appears less effective than Cotacachi resistance. However, resistance of Saraguro lines might be enough for the less adverse pathogenic conditions in Saraguro.

Taking into account evidence developed in this study, the potential crop vulnerability of common bean to rust in Cotacachi and Saraguro, due to movement of more virulent races (Brown, 2008), appears less important in common bean variety mixtures. Therefore, resistance adjusting to pathogen evolution observed especially in Cotacachi lines shows variety mixtures a resilient cropping system.

### **3.5 Conclusions**

- As in many studies in the tropics, diversity of *U. appendiculatus* in Ecuador using the standard differential varieties is significantly high, with a rapid evolution of pathogen population in response to resistance in the field. This aspect is particularly evident in northern valleys, where high frequency of virulence was found in response to the recently introduced Middle American resistance genes.

- Diversity within *U. appendiculatus* populations was observed among regions, being the pathogen highly virulent in northern highlands but specially in northern valleys, while in southern highlands and southern valleys the pathogen was less virulent.
- High richness of resistance genes were identified at community level in Cotacachi and Saraguro variety mixtures. However, the pathogen has already developed virulence to the resistance genes identified in this study. Nevertheless, some resistance genes were efficient to a high fraction of the isolates studied, and probably they are more durable.
- Rust resistance genes identified in this study are not useful for a classical breeding program oriented to develop completely resistant varieties for monoculture cultivation. However, they have been collectively useful for long time in variety mixtures, reducing disease epidemics in traditional agriculture.
- Resistance genes identified at the seedling stage contributed significantly to explain the resistance observed in the field in Cotacachi and Saraguro mixtures. Partial resistance (PR) is also operating, explaining complementarily the high level of resistance in the field, especially within the Cotacachi lines. Major gene resistance conferring high levels of resistance and minor gene resistance conferring PR are operating together in variety mixtures of Cotacachi and Saraguro.
- Integration of resistance appears dynamic in close association with pathogen evolution; therefore, the potential crop vulnerability due to new virulent races entering in the common bean cropping system is less likely.

## Chapter 4

### Common bean variety mixture intensification: a sustainable and resilient approach to improve food security in the highlands of Ecuador.

#### Abstract

Farmers in the highlands of Cotacachi-Ecuador have implemented variety mixture intensification to improve food security and sustain cultivation of common bean. The late-maturing *Chacra* belonging to growth habit IV and the early maturing *Allpa* belonging to growth habits I, II and III are planted together with maize in the same plot. The former type is planted with maize in the same planting site and the later type is planted between maize/*Chacra* plants. The effect of this intensification approach on pest and disease development, yield (kg/ha), and 100 seed weight were studied in Cotacachi (highland) and Tumbaco (valley). The land equivalent ratio (LER) index was used to establish the effect of the intensified approach on productivity (yield). In Cotacachi, LER of *Chacra* in any biotic constraint or climatic condition was higher than 0.5, being favoured in any condition by the intensification approach. *Allpa* on the other hand was slightly affected by the intensification approach at high rust and ALS epidemics, with a LER slightly lower than 0.5. Loss of *Allpa* due to disease epidemics was compensated by better performance of *Chacra*. In favourable conditions at low disease severity, LER of both *Chacra* and *Allpa* reached around 0.75, showing that *Chacra* and *Allpa* have co-adapted to the intensification approach. In Cotacachi, intensification was a compensatory system at high disease pressure, while a synergic system in favourable conditions. In Tumbaco, on the other hand the intensification approach was competitive, *Chacra* performed much better in the mixture than *Allpa* reaching a LER of around 1; however, *Allpa* performed better than *Chacra* as sole crop. Result of this study allowed us to conclude that *Chacra* is better adapted to the highland, while *Allpa* is better adapted to lowlands; and therefore, *Allpa* has been introduced to the highlands, and already co-adapted with *Chacra*, creating a compensatory and synergic system.

#### 4.1 Introduction

Common bean (*Phaseolus vulgaris* L) has long been the most important cultivated legume in Ecuador (Peralta et al., 1997). Phenologically diverse types ranging from determinate called bush (growth habit I and II) to indeterminate called climbing (growth habit III and IV) are grown along the highland; the former in the valleys and western foothills, and the later in the temperate highlands (Peralta et al., 2013). Within these types, phenotypically diverse varieties of the Nueva Granada and Perú races (Singh et al., 1991) are grown in diverse agroecological conditions, which have created a wide genetic structure (Bonilla, 2010; Jacome, 2017; Torres, 2012).

Ecuador is also part of a unique center of wild common bean diversity, intermediate between the Andean and Mesoamerican gene pools (Debouck et al., 1993; Freyre et al., 1996).

Bush types are grown in the valleys and the Andean foothills in traditional and modern cropping systems in around 24,374 ha (Peralta et al., 2013). In northern valleys, and in central and northwestern foothills, monoculture of improved varieties is an important agricultural activity (Garver et al., 2008; Subia et al., 2007). In these regions, varieties of the commercial type *Rojo Moteado* appreciated in the Colombian market are cultivated (Peralta et al., 2009; Subia et al., 2007). In southern valleys, on the other hand, varieties are primarily traditional and less devoted to commerce (Jiménez et al., 1996).

Climbing types are cultivated in the temperate highland intercropped with maize in an ancient cropping system on around 97,217 ha (Peralta et al., 2013). In this cropping system, climbing types have long been cultivated in a variety mixture approach, which is a combination of genotypes that are planted, harvested, consumed, and eventually commercialized together (Chapter 2). At present, *Canario*, a local yellow big-seeded population has replaced variety mixture cultivation in many common bean areas. *Canario* is the main commercial type grown also intercropped with maize, and sometimes with wire fence supports. In addition to *Canario*, the commercial varieties Bombolin and Cargamanto types are grown with wire fence supports in central-west Ecuador.

Despite the countrywide interest of *Canario*, variety mixtures continue to be an important common bean cultivation strategy in Cotacachi inhabited by the Imbaya native community in northern Ecuador (Chapter 2). In this region, variety mixtures are the main source of protein since they have less access to animal protein; but also, as *Canario* is part of the mixture (Chapter 2), variety mixtures also provide income for small scale farmers (Peralta et al., 2013, 1997). Therefore, variety mixtures are key for food security in northern Ecuador.

Two types, *Chacra* and *Allpa* variety mixtures are intercropped with maize in Cotacachi; the former a late maturing type belonging to growth habit IV, and the later an earlier type belonging to growth habit I, II and III. *Chacra* is planted together with maize in the same planting site and climbs on the maize plant, and *Allpa* is planted between maize/*Chacra* types, and plants of the growth habit III partially climbs on the maize plant (Chapter 2). Two complementary intensified approaches are clearly identified in this cropping system: 1) species intensification: maize and common bean, and 2) crop type intensification: *Chacra* and *Allpa*. This two way intensification approach is clearly oriented to food security, ensuring food provision during the crop season, *Allpa* providing food early in the season and *Chacra* providing food later in the season.



The two-way intensification approach is characterized by the improved food security, but also by incorporating *Chacra* and *Allpa* variety mixtures farmers aim sustainability and resilience. In participatory diagnostic studies, farmers agree that common bean variety mixtures are important strategies to reduce pest and disease epidemics (Chapter 2). Farmers' perception of the usefulness of variety mixtures reducing disease epidemics was supported by the high "mixture effect" to rust of variety mixtures reported by (Espinoza and Ochoa, 2012), and by the rich sources of resistance to rust found at the community level mainly in *Chacra* variety mixtures (Chapter 3).

Intensification is nowadays considered a key alternative to improve agricultural productivity to satisfy the increasing food demand. The main aim of intensification is to obtain a better output per production unit with efficiency of inputs (Struik et al., 2014). Under these considerations, Green Revolution is considered an intensified process; however, a more sustainable intensification approach is considered necessary to meet with production challenges. Sustainable intensification is defined as a "process or system where agricultural yields are increased without adverse environmental impact and without the conversion of additional non-agricultural land" (Pretty and Bharucha, 2014).

The intensification approach for major cereals (wheat, rice and maize) is suggested to concentrate in three main modern actions: improving yield to approach yield potential, improving soil quality, and exploiting precision agriculture (Cassman, 1999). For traditional agriculture in developing countries, intensification is primarily associated with efficient crop management to improve soil fertility, reduce tillage, a better crop rotation planning, and intercropping (Devkota et al., 2016; Mungai et al., 2016; Nyagumbo et al., 2016; Pradhan et al., 2016; Wani et al., 2016). However, less attention is paid to low input intensification, which is primarily considered to be achieved with genomic tools (Abberton et al., 2016).

Intensification approaches in modern and traditional agriculture are nowadays oriented to promote the use of inputs to maximize the yield potential, which is less applicable for the low input traditional agriculture. Intensified cultivation of common bean in Cotacachi adapted to local agroecological and socioeconomical conditions is a low input system that meets a sustainable food supply in adverse conditions. Traits and principles of this traditional intensification can be useful to help future interventions to conserve and improve intensification productivity, but may be also useful in planning intensification initiatives in modern agriculture.

Variety mixtures on the other hand, are mostly associated with sustainability and resilience; scientific evidence supports the contribution of variety mixtures to reduce crop disease

epidemics. According to Wolfe (1985), the main purpose of genetic mixtures (crop variety mixtures) for pest and disease management is to slow down pest and pathogen spread. Several recent studies have shown that a diverse genetic basis of resistance is beneficial for the farmer because it allows a more stable management of pest and disease pressure than a monoculture (Di Falco and Chavas, 2007; Finckh, 2003; Jarvis et al., 2007; Thinlay et al., 2000; Thurston et al., 1999; Trutmann et al., 1996). Most pest and disease management strategies concentrate on reducing the current or coming crop season's loss. Few crop management programmes are oriented to providing options that could reduce the risk to future crop loss, i.e., reducing genetic vulnerability within the farmers' fields. Vulnerability is intended here as the probability of crop loss due to a new biotype of pest or pathogen entering into the farmer's production system (Brown, 2008), a phenomenon more likely to occur in an area consisting of one or few varieties that share a very similar resistance structure.

Common bean disease surveys including farmers' opinion identify diseases as the main biotic constraint of climbing common bean cultivation in Ecuador (INIAP, 2003; Lepíz et al., 1995; Peralta et al., 2013). The main diseases identified in these surveys were rust (*U. appendiculatus*), anthracnose (*Colletotrichum lindemuthianum*) and ascochyta leaf spot (AsLS) (*Ascochyta phaseolorum*). Management of these diseases in Cotacachi has primarily relied on intercropping and variety mixtures (Chapter 2).

