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Diversity of Beans grown in Kyrgyzstan and Marker-aided Breeding for Resistance to *Bean Common Mosaic Virus* and Anthracnose

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Cover: Common bean flowering and different types of seeds
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Diversity of beans grown in Kyrgyzstan and marker-aided breeding for resistance to *Bean common mosaic virus* and anthracnose

Abstract

Common bean (*Phaseolus vulgaris* L.) is an important export crop in Kyrgyzstan since the end of the 20th century. Kyrgyzstan produces about 70,000 t of common beans per year, which provides jobs to 76% of the population in the Talas region. Information about genetic diversity of common beans helps to select appropriate genetic material to be used for breeding programs. Accessions originating from both Mesoamerican and Andean gene pools, including the main Kyrgyz common bean market types were analyzed using simple sequence repeats (SSR) and qualitative morphological traits. The similarity matrices generated from the molecular and morphological data were well correlated (r = 0.49**). The cluster analysis of both data sets grouped the accessions according to their gene pools of origin, where Mesoamerican accessions were more diverse than Andean accessions. Both SSR and qualitative morphological markers were suitable for assigning cultivars to their gene pools of origin. Furthermore, information about traits of interest for Kyrgyz farmers has been gained. We found that *Bean common mosaic virus* strain NL6 and anthracnose affect this crop and reduce its grain yield significantly in Kyrgyzstan. The susceptible Kyrgyz cultivars Ryabaya, Kytayanka and Lopatka were included as recurrent parents in a backcrossing breeding scheme for introducing host plant resistance to these diseases from donor cultivars. After the 4th backcross, seeds (color, shape, size) and pods (shape) were similar to the respective recurrent parent. The sequence characterized amplified region (SCAR) markers SW13, SBD5 and SCAreoli were used successfully in marker-aided backcrossing for pyramiding the *I*, *bc-1*^{2} and *Co-2* genes, which provide host plant resistance to BCMV and anthracnose, respectively. Inoculation tests with anthracnose races delta and gamma, virus strain NL3, and DNA markers confirmed the presence of resistance genes in the offspring.

Keywords: Phaseolus vulgaris, DNA markers, genetic diversity, microsatellites, backcross breeding

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Dedication

This thesis is dedicated to the memory of the late Professor Arnulf Merker, my grandmothers, the late Fekla Hegay and the late Elena Kim.

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Hegay, S., Geleta, M., Bryngelsson, T., Gustavsson, L., Persson Hovmalm, H. & Ortiz, R. (2012). Comparing genetic diversity and population structure of common beans grown in Kyrgyzstan using microsatellites. *Scientific Journal of Crop Science* 1(4), 63-75.

II Hegay, S., Geleta, M., Bryngelsson, T., Garkava-Gustavsson, L., Persson Hovmalm, H. & Ortiz, R. Genetic diversity analysis in *Phaseolus vulgaris* using morphological traits (submitted).

III Hegay, S., Ortiz, R., Garkava-Gustavsson, L., Persson Hovmalm, H. & Geleta, M. (2013). Marker-aided breeding for resistance to *bean common mosaic virus* in Kyrgyz bean cultivars. *Euphytica*. Doi: 10.1007/s10681-013-0928-9.

IV Hegay, S., Geleta, M., Bryngelsson, T., Garkava-Gustavsson, L., Persson Hovmalm, H. & Ortiz, R. Introducing host plant resistance to anthracnose in Kyrgyz beans through marker-aided and inoculation-based backcrossing (submitted).

The contribution of Sergey Hegay to the papers included in this thesis was as follows:

I Planned greenhouse experiments, requested germplasm, did research in a greenhouse, DNA extraction plus DNA marker fingerprinting in laboratory, analyzed data, wrote the manuscript with inputs and edits from co-authors

II Planned greenhouse experiments, requested germplasm, did research in greenhouse, data recording of morphological characters, analysed data, wrote the manuscript with inputs and edits from co-authors

III Planned greenhouse and field experiments, did planting, hybridization, inoculatins and screening in a greenhouse and in the field, plus laboratory work, analysed data, wrote the manuscript with inputs and edits from co-authors

IV Planned greenhouse and field experiments, did planting, hybridization, inoculation and screening in greenhouse and at the field, plus laboratory work, analysed data, wrote manuscript with inputs and edits from co-authors

Abbreviations

BCMNV	*Bean common mosaic necrosis virus*
BCMV	*Bean common mosaic virus*
FAO	Food and Agriculture Organization of the United Nations
NTSYS	Numerical taxonomy system
PCoA	Principal coordinate analysis
SAHN	Sequential agglomerative hierarchical nested cluster analysis
SCAR	Sequence characterized amplified region
Sida	Swedish International Development Agency
SSR	Simple sequence repeat
STS	Sequence-tagged site
UPGMA	Unweighted pair group method with arithmetic average

1 Introduction

1.1 Classification of *Phaseolus vulgaris*

Common beans (*Phaseolus vulgaris* L.) belong to the genus *Phaseolus,* subtribe Phaseolionae, tribe Phaseoleae, subfamily Papilionideae in the family Fabaceae (McClean *et al.*, 2004; Singh, 2001; Debouck, 1991). It includes dry beans, green beans, shelling beans and popping beans. The genus *Phaseolus* previously included more than 180 species (Ditmer *et al.*, 1937), but now the genus includes only 50 species (Delgado-Salinas *et al.*, 2006). All of them are Meso- and South American native species. Only five of these species have been domesticated, bred and widely used for human consumption, namely common bean (*P. vulgaris* L.), runner bean (*P. coccineus* L.), year bean (*P. dumosus* L.), tepary bean (*P. acutifolius* L.) and lima bean (*P. lunatus* Greenman) (Aragao *et al.*, 2011; Gepts *et al.*, 2008). All cultivated *Phaseolus* species and their wild relatives are diploid ($2n = 2x = 22$) and have 11 pairs of chromosomes (Mercado-Ruaro & Delgado-Salinas, 1998). Morphological seed characters (size, color, shape and hilum) are useful descriptors for distinction between cultivated *Phaseolus* species (CIAT, 1986a). Common bean accounts for above 85% of world production among cultivated *Phaseolus* species (Singh, 2001).

The species in the genus *Phaseolus* were classified into primary, secondary, tertiary and quaternary gene pools related to *Phaseolus vulgaris*. The primary gene pool includes wild and cultivated forms of *P. vulgaris* as their fertile hybrids (Singh *et al.*, 1995). The secondary gene pool comprises cultigens of *P. coccineus*, *P. costaricensis* and *P. polyanthus.* Hybrids among these species are feasible but the hybrids between them and common bean may be sterile. The tertiary gene pool consists of *P. acutifolius* and *P. parvifolius*. Their hybrids after crossing with common bean, can be obtained through

embryo rescue (Andradf-Aguilar & Jackson, 1988). *P. lunatus, P. fuliformis* and *P. angustissimus* belong to the quaternary gene pool. So far none of the hybrids between common bean and these species are known to be fertile (Singh, 2001).

Phaseolus vulgaris (common bean) was first described by Linnaeus in 1753 (Brücher, 1988). *Phaseolus vulgaris* var. *aborigineus*, which was found in west and central Mexico, Central America and northwestern Argentina, was first proposed as a wild relative of common bean (Kaplan, 1981; Berglund-Brücher & Brücher, 1976; Gentry, 1969). Further research on hybrids between wild and cultivated forms of common beans indicated that they belong to the same species, *P. vulgaris* (Burkart & Brücher, 1953).