Intensifying with intercrops is an efficient strategy to reduce disease epidemics by reciprocal barriers (Finck and Wolfe, 2006), which was also clear to farmers who consider intercrops the main strategy to cope with disease epidemics (Chapter 2). However, intensification of the same species as *Chacra* and *Allpa*, hosts of the same pathogens appears less promising from the epidemiological point of view, because for most diseases, high plant population provides more conducive conditions.

*Chacra* and *Allpa* intensification in Cotacachi would seem associated mostly with food provision rather than yield benefits. However, genetic diversity of *Chacra* and *Allpa* variety mixtures in which resistance is operating (Chapter 3) producing an important mixture effect (Espinoza and Ochoa, 2012), appears to be a complement that sustains this intensification approach and provides cropping system resilience.

Despite the importance of common bean intensified variety mixtures approach, little attention has been received this ancient cropping system. The aim of this study was to analyze productivity, sustainability, and resilience of the common bean intensified variety mixtures in Cotacachi.

**Main objective of this study were:**

- a) Assess the epidemiological development of pest and diseases within and among variety mixtures.
- b) Establish the epidemiological effect of common bean intensification on pest and disease development.
- c) Assess productivity of common bean intensification in terms of yield and seed size parameters.

**4.2 Materials and methodology**

**4.2.1 Site information**

The study was undertaken in Cotacachi in 2009 and 2016, and in Tumbaco-Quito in 2016. In Cotacachi, the study was conducted in Morochos and Cumbas Conde in 2009, and in Cumbas Conde in 2016. The Morochos plot was located at 78° 18' 50" W, 0° 15' 56" N, and at altitude of 2793 masl. The Cumbas Conde plot was located at 78° 18' 51" W, 0° 15' 59" N, and at altitude of 2694 masl. The Tumbaco plot was located at 78° 22' 0" W, 0° 13' 16" N, and at an altitude of 2480 masl. Cotacachi is a temperate region, to which climbing common bean types have been adapted by farmers a long time ago. In contrast, Tumbaco is a warmer valley where mostly bush types are cultivated.

**4.2.2 Experimental material and sites**

In 2008, three random samples of *Chacra* and *Allpa* variety mixtures were selected for 2009 experiments in a common bean diversity survey in Cotacachi. In a similar common bean survey in 2015, four random samples of *Chacra* and *Allpa* variety mixtures were selected for 2016 experiments (Table 1). In the 2009 experiments, variety mixtures were evaluated in farmers' plots in Morochos and Cumbas Conde (Cotacachi). In the 2016 experiments, variety mixtures were evaluated in Cumbas Conde (same plot of 2009) and in Tumbaco–Quito at the CADET Station of the Agricultural Science Faculty of the Central University of Ecuador.

**Table 1. Information of *Chacra* and *Allpa* variety mixtures**

<i>Chacra</i>	Farmer origin	<i>Allpa</i>	Farmer origin
<b>2009</b>			
A	Martha Gualsaqui	A	Martha Gualsaqui
B	Angel Cumba	B	Angel Cumba
C	Camilo Flores	C	Camilo Flores
<b>2016</b>			
A	Cecilia Cumba	A	Jose Bonilla
B	Angel Cumba	B	Angel Cumba
C	José Lima	C	José Lima
D	Francisca Panamá	D	Francisca Panamá

### 2.3 Treatments and experimental design

In 2009 and 2016 experiments, variety mixtures listed in Table 1 were evaluated in two crop management approaches: 1) Sole Variety Mixture (SVM) and 2) Intensified Variety Mixture (IVM). The SVM consisted in planting only *Chacra* or *Allpa* variety mixtures, whereas the IVM consisted of planting *Chacra* and *Allpa* variety mixtures in the same plot with high plant density (twice the SVM), as most farmers do in Cotacachi (Chapter 2).

In all sites and years, experiments consisted of a three way factorial: crop management approach, crop type and variety mixtures. The **crop management approach** (SVM and IVM), and the **crop type** (*Chacra* and *Allpa*) were similar in 2009 and 2016 experiments. The **variety mixture** consisted of three varieties in 2009, and four varieties in 2016 (Table 1). Experiments consisted of 12 and 16 treatments in 2009 and 2016, respectively.

In the field, the factorial was implemented in a split plot design with three replications. Each replication consisted of three whole plots in which the interaction between **crop management** and **crop type** were set up: 1) *Chacra* (SVM), 2) *Allpa* (SVM), and 3) *Chacra* + *Allpa* (IVM). In the IVM plot, *Chacra* and *Allpa* were treated as different treatments. In the whole plots, the variety mixtures were set up in the sub-plots. In the IVM subplots, *Chacra* and *Allpa* variety mixtures of the same letter (Table 1) were set up. This experimental design allowed creating a uniform environmental condition for pest and diseases within each crop management approach (whole plot).

Experimental plots in 2009 consisted of five rows, 6 m-long spaced by 0.80 m, while experimental plots in 2016 consisted of six rows, 6 m-long spaced by 0.8 m. In *Chacra* SVM plots, two seeds of *Chacra* with two seeds of the maize variety Killu Sara were planted in the same

planting site, and planting sites were separated every 0.8 m. In *Allpa* SVM plots, two seeds of *Allpa* were planted between maize plants of variety Killu Sara every 0.8 m. In IVM plots, *Chacra* and *Allpa* were planted together in the same way as in SVM, having twice as many plants as SVM plots. To keep similar genotype frequency of variety mixtures among experiments, seeds of all experiments were derived from the same seed population. The planting system described was similar as farmers do in Cotacachi, and planting was done together with experienced farmers.

For evaluation, 10 planting sites per plot (treatment) were randomly selected in the three inner rows in 2009, and 15 planting sites per plot (treatment) were randomly selected in the four inner rows in 2016. The same number of planting sites were selected in *Chacra* and *Allpa* variety mixtures in SVM as well as in IVM. Only one common bean plant was allowed to grow in each planting site at V3 plant stage (Fernández et al., 1986).

Soil preparation, planting, and crop practices were done as farmers do in Cotacachi. Since soil fertility in Cotacachi is relied primarily on plant debris, fertilization was not applied in any site and year. Soil nutritional analysis reported a very similar nutritional content in Morochos and Cumbas Conde plots. A high content was reported for P, K, and Fe; medium content was reported for N, Ca, Mg, and Cu; and low content was reported for S, Zn, Mn, and B. In Tumbaco, although fertilization was not applied, soil nutrition was higher than in Cotacachi. Soil analysis reported high content of N, P, K, Ca, Mg, Cu, and Fe, and medium content was reported for S, Zn, and Mn.

### **4.2.3 Evaluation**

#### **4.2.3.1 Diseases and spider mite evaluation**

Experiments conducted in farmers' plots in Cotacachi and Tumbaco-Quito were exposed to natural infestations of pest and diseases. Development of pest and diseases were monitored during the crop season and evaluation was initiated as soon as the epidemic was uniform. Disease severity (DS) and spider mite damage was evaluated every 2 to 3 weeks using the Cobb modified scale (James, 1972), which was adjusted in this study as suggested by Stavely (1983). These scales varied from 0-100%, 0% meaning absence of disease symptoms and 100% complete coverage of the foliar by the disease. At the time of evaluation, plant growth stage was also recorded (Fernández et al., 1986).

#### **4.2.3.2 Yield and weight of 100 seeds**

Selected plants from the inner rows were independently harvested, and yield and weight of 100 seeds assessed. Yield of the selected plants per plot (treatment) were collectively tabulated to

yield/hectare (kg/ha) by using 0.64 m<sup>2</sup> as the average area covered by each plant. The weight of 100 seeds was calculated for each plant.

#### 4.2.3.3 Phenotype population frequency

All plants of the plot were harvested and then classified phenotypically taking into account size, shape and color of the seed, as farmers do for variety mixtures phenotypes in Cotacachi (Chapter 2). With this information, frequency of each phenotype within the plot was tabulated.

#### 4.2.4 Data analysis

Average, range, and standard deviation of DS and spider mite damage were calculated for each *Chacra* and *Allpa* variety mixture. Using phenotype frequency, richness (number of phenotypes in the plot) and evenness estimated as a complement of D (1 – D) where D is the Simpson measure of dominance (Jarvis et al., 2008; Magurran AE, 2004) were calculated for each treatment.

The Land Equivalent Ratio (LER), defined as the relative land area required as SVM to produce the same yield as in the IVM was calculated for each *Chacra* and *Allpa* variety mixture, and then the total (tLER) (LER of *Chacra* + LER of *Allpa*) was tabulated as proposed by Mead and Willey (1980).

LER is of particular importance, because it assesses and characterizes common bean intensification in Cotacachi. This index is suitable (Mead and Willey, 1980) to assess components of intercroops (*Chacra* and *Allpa* in this case) with a common objective. In this case, main objective of *Chacra* and *Allpa* production is food for self-consumption.

LER was calculated as follow:

$$tLER = L_{Ch} + L_{All} = IVM_{Ch}/SVM_{Ch} + IVM_{All}/SVM_{All}$$

Where:  $L_{Ch}$  and  $L_{All}$  are individual LER of *Chacra* and *Allpa*, respectively.  $IVM_{Ch}$  and  $IVM_{All}$  are the yield of *Chacra* and *Allpa* variety mixtures in the IVM, respectively; and  $SVM_{Ch}$  and  $SVM_{All}$  are the yield of *Chacra* and *Allpa* variety mixtures in the SVM, respectively. An individual LER over 0.5 is considered an advantage for the individual component if the tLER is at least 1. A tLER over 1 is considered an advantage for the intensified approach.

The most accurate DS and spider mite damage evaluations were analyzed statistically. ANOVA was performed for DS and spider mite damage with data transformed using Arcsine. ANOVA was also performed for grain yield (kg/ha), 100 seed weight, richness, evenness and LER. Statistical differences among **sites**, among **types** and among **crop management** were derived from the

ANOVA tests, while statistical differences among **variety mixtures** were defined using the Duncan mean test at  $P < 0.05$ . The Pearson correlation coefficient was performed for DS, spider mite damage, yield and 100 seed weight with richness, evenness, and yield. Data of all studies were processed in the MS excel (Microsoft Office Professional Plus, 2013) computer program and the statistical analysis were done using the R statistical program version 3.4.

### **4.3 Results**

#### **4.3.1 Disease severity and spider mites damage**

Epidemics of rust, ALS, and AsLS were consistently recorded in all sites and years, spider mite damage was consistently recorded in Cotacachi and Tumbaco in 2016, and anthracnose was only recorded in Cotacachi in 2009. Patterns of disease epidemics and spider mite damage were common across sites and years. Thus, rust, ALS, and anthracnose epidemics initiated early in the crop season and overlapped with epidemics of AsLS and spider mites in Cotacachi, while in Tumbaco, all diseases and the spider mites initiated simultaneously. In Cotacachi in 2009, rust, ALS and anthracnose were recorded at growth stage R5 for *Chacra*, and growth stage R6 for *Allpa*. AsLS epidemics and spider mite damage were recorded at growth stage R8 for *Chacra* and R9 for *Allpa*. In Cotacachi in 2016, epidemics started later than in 2009 in such a way that *Allpa* escaped infection of AsLS and spider mites. In Tumbaco in 2016, epidemics of rust, ALS, and AsLS, and spider mite damage were initiated at growth stages R6 for *Chacra* and R7 for *Allpa*.

Epidemics of diseases and spider mites damage varied among sites and years (Table 2). DS of rust varied in 2009 from low in Morochos to intermediate in Cumbas Conde, while DS of rust was low in 2016 in both, Cumbas Conde and Tumbaco. DS of ALS was low in all sites and years, except in Cumbas Conde in 2016, with intermediate DS values. DS of anthracnose and AsLS in Cotacachi sites varied from intermediate in 2009 to low in 2016. Damage of spider mites varied in 2016 from high in Cumbas Conde to intermediate in Tumbaco.

Average of DS and spider mite damage for *Chacra* and *Allpa* variety mixtures across sites and years are also shown in Table 2. DS of rust was statistically similar among *Chacra* and *Allpa* variety mixtures in 2009, but statistically higher in *Allpas* in 2016. DS of ALS was in 2009 statistically higher in *Chacras* in Morochos and statistically lower in Cumbas Conde. In Tumbaco in 2016. DS of ALS was statistically similar among *Chacra* and *Allpa*. DS of anthracnose was statistically similar among types, and statistically higher in Cumbas Conde than in Morochos. DS of AsLS was lower in *Allpa* than *Chacra* in Morochos and the opposite in Cumbas Conde in 2009. Damage from spider mite was statistically similar in both *Chacra* and *Allpa* variety mixtures in 2009, but statistically higher in Cumbas Conde than in Tumbaco in 2016.

Table 2. Disease severity (DS) average, spider mite damage, yield, and 100 seed weight of variety mixtures among experimental sites in two cropping seasons.

Variety mixture	2009		2016		Variety mixture	2009		2016	
	Morochos	C. Conde	C. Conde	Tumbaco		Morochos	C. Conde	C. Conde	Tumbaco
<b>DS of rust</b>					<b>DS of AsLS</b>				
Chacra	16.9	40.2	5.6 a	3.2 a	Chacra	65.6 b	63.7 a	5.9	17.3 b
Allpa	14.3	31.8	10.1 b	6.9 b	Allpa	53.8 a	67.5 b	(-)	13.5 a
<b>Average</b>	<b>16.3 a</b>	<b>36 b</b>	<b>7.8</b>	<b>5.1</b>	<b>Average</b>	<b>59.2 a</b>	<b>65.6 b</b>	<b>5.9 a</b>	<b>15.4 b</b>
<b>DS of ALS</b>					<b>Spider mite damage</b>				
Chacra	14.4 b	11.2 a	30.6	2.8	Chacra	-	-	53.8	33.6
Allpa	10.5 a	17.9 b	31.2	2.9	Allpa	-	-	(-)	30.2
<b>Average</b>	<b>11.9</b>	<b>14.5</b>	<b>30.9 b</b>	<b>2.85 a</b>	<b>Average</b>			<b>53.8 b</b>	<b>31.9 a</b>
<b>DS of anthracnose</b>					<b>100 seed weight</b>				
Chacra	30.9	37.9	-	-	Chacra	42.6	41.9	50.8	47.3
Allpa	34.5	39.4	-	-	Allpa	39.4	42.5	40.3	39.8
<b>Average</b>	<b>31.6 a</b>	<b>38.6 b</b>			<b>Average</b>	<b>41.2</b>	<b>42.2</b>	<b>45.5 a</b>	<b>43.5 b</b>
- Symptoms were not evident in the field.					<b>Yield (kg/ha)</b>				
(-) - scape infection.					Chacra	521.8 a	271.9 a	607.3 a	620.4
Values having different letters are significantly different at Duncan					Allpa	271.3 b	167.6 b	317.2 b	625.8
P < 0.05.					<b>Average</b>	<b>366.6 a</b>	<b>219.7 b</b>	<b>462.2 b</b>	<b>623.1 a</b>

DS and spider mite damage of *Chacra* variety mixtures evaluated in SVM and IVM are shown in Table 3 (2009 experiments) and Table 4 (2016 experiments). Statistical differences in DS for most diseases and spider mite damage among variety mixtures were only found at IVM in Morochos in 2009, and SVM in Cumbas Conde in 2016. In Tumbaco, on the other hand, DS of all diseases and spider mite damage among variety mixtures were statistically similar in SVM as well as IVM. The overall DS average between SVM and IVM were statistically similar, except for ALS in Cumbas Conde in 2016, where the DS average at IVM was higher than at SVM (Table 5).

Disease and spider mite epidemics among *Allpa* variety mixtures evaluated in SVM and IVM are shown in Table 6 (2009 experiments) and Table 7 (2016 experiments). In 2009, statistical differences were observed for DS of all diseases and spider mite damage at both SVM and IVM, except for anthracnose. In 2016, differences in DS between *Allpa* variety mixtures were only found for rust and ALS in SVM in Cumbas Conde. Unlike *Chacra*, the average of *Allpa* rust DS at IVM was statistically higher than at SVM in Cumbas Conde in 2009 and 2016, and for AsLS in Tumbaco in 2016 (Table 5).

Range and standard deviation in this study allowed establishment variation in resistance among plants evaluated in variety mixtures. Low to intermediate DS, wide range, and high standard deviation of rust and ALS particularly for *Chacra* (Table 3 and 4), and to a lesser extent for *Allpa* (Table 6 and 7), show variation in the expression of resistance of plants within variety mixtures; range for rust and ALS for *Chacra* were very wide in Cumbas Conde in 2009, DS of rust varied from 0 to 70%, and DS of ALS varied from 0 to 80%.



**Table 3. Average, range and standard deviation of disease severity (DS) of rust, ALS, AsLS, and anthracnose, and 100 seed weight for *Chacra* variety mixtures evaluated in sole variety mixtures and intensified variety mixtures in Cotacachi in 2009.**

Variety Mixture	Morochos-Cotacachi						Cumbas Conde-Cotacachi					
	Sole variety mixture			Intensified variety mixture			Sole variety mixture			Intensified variety mixture		
	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev
<b>DS of rust</b>												
A	13.8 a	1-30	10.9	14.7 a	0-40	11.3	41.5	0-70	24.7	47.5	0-70	22.0
B	15.7 a	0-33	11.7	14.0 a	0-37	12.4	46.0	0-60	29.1	39.0	0-70	26.9
C	23.2 b	5-47	12.7	17.5 b	0-40	12.8	40.0	0-70	25.8	36.0	0-70	26.7
<b>DS of ALS</b>												
A	15.7	2-25	8.1	8.2 a	0-23	8.4	14.0 b	0-33	11.4	11.5	0-27	8.8
B	16.2	2-30	9.5	17.2 b	3-30	8.2	5.0 a	0-20	6.9	11.0	0-27	7.9
C	15.6	2-30	8.0	13.0 b	3-27	8.2	10.0 ab	0-20	7.3	15.5	0-33	12.4
<b>DS of AsLS</b>												
A	68.0	43-83	15.5	73.6 b	50-87	14.8	62.2	33-77	12.7	66.6	63-77	6.2
B	71.1	56-87	16.4	69.7 b	37-83	18.6	64.4	50-77	8.5	64.4	50-77	8.5
C	70.0	44-87	16.3	46.4 a	17-87	21.0	63.8	50-77	7.8	60.6	50-73	9.9
<b>DS of anthracnose</b>												
A	28.8	23-40	7.1	30.8	23-43	8.14	38.9	33-57	9.9	37.2	33-43	5.4
B	33.3	23-60	11.8	33.9	23-43	7.9	38.9	33-43	5.7	38.9	33-50	6.7
C	28.4	17-33	7.9	32.5	27-40	7.3	35.0	33-43	4.1	40.6	28-43	6.5
<b>100 seeds weight (g)</b>												
A	44.0	40-58	7.8	40.9	23-61	17.7	41.8	25-63	11.3	43.3 ab	33-65	14.1
B	48.9	34-63	16.5	39.4	29-61	8.9	43.9	26-60	12.4	44.2 a	26-70	14.6
C	41.6	36-58	7.6	41.1	30-57	7.3	40.5	26-60	13.7	37.8 b	23-62	15.9

<sup>1</sup>Values having different letters are significantly different at Dunca P < 0.05.

<sup>2</sup>Average of the lowest and highest values of replicates.

In contrast, high average DS, short range, and low standard deviation of AsLS for *Chacra* and *Allpa* in 2009 (Table 3 and 6) showed low levels of resistance in the plants evaluated within variety mixtures. DS of AsLS for *Chacra* varied from 50 to 70%, but for *Allpa* was more variable, especially across sites showing in *Allpa* some levels of resistance to AsLS. Low DS of AsLS in 2016, especially in Cumbas Conde (Table 4 and Table 7) was due to late pathogen infection, and unfavorable conditions.