1.2 Origin of common beans

Carl Linnaeus or Carl von Linné (after his ennoblement), was a Swedish botanist, physician, and zoologist, who laid the foundations for the modern scheme of binomial nomenclature for species. Linnaeus proposed in 1753 India as the origin of common beans when he did his classical work of plant taxonomy in Uppsala, Sweden (Brücher, 1988). Vavilov (1931) corrected this hypothesis and indicated Mesoamerica as the center of origin for common bean based on the crop diversity and the presence of crop wild relatives. Later, Burkart & Brücher (1953) found wild common bean species, *Phaseolus aborigineus* in northwest Argentina. Gentry (1969) also noticed wild and domesticated species of common beans in Mexico and Guatemala. The wild types of *P. vulgaris* and crop wild relatives found in Mexico, Central America, and South America support that this crop is originated in Latin America (Torres *et al.*, 2004; Kaplan & Lynch, 1999; Freyre *et al.*, 1996; Brücher, 1988; Berglund-Brücher & Brücher, 1976; Kaplan & MacNeish, 1960; Kaplan, 1956).

Evidence from morphology and history suggest that common beans were domesticated independently at least 7000 years ago in Mesoamerica and the Andes (Kaplan, 1981; Kaplan *et al.*, 1973). Debouck (1986) further suggested three centers of diversity for cultivated *Phaseolus* species in the American continent. The hypothesis of two independent gene pools was later confirmed by researching on the F1-hybrid weakness, agro-morphology, seed storage protein phaseolin and enzyme polymorphism (Koenig & Gepts, 1989; Singh, 1989; Gepts & Bliss, 1986; Gepts & Bliss, 1985). Bitocchi *et al.* (2012) recently proposed that common beans have three gene pools (Mesoamerica,

Andean and northern Peru-Ecuador) and wild types of common beans were first domesticated in central Mexico (Mesoamerican gene pool) and later migrated to South America.

1.3 Distribution and adaptation of common beans

Common beans can adapt to extreme environments, grow at different altitudes (50–3000 m above sea level) in most continents. The crop likes temperatures ranging from 14 to 26°C, an annual precipitation between 400 and 1600 mm year^{-1}, a slightly acid soil pH (average 5-6). They show a wide range for days to maturity (70–330) and grain yield potential (400–5000 kg ha^{-1}) (Wortmann *et al.*, 1998; Debouck, 1994). After Columbus's voyages, common beans were introduced from its native Latin America to many regions in the world. Dry and green beans were grown in 30.75 million ha with its harvest totaling 43.6 million t in 2011 (FAOSTAT, 2013), thereby providing an important food staple to millions of people worldwide (Aragao *et al.*, 2011; CIAT, 1989). The global average yield of dry and green beans are 0.8 and 1.3 t ha^{-1}, respectively. Figure 1 shows the most important producers of dry beans worldwide.

Consumers eat edible dry grains and fresh (green) pods. The dry grains are source of calories and fiber and rich on dietary protein (18-40% of seed weight), which humans need for their daily activity (Bliss, 1990).

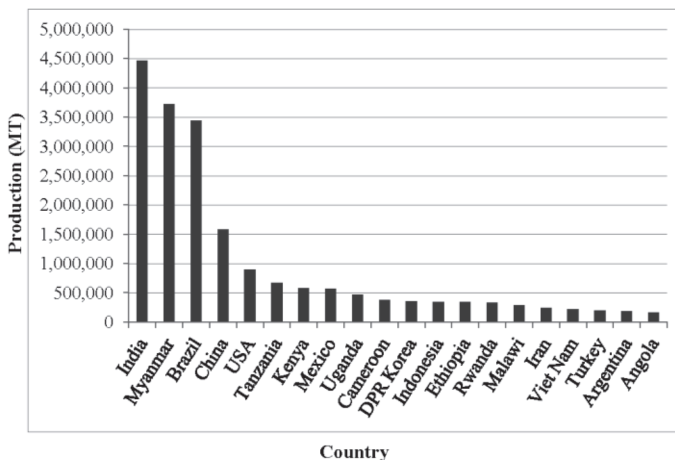


Figure 1. World leading countries producers of dry common bean sorted by decreasing order of estimated production volume. Approximately 18.3 million t of dry common beans are produced annually worldwide with an average yield of 760 kg/ha^{-1} (FAO, 2011).