**Table 4. Average, range, and standard deviation of disease severity (DS) of rust, ALS, AsLS, and anthracnose, spider mite damage and 100 seed weight for *Chacra* variety mixtures evaluated in sole variety mixtures and intensified variety mixtures in Cotacachi in 2016.**

Variety Mixture	Cumbas Conde-Cotacachi						Tumbaco-Quito					
	Sole variety mixture			Intensified variety mixture			Sole variety mixture			Intensified variety mixture		
	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev
<b>DS of Rust</b>												
A	4,8 b	0-30	8.6	8.8	0-33	12.4	3.2	0-20	6.5	3.2	0-23	7.9
B	5,1 b	0-23	8.8	6.0	0-27	9.3	3.6	0-17	7.4	4.9	0-27	9.7
C	3,7 ab	0-23	7.9	5.8	0-30	9.5	2.3	0-10	4.0	4.3	0-30	8.9
D	1,4 a	0-13	4.6	7.3	0-27	11.0	1.0	0-10	3.6	3.1	0-20	7.9
<b>DS of Angula Leaf Spot (ALS)</b>												
A	28.1 b	10-63	21.4	46.1	13-80	21.0	3.2	0-20	6.7	2.2	0-20	5.8
B	21.7 a	5-50	12.0	30.9	8-60	17.0	4.0	0-23	8.0	2.6	0-20	6.1
C	28.4 b	5-67	21.3	41.5	8-70	22.4	3.7	0-20	7.3	2.3	0-16	5.4
D	21.0 a	7-47	11.8	28.9	17-85	20.0	2.7	0-15	5.1	1.6	0-16	4.8
<b>DS of Ascochyta Leaf Spot (AsLS)</b>												
A	5.8	0-15	4.9	6.4	5-13	0.0	16.2	0-30	13.2	13.6	0-33	13.2
B	4.5	0-13	6.1	8.0	0-15	7.0	16.4	1-33	14.0	15.2	0-30	11.8
C	6.5	0-17	6.2	6.4	0-15	6.6	18.2	0-33	13.0	17.2	5-37	11.8
D	4.5	0-23	7.3	5.9	5-20	4.7	22.0	5-33	10.5	19.4	0-30	12.0
<b>Spider Mites Damage</b>												
A	58,6 b	33-67	15.9	59.1	37-70	17.3	32.8	0-53	21.4	25,4 a	0-47	20.4
B	51,2 ab	33-67	15.4	59.4	40-70	15.5	29.7	5-57	23.6	29,3 ab	5-50	17.3
C	50,9 ab	30-63	16.3	59.8	33-65	12.7	34.5	0-57	22.3	35,8 ab	0-50	16.7
D	42,7 a	17-60	18.0	48.7	23-70	21.8	41.9	5-53	15.3	39,7 b	10-53	17.5
<b>100 seed weight (g)</b>												
A	50,7 b	36-65	8.7	46.9	33-61	8,8	49,1 ab	39-62	8.7	47.6	36-61	7.8
B	48,0 b	38-70	8.8	46.9	35-59	7.6	45,6 b	27-63	10.4	44.9	32-61	11.6
C	49,6 b	37-63	9.8	50.4	37-67	8.5	44,5 b	32-59	8.3	45.4	31-61	8.3
D	59,6 a	42-76	10.5	54.8	39-73	14.8	55,5 a	41-67	10.9	45.6	34-64	9.4

<sup>1</sup>Values having different letters are significantly different at Duncan P < 0.05.

<sup>2</sup>Average of the lowest and highest values of replicates,

A differential response among sites was observed for spider mite damage of *Chacra* in 2016. Thus, high damage, short range and low standard deviation of spider mite damage was observed in Cumbas Conde, whereas low damage, wide range, and high standard deviation of spider mite was observed in Tumbaco (Table 4). Results were similar for *Allpa*, showing that resistance to spider mites in variety mixtures appears efficient to Tumbaco spider mite population, but inefficient to Cumbas Conde spider mite population.

DS of anthracnose in Morochos and Cumbas Conde in 2009 was uniform with intermediate DS values among variety mixtures in SVM and IVM across sites. The DS range was short and the standard deviation low for *Chacra* (Table 3); whereas for *Allpa*, the DS range was wide, showing presence of resistance in *Allpa* variety mixtures (Table 6). Uniform reactions of *Chacra* for anthracnose show that conditions were either not conducive to the disease and/or most genotypes of variety mixtures carry quantitative resistance.

### 4.3.2 One Hundred seed weight

Average 100 seed weight was similar among sites in Cotacachi in 2009, higher in Cumbas Conde than in Tumbaco in 2016, and similar in all years among *Chacra* and *Allpa* (Table 2). For *Chacra*, differences of 100 seed weight among variety mixtures were observed at IVM in Cumbas Conde in 2009 (Table 3) and at SVM in Tumbaco in 2016 (Table 4). For *Allpa*, differences among variety mixtures were only observed at SVM in Tumbaco in 2016 (Table 7). These results show that differences of 100 seed weight among *Chacra* variety mixtures are expressed at high plant density and high disease pressure in Cumbas Conde in 2009; but also under favorable conditions, low plant density, and low disease pressure in Tumbaco in 2016 (Table 2). Wide range and low standard deviation were recorded for both *Chacra* and *Allpa* variety mixtures in 2009 and 2016 (Table 3 and 4), showing similar variation in seed size among the variety mixtures.

**Table 5. Average disease severity (DS) of rust, ALS, anthracnose, and AsLS, spider mite damage, and the 100 seed weight of *Chacra* and *Allpa* variety mixtures evaluated in sole variety mixture (SVM) and intensified variety mixtures (IVM) in 2009 and 2016.**

Crop management approach	Crop season of 2009				Crop season of 2016			
	Morochos		Cumbas Conde		Cumbas Conde		Tumbaco	
	chacra	Allpa	chacra	Allpa	chacra	Allpa	chacra	Allpa
<b>DS of rust</b>								
SVM	20.3	12.8	42.5	22.8 a	3.8	6.5 a	2.5	8.6
IVM	16.9	15.0	37.9	40.8 b	7.4	13.7 b	3.9	5.2
<b>DS of ALS</b>								
SVM	14.7	9.5	9.7	17.7	24.8 a	33.8	3.4	3.3
IVM	12.8	10.6	12.7	18.0	36.3 b	28.6	2.2	2.4
<b>DS of anthracnose</b>								
SVM	29.4	31.9	37.0	39.0	(-)	(-)	(-)	(-)
IVM	31.1	36.0	38.9	39.9	(-)	(-)	(-)	(-)
<b>DS of AsLS</b>								
SVM	71.8	52.0	63.5	68.0	5.3	-	18.2	6.5 a
IVM	61.7	51.7	63.9	67.0	6.6	-	16.3	20.5 b
<b>Damage of spider mites</b>								
SVM	(-)	(-)	(-)	(-)	50.9	-	34.7	15.6 a
IVM	(-)	(-)	(-)	(-)	56.8	-	32.5	44.8 b
<b>100 seeds weight (g)<sup>1</sup></b>								
SVM	44.8	44.0 a	42.1	43.9	51.9	40.7	48.7	40.5
IVM	40.5	36.0 b	41.8	41.1	49.8	39.8	45.9	39.1

(-) Symptoms were erratic in the field

- Scape infection

Values having different letters are significantly different at Duncan P < 0.05.

The effect of SVM and IVM on 100 seed weight is shown in Table 5. Weight of 100 seeds was slightly greater in SVM than IVM for *Chacra* and *Allpa* mixtures in all sites and years; however, these differences were not statistically different. Nevertheless, differences of 100 seed weight was statistically greater in Cumbas Conde than in Tumbaco in 2016 (Table 2).

### 4.3.3 Yield and Land Equivalent Ratio

Yield performance of variety mixtures varied among sites, and among *Chacra* and *Allpa* types (Table 2). In 2009, the average yield in Morochos was greater than in Cumbas Conde; and in 2016, the average yield of Tumbaco was greater than in Cumbas Conde. The average yield of *Chacra* was higher than *Allpa* in Cotacachi in both 2009 and 2016; however the average yield of *Chacra* and *Allpa* were similar in Tumbaco.

Table 6. Average, range and standard deviation of disease severity (DS) of rust, ALS, AsLS, and anthracnose, and 100 seed weight of *Allpa* variety mixtures evaluated in sole variety mixture and intensified variety mixture in Cotacachi in 2009.

Variety mixture	Morochos-Cotacachi						Cumbas Conde-Cotacachi					
	Sole variety mixture			Intensified variety mixtures			Sole variety mixture			Intensified variety mixtures		
	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev
<b>DS of Rust</b>												
A	1.0 a	0-2	2.2	10.2 a	0-30	10.8	1.0 a	0-10	4.5	44.0 a	0-70	24.6
B	30.2 c	5-53	17.2	15.6 b	2-33	11.8	33.5 b	0-77	30.6	37.2 b	0-67	28.2
C	9.9 b	0-23	11.9	19.7 b	2-40	13.9	33.8 b	0-67	25.3	32.5 b	0-70	32.7
<b>DS of ALS</b>												
A	6.5 a	0-20	8.2	6.8 a	0-10	5.8	10.7 a	0-30	11.7	14.5 a	5-30	8.9
B	12.8 b	2-20	7.2	11.7 b	0-30	10.1	21.0 b	5-50	14.5	9.6 a	0-20	8.6
C	8.8 a	0-15	7.3	13.3 b	0-30	10.3	21.3 b	10-40	10.0	30.0 b	10-53	14.8
<b>DS of AsLS</b>												
A	42.1 a	33-57	8.0	50.2	27-77	19.7	65.5	50-77	8.8	66.1	57-77	8.4
B	66.9 c	37-83	16.6	44.4	33-37	16.3	71.1	67/83	6.6	67.2	57-73	6.64
C	55.4 b	28-83	22.0	61.9	27-87	23.0	67.3	53-77	7.8	67.7	57-83	9.9
<b>DS of Anthracnose</b>												
A	36.6	30-43	5.6	39.7	13-43	6.7	39.4	33-43	5.7	37.2	27-37	6.5
B	31.1	23-43	8.8	37.8	27-43	6.6	38.3	33-43	5.7	40.8	33-43	6.9
C	32.4	23-43	7.6	31.5	23-37	7.0	40.7	33-43	5.4	41.6	33-43	4.8
<b>100 seed weight (g)</b>												
A	44.4	29-57	9.0	38.9	18-52	16.7	41.9	25-62	11.6	43.3	31-57	14.0
B	47.8	29-65	13.2	36.7	41-52	14.0	48.9	20-58	-	38.8	19-52	12.6
C	37.8	25-52	13.0	37.8	26-52	9.6	41.1	20-55	14.8	41.3	25-62	12.5

<sup>1</sup>Values having different letters are significantly different at Duncan P < 0.05.

<sup>2</sup>Average of the lowest and highest values of replicates.

Similarly, statistical differences in yield were observed among *Chacra* and *Allpa* variety mixtures in both management approaches in 2009 and 2016 (Tables 8 and 9). The average yield of *Chacra* was greater than *Allpa* with all management approaches, site, and year in Cotacachi. In Tumbaco, the average yield of *Allpa* was greater than *Chacra* at SVM, but the opposite at IVM (Table 9). These results show that *Chacra* performs better in temperate areas (Cotacachi) under any crop management approach, whereas *Allpa* are better adapted to the warmer valley (Tumbaco), only at SVM. In Tumbaco, *Chacra* plants had a more robust development affecting *Allpa* plants at IVM.

*Chacra* and *Allpa* variety mixtures performed differentially within both SVM and IVM in 2009 (Table 8). *Chacra* B performed better at SVM, while *Chacra* A performed better at IVM. Similarly, *Allpa* B performed better at SVM, while *Allpa* C performed better at IVM. In 2016 on the other hand, *Chacra* and *Allpa* variety mixtures performed similarly at both SVM and IVM, except for *Allpa* A and *Allpa* B, which performed better at IVM (Table 9).

LER of all *Chacra* variety mixtures was higher than 0.5 in Cumbas Conde and Morochos in 2009 (Table 8) and in Cumbas Conde and Tumbaco in 2016 (Table 9), showing that the IVM approach favors *Chacra* in any condition. LER for *Allpa* was also generally higher than 0.5 in Cumbas Conde and Morochos in 2009 and 2016, since tLER is over 1 (Table 8 and 9); however in Tumbaco in 2016 *Allpa* LER was slightly lower than 0.5 (Table 9). These results show that the IVM approach also favors *Allpa*, but only in Cotacachi, and the IVM approach is not favorable for *Allpa* in Tumbaco.

**Table 7. Average, range, and standard deviation of disease severity (DS) of rust, ALS, and spider mite damage, and 100 seed weight of *Allpa* variety mixtures evaluated in sole variety mixture and intensified variety mixture in Cumbas Conde and Tumbaco in 2016.**

Variety mixture	Cumbas Conde-Cotacachi						Tumbaco-Quito					
	Sole variety mixture			Intensified variety mixture			Sole variety mixture			Intensified variety mixture		
	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev	Avg <sup>1</sup>	Range <sup>2</sup>	St. dev
<b>DS of Rust</b>												
A	0.7 b	0-10	13.8	16.5	0-43	16.1	7.8	0-40	13.8	5.6	0-37	14.1
B	8.9 b	0-40	12.0	12.8	0-40	13.8	7.1	0-40	15.0	5.3	0-27	12.8
C	5.3 b	0-30	11.0	13.0	5-35	13.4	8.9	0-20	16.2	4.7	0-30	12.4
D	0.7 a	0-30	4.7	12.2	0-37	15.3	9.7	0-47	13.9	5.2	0-30	10.3
<b>DS of ALS</b>												
A	37.0 b	1-70	28.2	35.7	0-73	31.4	3.0	0-17	6.4	3.3	0-23	6.7
B	46.2 b	0-73	30.5	23.9	0-53	21.2	1.6	0-20	4.3	1.8	0-13	4.6
C	21.3 a	0-73	26.1	21.6	2-55	25.1	4.8	0-20	8.5	2.4	0-13	4.5
D	30.6 b	0-80	25.4	30.7	0-80	28.7	3.7	0-13	5.6	1.8	0-10	4.2
<b>DS of AsLS</b>												
A	-	-	-	-	-	-	-	-	-	15.4	0-20	12.6
B	-	-	-	-	-	-	5.9	0-13	10.7	15	0-15	7.1
C	-	-	-	-	-	-	5.6	0-20	9.3	25.8	1-20	8.6
D	-	-	-	-	-	-	7.5	0-20	11.5	-	-	-
<b>Spider mite damage</b>												
A	-	-	-	-	-	-	21.5	0-43	20.1	37.8 a	10-50	17.8
B	-	-	-	-	-	-	10.4	0-43	18.7	41.4 a	13-60	14.6
C	-	-	-	-	-	-	14.8	0-43	20.0	46.7 ab	10-57	16.8
D	-	-	-	-	-	-	15.8	0-47	19.3	53.3 b	17-70	22.4
<b>100 seed weight</b>												
A	38.3	24.4-43.7	7.8	41.6	18.8-36.4	7.5	41.5 ab	30.7-49.4	5.6	40.9	29.5-52.7	7.1
B	41.7	28.6-54.8	8.6	41.8	24.5-51.0	15.6	43.6 a	34.6-51.8	6.3	41.7	32.1-50.9	6.3
C	34.3	27.0-46.6	4.4	38.9	28.5-47.1	7.2	36.5 b	27.0-45.6	8.5	36.3	26.8-47.3	8.1
D	48.6	30.1-57.0	13.9	36.8	29.0-54.7	9.6	40.4 ab	33.4-53.7	7.1	37.7	27.5-57.3	8.9

- Symptoms were erratic in the field

<sup>1</sup>Values having different letters are significantly different at Duncan P < 0.05.

<sup>2</sup>Average of the lowest and highest values of replicates

LER varied significantly among *Chacra* and *Allpa* variety mixtures in 2009 (Table 8). LER tendency among variety mixtures were similar in Morochos and Cumbas Conde for both mixture types, with higher LER for *Chacra* C and *Allpa* A. In 2016, on the other hand, LER of *Chacra* and *Allpa* variety mixtures were similar in both sites (Table 9). These results show that LER differences among variety mixtures are expressed under less favorable conditions as in Cotacachi in 2009, where disease epidemics were severe.

In this study, the common negative association of LER with yield at SVM, and the positive association of LER with yield at IVM was observed for *Chacra* and *Allpa* in 2009 and 2016 (Figs. 1 and 2). These results show that *Chacra* and *Allpa* intensification is a favorable approach when yield is threatened. Another important LER trait was the *Chacra* and *Allpa* LER compensation;

thus in 2009, LER of *Chacra* (0.76) was lower than the LER of *Allpa* (1.07) in Morochos, but LER of *Chacra* (0.93) was higher than LER of *Allpa* (0.49) in Cumbas Conde (Table 8). In this case, LER compensation was closely associated with *Allpa* damage due to diseases; therefore, *Allpa* performs better in IVM at lower disease epidemics as in Morochos in 2009, and high disease epidemics appears affect the performance of *Allpa* in IVM, as in Cumbas Conde-in 2009. At low disease epidemics in Cumbas Conde in 2016, LER of *Chacra* (0.79) and LER of *Allpa* (0.72) were similar (Table 9). This balanced *Chara* and *Allpa* LERs of around 0.75 for each type, reaching a tLER of around 1.5 appear the potential LER in Cotacachi.

**Table 8. Yield and Land Equivalent Ratio (LER) of *Chacra* and *Allpa* variety mixtures evaluated in sole variety mixture (SVM) and Intensified Variety Mixture (IVM) in Cotacachi in 2009.**

Sole variety mixture (SVM) and intensified variety mixture (IVM) in Cotacam in 2009.						
Variety mixture	Morochos			Cumbas Conde		
	Yield (kg/ha)		LER	Yield (kg/ha)		LER
	SVM	IVM		SVM	IVM	
<b>Chacra</b>						
A	564.4 b	463.7 a	0.82 a	288.3 a	399.5 a	1.39 ab
B	711.2 a	414.0 b	0.58 b	243.1 a	237.3 b	0.98 b
C	519.3 c	457.8 a	0.88 a	159.1 b	304.4 b	1.99 a
<b>Allpa</b>						
A	264.7 b	243.7	1.04 b	170.0 b	129.5	0.76
B	352.3 a	213.4 b	0.62 c	204.5 ab	88.0	0.43
C	227.5 c	351.7 a	1.54 a	271.8 a	142.2	0.52
<b>Average</b>						
Chacra	598.3 a	445.2 a	0.76 b	230.2 a	313.7 a	1.45 a
Allpa	281.5 b	282.6 b	1.07 a	215.4 b	119.9 b	0.57 b
<b>Total Average</b>		<b>727.8</b>	<b>tLER 1.83</b>		<b>433.6</b>	<b>tLER 2.02</b>

Values having different letters are significantly different at Duncan < 0.05.

In Tumbaco, the nature of intensification was different. The average LER of *Chacra* (0.93) in Tumbaco was significantly higher than of *Allpa* (0.49)(Table 9), even at low disease pressure, showing that *Chacra* had a competition effect on *Allpa*, and *Allpa* did not have any effect on *Chacra*. Nevertheless, the total LER is still high, also around 1.5.

Independent of disease epidemics and agroecological conditions, the tLER (LER *Chacra* + LER *Allpa*) were higher than 1.4, showing benefits of *Chacra* and *Allpa* intensification. Intensification was more beneficial under high disease pressure as in Morochos (tLER of 1.83) and Cumbas Conde (tLER of 2.02) in 2009 (Table 8). At less severe disease pressure the tLER tended to be around 1.5, as in Cumbas Conde (tLER of 1.5) and Tumbaco (tLER of 1.42) in 2016 (Table 9).

Yield of *Chacra* and *Allpa* variety mixtures were negatively correlated with disease epidemics and spider mite damage (Table 10). Yield of *Chacra* was negatively correlated with rust and anthracnose in Cotacachi in 2009 and 2016, and with AsLS and spider mites in Tumbaco in 2016.

Yield of *Allpa* was negatively correlated with rust in Cumbas Conde in 2016, and with spider mites in Tumbaco in 2016. Yield of *Chacra* was positively correlated with 100 seed weight in Cotacachi in 2009, and yield of *Allpa* was positively correlated with 100 seed weight in Cotacachi and Tumbaco in 2016.

**Table 9. Yield and land equivalent ratio (LER) of *Chacra* and *Allpa* variety mixtures evaluated in sole variety mixture (SVM) and intensified variety mixture (IVM) in Cotacachi in 2016.**

Variety mixture	Cumbas Conde-Cotacachi			Tumbaco- Quito		
	Yield (kg/ha)		LER	Yield (kg/ha)		LER
	SVM	IVM		SVM	IVM	
<b>Chacra</b>						
A	697.8	480.5	0.69	699.0	765.9	1.09
B	593.1	550.2	0.93	715.2	657.8	0.92
C	679.6	508.5	0.75	578.0	537.8	0.93
D	761.0	588.7	0.77	575.0	528.5	0.92
<b>Allpa</b>						
A	367.0	212.6	0.58	737.0	488.0 a	0.66
B	390.4	313.5	0.83	1021.0	411.9 ab	0.40
C	286.7	251.1	0.88	749.4	353.6 b	0.47
D	447.7	258.6	0.58	885.7	359.8 b	0.41
<b>Average</b>						
Chacra	682.9 a	531.9 a	0.79	641.8 b	599.1 a	0.93 a
Allpa	372.9 b	261.4 b	0.72	848.3 a	403.3 b	0.49 b
<b>TOTAL AVERAGE</b>		<b>793.3</b>	<b>tLER 1.5</b>		<b>1002.4</b>	<b>tLER 1.42</b>

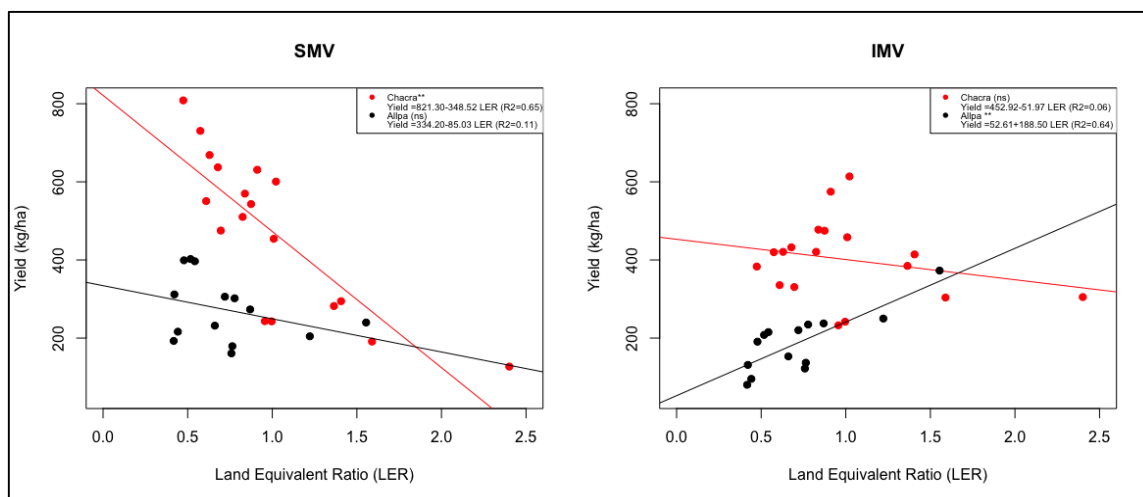
Values having different letters are significantly different at Duncan P < 0.05.

#### 4.3.4 Richness and evenness

Richness and evenness of *Chacra* and *Allpa* in 2009 varied among variety mixtures across crop management approaches (Table 11). Richness of *Chacra* varied from 4.3 to 6.1, and richness of *Allpa* varied from 3.5 to 6.5. Evenness also varied among variety mixtures; but unlike richness, differences were not consistent across crop management approaches and sites. Evenness for *Chacra* varied from 0.49 to 0.69, and evenness for *Allpa* varied from 0.51 to 0.73.

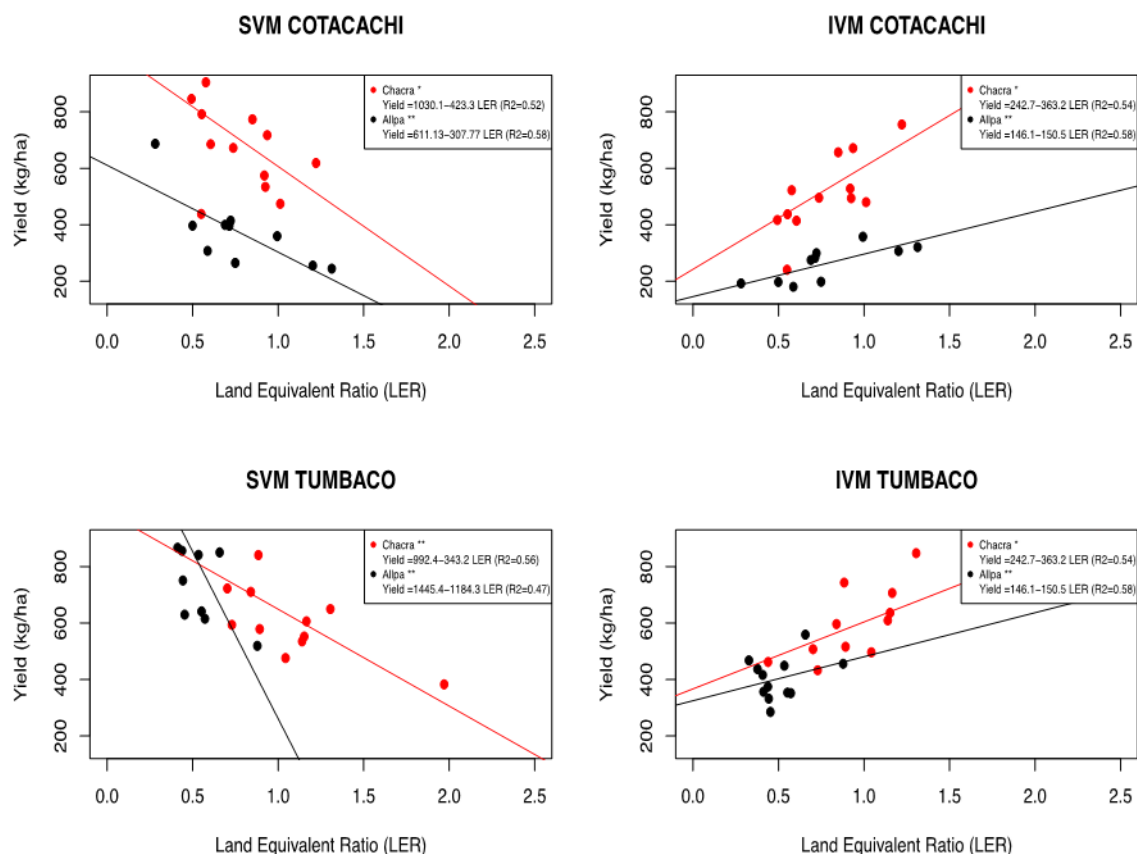
Richness and evenness of *Chacra* and *Allpa* variety mixtures in 2016 are shown in Table 12. Statistical differences of richness of *Chacra* variety mixtures was only observed in Cumbas Conde at IVM, and statistical differences of richness of *Allpa* variety mixtures was only observed in Tumbaco at SVM. Richness of *Chacra* varied from 9 to 11.2, and richness for *Allpa* varied from 10.3 to 11.2. Evenness, on the other hand, varied among *Chacra* variety mixtures in Cumbas Conde at both SVM and IVM, and among *Allpa* variety mixtures in Cumbas Conde at SVM. Evenness of *Chacra* varied from 0.78 to 0.88, and evenness for *Allpa* varied from 0.82 to 0.88.

In this study, richness and evenness were negatively correlated with DS and spider mite damage (Table 10). For *Chacra*, richness and evenness were negatively correlated with rust and ALS in Cotacachi in 2009, while richness and evenness were negatively correlated with rust, ALS and AsLS in Tumbaco in 2016. For *Allpa*, richness and evenness was negatively correlated with rust in Tumbaco in 2016. Surprisingly, a positive correlation coefficient was recorded for richness and evenness with spider mite damage in Cumbas Conde in 2016. Richness and evenness were poorly correlated with yield and weight of 100 seeds.



**Figure 1. Yield and Land Equivalent Ratio association of *Chacra* and *Allpa* variety mixtures evaluated in Sole variety mixture (SVM) and Intensified Variety Mixture (IMV) in Cotacachi in 2009.**





**Fig 2. Yield and Land Equivalent Ratio association of Chacra and Allpa variety mixtures evaluated in Sole Variety Mixture (SVM) and Intensified Variety Mixture (IVM) in Cotacachi and Tumbaco in 2016.**

#### 4.4 Discussion

##### 4.4.1 Variety mixture reactions to diseases and spider mites

Since the study was implemented in different agroecological conditions, variety mixtures were exposed to a wide range of pathogens and spider mite populations. Rust, ALS and AsLS were the main diseases in Cotacachi and Tumbaco in 2009 and 2016. Spider mites were recorded in Cotacachi and Tumbaco only in 2016, and anthracnose was only recorded in Cotacachi in 2009. Pest and diseases reported in this study are consistent with other reports for climbing common bean in Ecuador (Lepíz et al., 1995; Peralta et al., 2009). Rust has been consistently reported along the highlands as the most important disease in bush and climbing types (Jiménez et al., 1996; Lepíz et al., 1995; Peralta et al., 2009), and resistance to this disease has been a main objective of common bean breeding programs in Ecuador (Murillo et al., 1999; Ochoa et al., 1999; Peralta et al., 2009). ALS has on the other hand been considered primarily a bush type disease of the valleys (Peralta et al., 2007); however, ALS was important in the valley but also in the highland in this study. Anthracnose has been reported as an important disease of climbing

types (Lepíz et al., 1995; Murillo et al., 1997; Peralta et al., 2007); however, in this study, the disease was only present in Cotacachi in 2009. Spider mites reported in bush types (Peralta et al., 2013, 2007) have not been reported as a constraint to climbing types, and although late in the season, spider mite damage was severe in 2016 in Cotacachi. Dynamics of disease and spider mite epidemics observed in this study in regards to previous reports appears associated with changes in conducive conditions to pathogens, which are apparently becoming less conducive to anthracnose, and more conducive to ALS and spider mites.

Rust epidemics initiated early in the season in all sites and years, and progressed with a wide variation in DS among the plants evaluated in *Chacra* (Tables 3 and 4) as well as *Allpa* (Tables 6 and 7) variety mixtures. Large variation of DS among plants is associated with resistance operating in variety mixtures. High richness of resistance of Cotacachi genotypes components of variety mixtures was identified in greenhouse and field studies (Chapter 3). This resistance was shown functional, with a high “mixture effect” (Espinoza and Ochoa, 2012), where the average DS of lines grown in monoculture was 45% and the average DS of lines grown in the mixture was 26.1 %. Similarly, Mundt and Leonard (1986) report a reduction from 25 to 50% with different proportions of rust resistance in the mixture. Therefore, diversity of variety mixtures in the common bean cropping system studied is functional, as defined by Finck and Wolfe (2006)

**Table 10. Pearson coefficient of correlation between disease severity (DS) of rust, ALS, anthracnose, and AsLS, spider mite damage, 100 seed weight and yield with richness, evenness and yield in Cotacachi and Tumbaco in 2009 and 2016**

	Cotacachi 2009 <sup>1</sup>			C. Conde- Cotacachi 2016			Tumbaco-Quito 2016		
	Richness	Evenness	Yield	Richness	Evenness	Yield	Richness	Evenness	Yield
<b>Chacra</b>									
DS of rust	-0.35*	-0.10	-0.64**	-0.03	0.20	-0.59**	-0.34*	0.05	0.09
DS of ALS	-0.32*	-0.45	0.45	0.36	0.30	-0.31	-0.44*	-0.45*	0.16
DS of anthracnose	0.21	-0.14	-0.49**	-	-	-	-	-	-
DS of AsLS	0.12	0.09	0.18	0.01	0.03	-0.07	-0.27	-0.36*	-0.72***
Spider mites damage	-	-	-	0.68**	0.57*	-0.27	-0.04	-0.06	-0.80***
100 seed weight	0.31	0.25	-0.11	-0.44*	-0.41*	0.57**	-0.28	-0.61**	-0.02
Yield	-0.37	-0.40	-	-0.30	-0.33	-	0.10	0.07	-
<b>Allpa</b>									
DS of rust	0.30	0.42**	-0.13	-0.14	-0.21	-0.60**	0.21	-0.34*	0.31
DS of ALS	0.21	0.40	-0.13	-0.24	-0.08	-0.01	-0.19	-0.16	0.14
DS of anthracnose	-0.13	-0.01	-0.69	-	-	-	-	-	-
DS of AsLS	0.04	0.28	-0.01	-	-	-	-0.26	-0.26	0.79*
Spider mites damage	-	-	-	-	-	-	-0.27	-0.10	-0.88**
100 seed weight	-0.14	-0.26	0.14	0.26	0.08	0.62**	0.09	-0.07	0.38*
Yield	0.20	0.09	-	0.20	0.13	-	0.21	0.04	-

<sup>1</sup>Calculations using data from Cumbas Conde and Morochos

AsLS and spider mites began late in Cotacachi, and although severe epidemics were recorded, they did not correlate with yield. In Tumbaco on the other hand, AsLS and spider mites began earlier and correlated negatively with yield, suggesting that AsLS and spider mites will remain of

low importance in Cotacachi as long as they begin late. These results also show differential importance of diseases among common bean regions. Rust and ALS are more important in temperate regions, while AsLS and spider mites are more important in the valleys.

**Table 11. Richness and evenness of *Chacra* and *Allpa* variety mixtures evaluated in sole variety mixture (SVM) and intensified variety mixture (IVM) in Morochos and Cumbas Conde (Cotacachi) in 2009.**

mixture (IVM) in Morochos and Cumbas Conde (CotaCachi) in 2005.										
Variety mixture	Richness				Average richness	Evenness				Average evenness
	Morochos		Cumbas Conde			Morochos		Cumbas		
	SVM	IVM	SVM	IVM		SVM	IVM	SVM	IVM	
Chacra										
A	4.3 b	5.0	3.5 b	4.5 b	4.3	0.48	0.56 a	0.29 b	0.65 b	0.49
B	6.0 a	4.4	7.0 a	7.0 a	6.1	0.64	0.49 c	0.81 a	0.81 a	0.69
C	5.0 ab	4.5	8.0 a	6.0 ab	5.9	0.58	0.64 b	0.68 a	0.68 ab	0.64
Allpa										
A	2.5 b	5.0 b	3.0 b	3.5	3.5	0.26 b	0.72	0.60	0.44 b	0.51
B	6.5 a	7.0 a	8.0 a	4.5	6.5	0.74 a	0.81	0.78	0.61 ab	0.73
C	5.5 a	4.0 b	5.5 a	3.5	4.6	0.61 a	0.55	0.77	0.63 a	0.64

Values having different letters are significantly different at Duncan P < 0.05.

Conducive conditions together with early infection has resulted in severe rust pressure that has allowed farmers to select the diverse sources of resistance identified in Cotacachi variety mixtures (Chapter 3). This diversity, as mentioned before, is functional contributing significantly to rust control in Cotacachi. The high DS and low standard deviation for AsLS and spider mites show lack of resistance in genotypes components of variety mixtures, which explains the severe epidemics observed in most experiments.

**Table 12. Richness and evenness of *Chacra* and *Allpa* variety mixtures evaluated in sole variety mixture (SVM) and intensified variety mixture (IVM) in Cumbas Conde (Cotacachi) and Tumbaco (Quito) in 2016.**

mixture (IVM) in Cumbas Conde (Cotacachi) and Tumbaco (Quito) in 2010.										
Variety mixture	Richness				Average richness	Evenness				Average evenness
	C Conde		Tumbaco			C. Conde		Tumbaco		
	SVM	IVM	SVM	IVM		SVM	IVM	SVM	IVM	
Chacra										
A	10.3	13.0 a	10.3	13,3	11.2	0.82 ab	0.90 a	0.86	0.9	0.87
B	11.3	14,3 a	10.0	11.3	10.9	0.87 a	0.90 a	0.87	0.87	0.88
C	10.3	14.0 a	10.3	9.3	11.0	0.88 a	0.91 a	0.86	0.86	0.88
D	8.3	8.0 b	9.7	10.0	9.0	0.70 b	0.78 b	0.79	0.87	0.78
Allpa										
A	8.0	10.3	14.0 a	9.0	10.3	0.72 b	0.83	0.89	0.84	0.82
B	11.3	10.0	9.7 b	12.3	10.8	0.83 a	0.86	0.84	0.90	0.86
C	11.0	13.0	11.7 ab	9.3	11.2	0.88 a	0.91	0.88	0.84	0.88
D	11.0	9,67	11.7 ab	10.3	11.0	0.88 a	0.87	0.87	0.87	0.87

Values having different letters are significantly different at Duncan P < 0.05.

In this study, richness and evenness were negatively correlated with rust, ALS, and AsLS, which is closely associated with richness of resistance operating in variety mixtures; therefore, these results show the importance of maintaining variety mixtures diversity. Richness and evenness did not correlate with yield, showing that maintenance of variety mixtures at farm are mostly associated with disease management and other factors that influence yield.

#### 4.4.2 Biotic stresses and intensification

Crop intensification (IVM in this study) of the same crop (high plan density) is expected to create conducive conditions to most pest and diseases, and therefore higher disease epidemics are

expected. This hypothetical effect was observed in this study for rust and ALS only in *Allpa*, which is likely due to the early exposure of *Allpa* to these diseases. Nevertheless, the inoculum increment due to higher DS of *Allpa* in the IVM did not increase DS in *Chacra*, showing that resistance operating in *Chacra* is buffering the epidemics of these diseases. For late infecting diseases such as AsLS and spider mites, *Allpa* often escaped infection, which is an important mixture advantage.

Positive correlation of richness and evenness with spider mite damage might be associated with late infections not affecting yield, complemented by the preference of spider mites for healthy plants, which are higher at high richness.

#### **4.4.3 Variety mixture performance at intensification**

LER of *Chacra* was for any variety mixture in Cumbas Conde and Tumbaco higher than 0.5 and the tLER was always over 1, showing that *Chacra* is favored by the intensification approach under all conditions, but particularly at low *Allpa* LER; therefore, lower yield of *Allpa* in IVM is compensated by the increased yield of *Chacra*. Lower yield of *Allpa* was in turn due to increased disease epidemics as in Cumbas Conde in 2009. Under less severe disease epidemics, LER of *Chacra* and *Allpa* were on the other hand balanced, as in Cumbas Conde in 2016, where *Chacra* LER of 0.79 and *Allpa* LER of 0.72 were statistically similar. This balanced interactions that favored both *Chacra* and *Allpa* applied only in Cotacachi, showing that *Chacra* and *Allpa* co-adapted in the intensification practice, creating a beneficial cropping system based on compensation and synergism. *Chacra* and *Allpa* compensation observed in this study is the key trait suggested by Davis and Woolley (1993) for intercropping.

In Tumbaco on the other hand, *Chacra* competed over the *Allpa*, however intensification was still beneficial with an average tLER of 1.42. Therefore, intensification in this case is beneficial if *Chacra* is the most important type in the intensification, which is not affected by *Allpa*, yielding in IVM similarly than in SVM. Although *Allpa* is not benefited in the intensification with an average LER of 0.49, however *Allpa* practically reached the neutral LER (0.5), which is an additional yield considering that *Chacra* already produces its potential.

An important aspect of common bean intensification was the negative association of LER with yield in the SVM approach, and the positive association of LER with yield in the IVM, showing that intensification is particularly important when yield is threatened, as in Cotacachi in 2009 where LER of *Chacra* and *Allpa* were complementarily high, reaching a LER of 2.02 in Cumbas Conde and a LER of 1.83 in Morochos. This aspect is particularly important in rain fed agroecosystems as in Cotacachi, where production depends entirely on rainfall uncertainties;

therefore, this intensification approach appears key to cope with uncertainties of climate change.

In the literature, LER is primarily used to assess intercropping, as the maize/common bean intercrop (Gebeyehu et al., 2006). Beneficial LER values (over than 1) in that study depended on common bean varieties in the intercrop, and therefore development of varieties suitable for intercropping is recommended (Baudoin et al., 1997; Davis and Garcia, 1983). This recommendation appears applicable for crop intensification in Tumbaco, where less aggressive climbing varieties could improve intensification. However, in Cotacachi, *Chacra* and *Allpa* appear already balanced in the mixture.

Yield performance of *Chacra* and *Allpa* variety mixtures depended of the interaction region x management approach. Thus, *Chacra* in Cotacachi performed better than *Allpa* in both SVM and IVM, while *Chacra* in Tumbaco performed better than *Allpa* only in IVM due to *Chacra* overgrowth, and *Allpa* performed better than *Chacra* in Tumbaco in the SVM. These results show that *Chacra* is better adapted than *Allpa* to the highland temperate region, and *Allpa* is better adapted than *Chacra* to the warmer valleys. These results show also that *Allpa* variety mixtures have been introduced into Cotacachi from the warmer valley, where they have already co-adapted with *Chacra* to the intensified approach, which is a clear farmers' reaction to improve food security.

Yield differences among Cotacachi sites in 2009 appear primarily due to rust epidemics, since it was the main difference among Cumbas Conde and Morochos, since soil nutrition was similar among sites. Negative correlation of rust DS with yield in Cotacachi (Table 11) also supports yield differences are mainly due to rust disease epidemics.

Yield performance in the intensified approach was beneficial for any biotic constraint and climatic condition. In the worse scenario with high disease pressure, as in Cumbas Conde in 2009, the average yield of the intensified approach reached 433.6 kg/ha, while in more favorable conditions, as in Morochos in 2009 and Cumbas Conde in 2016, the average yield of the intensified approach reached 727.8 kg/ha and 793.3 kg/ha, respectively. These yields appears roughly the yield potential of the intensified common bean cropping system in Cotacachi. Yield obtained in this low input cropping system under important biotic constraints is higher than the average country yield of among 300 kg/ha for climbing types (Lepíz et al., 1995; Peralta et al., 1997).

The intensification approach did not significantly affect 100 seed weight in Cotacachi, which also shows that competition is not taking place in intensified mixtures in Cotacachi. On the other

hand, 100 seed weight was affected by the intensification approach in Tumbaco, where competition of *Chacra* over *Allpa* plants was evident also for yield. These results suggest that intensification might affect common bean production in the valleys if it is devoted to commerce.

Contribution of the intensification approach to crop security is not only related with a better adaptation of the variety mixture to biotic and abiotic stresses, and/or due to the synergic compensation to ensure yield, but also to ensure food availability. *Chacra* and *Allpa* due to diverse crop cycles, collectively provide food to the household during the entire year.

Common bean intensification in Cotacachi is important for subsistence agriculture to satisfy household food demand; however, it could be also valid for commercial purposes. Similar outcomes could be obtained using climbing and bush commercial types in the intensification approach studied. Commercial intensification could be even better achieved with less aggressive climbing varieties as INIAP TOA in the warmer valleys (INIAP, 1993), which is the main objective of modern breeding for climbing type varieties (Baudoin et al., 1997).

Most sustainable intensification approaches for subsistence agriculture found in the literature are proposals to improve crop productivity (Devkota et al., 2016; Mungai et al., 2016; Nyagumbo et al., 2016; Pradhan et al., 2016; Wani et al., 2016). Some studies even question achievement especially for food-inadequate households in Africa (Ritzema et al., 2017). Furthermore, sustainability of intensification is in debate to meet the challenge to “increase agricultural output while keeping the ecological footprint as small as possible”, but also to meet the challenge to produce more with much fewer resources (Struik et al., 2014). Intensification approach farmers implement in Cotacachi in many ways fit with intensification required for subsistence agriculture. It is a low input intensification approach, which has been sustainable over time, showing resilience to high diseases pressure.

#### **4.5 Conclusions**

- Patterns of disease epidemics are changing over time in the climbing common bean cropping system. Thus, anthracnose considered an important disease in the past was marginally important in this study. Although they appeared late in the season, spider mites, not reported in the past, caused severe epidemics. Changes of environmental conditions probably explain changes in disease epidemic patterns. AsLS and spider mites can be important threats to climbing types if epidemics start early, since low levels of resistance to these diseases are operating in variety mixtures.
- In Cotacachi, *Chacra* and *Allpa* intensification showed compensatory interactions; co-adaptation of these common bean types was beneficial for the cropping system. In

Tumbaco on the other hand, intensification showed primarily competition of *Chacra* over *Allpa*; however, the global intensification was still beneficial. In Cotacachi, compensation of *Chacra* and *Allpa* variety mixtures was observed when *Allpa* was affected by early diseases (rust and ALS). In this case, although epidemics affected *Allpa*, *Chacra* compensated this loss and the global yield (*Chacra* and *Allpa*) was not actually affected. *Allpa* was additionally less affected under less conducive conditions to early diseases since regularly escape late diseases. In favorable conditions, as *Allpa* is early covers efficiently the lower horizontal space, while *Chacra* covers later the vertical space; therefore, the interaction is low competitive, and both types progress efficiently reaching an individual LER of around 0.75, and collectively a tLER of around 1.5.

- Intensification was beneficial for yield to enhance fitness to any biotic constraint or climatic conditions, but it was especially important when these conditions threatened the yield. Intensification is particularly important in rain fed agro-ecosystems, where production depends entirely on rainfall uncertainties, and therefore it is a key approach to cope with climate change uncertainties.
- In this study, *Chacra* was better adapted than *Allpa* to the temperate highlands, while *Allpa* was better adapted than *Chacra* to the warmer valleys, suggesting that *Allpa* has been introduced into the highland, where it has co-adapted with *Chacra* to the intensified approach.
- Weight of 100 seeds was not significantly affected by the intensified approach in Cotacachi, while it was affected in Tumbaco, confirming the compensatory nature of intensification in Cotacachi and the competition nature of intensification in Tumbaco.
- Contribution to crop security of *Chacra* and *Allpa* variety mixture intensification is not only related with a better crop adaptation to adverse conditions and yield improvement, but also improving food provision. *Chacra* and *Allpa* due to diverse crop cycles, collectively provide food to the household during the entire year.
- Intensification is important in traditional agriculture to satisfy household food demand; however, it could be also valid for commercial purposes through commercial varieties introduction within the mixture. It is also valid for warmer valleys by using less aggressive *Chacra* varieties.
- Traits for intensifying common bean mixtures fit in several ways with a sustainable intensification that is required nowadays for traditional agriculture. This low input intensification approach has provided food to households in a sustainable manner since a long time. In addition, components of intensification adapts each other to adverse conditions in a flexible manner, showing also resilience to several constraints.

## CHAPTER 5

### Overall conclusions and recommendations

- Farmers are analytical in relation to evidences farmers obtain from the agro ecosystem. Thus, they name in an analytical way most phenotypes components of variety mixtures, they perceive main contribution of interspecific and intraspecific diversity in regards to biotic stresses, and they differentiate among diseases and perceive disease transmission, resistance and its durability. This knowledge should be adapted to participatory initiatives implemented by the governmental extension services.
- The maize/common bean intercrop has been a key component of the temperate highland agroecosystem since these crops were domesticated or adapted to the temperate highlands of Ecuador long time ago. The cropping system has been the key for food security in the region long before Europeans arrived to America. This cropping system is still key for food security in the local communities of Cotacachi and Saraguro. Although the genetic background of maize and common bean appears dynamic to adapt to biological and socioeconomical conditions, the cultivation approaches to maintain crop diversity and sustainability appear similar since its initial implementation.
- The two way intensification approach: 1) maize and common bean (interespecific) and 2) *Chaca* and *Allpa* (intraspecific) in Cotacachi and *Chacra* and *Popayán* (interespecific) in Saraguro, should be enhanced and promoted to a wider scale, and transferred to other regions, as well as the management principles apply to other cropping systems.
- Commercial cultivation of both maize and common bean however have modified significantly the intercrop. In some areas maize is cultivated for cob production as sole crop, and although the commercial cultivation of the common bean *Canario* is done primarily intercropped with maize, sole crop of *Canario*, *Bombolin* and *Cargamanto* varieties with wire fence like supports are presently being popular.
- The main argument about difficulties of maize and common bean commercial cultivation as intercrops is the mutual plant competition, which has been documented experimentally in other regions, showing different degrees of effects depending on the maize and common bean varieties. The competition effect with the diverse Ecuadorian crop management conditions and crop diversity has not been studied, an important aspect to investigate for improvement of commercial and traditional cultivation of maize and common bean in Ecuador.
- A very important feature of this cropping system is bean cultivation as variety mixtures of *P. vulgaris* and *P. coccineus*, which appears to be implemented since domestication,



as wild types found in nature, which additionally explains the high genetic diversity found in Cotacachi and Saraguro.

- Common bean variety mixture cultivation has been a common practice along the highlands of Ecuador, and this traditional practice is only presently maintained by the old native communities of Imbayas (Cotacachi) and Saraguros (Saraguro) in southern and northern Ecuador. In the rest of common bean areas, this cultivation approach has been replaced by the commercial variety *Canario*.
- High genetic diversity found in Cotacachi and Saraguro mixtures should be conserved by an improved use, which can be achieved by studying other benefits as resistance to other pest and diseases not included in this study, drought resistance, nitrogen fixation, and adaptation to low nutrient soil content.
- In Cotacachi and Saraguro, cultivation of variety mixtures should be further enhanced to improving productivity. Although benefits are evident by the reduced crop vulnerability, sustainability and resilience are evident, improvement of cultural practices as improving soil fertility by incorporating organic fertilizers can significantly improve crop productivity.
- Benefits of variety mixture cultivation shown in this study should promote the recovery of variety mixture cultivation in other areas in a practical context, by adapting mixtures to commercial cultivation, and by promoting mixtures in other subsistence areas out of Cotacachi and Saraguro. Studies are necessary to approach both actions, among others the improvement of *Canario* mixtures, since *Canario* is already a genetically diverse population. The mixture diversity from Cotacachi and Saraguro can be adapted to other subsistence regions of climbing common bean cultivation.
- Farmers from the highlands with the aim to enhance food security has introduce *Allpa* from the valleys, which together with *Chacra* has created an intensification approach that is already adjusted to the temperate highland (Cotacachi). Similar initiatives can be promoted to other cropping system to diversify other agroecosystems.
- Variety mixtures integrated in the intensified approach (*Chacra* and *Allpa*) not only improves food security through a better food supply, but also improves productivity and resilience in unfavourable biotic conditions by a compensatory interactions of *Chacra* and *Allpa* variety mixtures. This benefits should be scaled up at country level,
- The high genetic diversity of variety mixtures resulting from the continuous recombination among genotypes components of the mixtures can be utilized by common bean breeders. Lines derived from this improvement can be immediately promoted after low costly conventional agronomical assessments.

- Pathogen evolution in commercial agriculture is detrimental to yield, and many efforts have been made to achieve durable resistance, with partial success. On the other hand, pathogen evolution in traditional agriculture is not a serious threat, since major gene and partial resistance is balanced with the pathogen population (rust in this study), creating a positive mixture effect that has been for long time reducing common bean vulnerability in the highlands of Ecuador.

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