Dry beans were grown in the USA on approximately 790,000 ha and with an estimated harvest close to 1.5 million t, of which 80% were from 6,236 farms in North Dakota, Michigan, Nebraska, Minnesota and Idaho in 2010. According to the 2007 Census of Agriculture, nearly 14% of the US population eats dry beans every day, which translates into an estimated annual per capita of 3 kg year^{-1} (USDA, 2013). Canada produced approximately 300,000 t of dry beans in 2010 but this country ranks among the top five exporters of this crop. It was not surprising therefore that 70% of its production is grown for trade markets. About 110,000 ha of land were used for common bean production in Alberta, Manitoba and Ontario in Canada (Beebe *et al.*, 2011; FAO, 2011).

Latin America grew 6.8 million ha with a total harvest of 6 million t in 2010. Brazil and Mexico are the main producers of common beans in this region, and their annual production was 4.1 million t (Beebe *et al.*, 2011). Most Latin American farmers (except in Argentina) who grow common beans are smallholders whose fields range between 1 and 10 ha. Common bean, together with maize, potato and rice are staple food in Latin America. Bean consumption per capita (grains and green pods) in several Latin American countries range from 6 to 18 kg year^{-1} (Broughton *et al.*, 2003), thereby showing the importance of this crop in human diets in the center of diversification of this crop. For example, Brazil is the third leading country producing common beans worldwide but exports less than 0.1% of its harvest. Common beans provide 9% protein to consumers of this crop in this country (Gepts *et al.*, 2008).

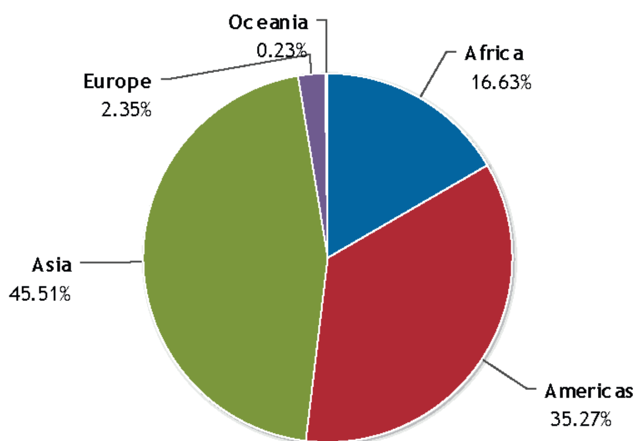


Figure 2. World production regions of common beans (FAO, 2011).

Asia is the largest continent producing common beans and exporting this crop in the world. About 46% of common beans are produced in Asia (Fig. 2). Spanish traders introduced common beans from the American continent to China 400 years ago. Currently China is one of the largest producers (1.6 million t year^{-1}) of common beans worldwide (FAO, 2011). In 2010 China produced around 9.8 million t of dry beans (FAO, 2011). The crop is grown on 1.2 million ha of family farms in various agrosystems (mainly in Guizhou, Heilongjiang, Neimenggu, Sichuan and Yunnan provinces) of the country (Zhang *et al.*, 2008). China is also one of the largest world exporters (800,000 t) of common bean (FAO, 2011). In India, common beans are grown by smallholders, who grow crop mainly by intercropping with other crops (Bakker, 2011). Common beans are both an important staple food and export crop in Myanmar. Production and export of common beans increased throughout the last decade, reaching the annual exports of 1.1 million t (Dapice *et al.*, 2011).

Table 1. *Common bean production (t) estimated by continent indicating major producers and their rankings from 1961-2009. Source: (FAO, 2011).*

	2000-09	1990-99	1980-89	1970-79	1961-69
Major producing countries					
Brazil	3,142,163	2,688,221	2,330,363	2,283,883	3,323,742
India	3,114,750	3,314,360	3,256,200	2,354,880	1,850,148
Myanmar	2,208,726	723,841	302,477	163,353	173,222
China	1,745,602	1,342,517	1,647,085	1,718,741	2,095,153
Mexico	1,144,891	1,194,116	993,917	885,526	832,468
United States of America	1,106,470	1,320,160	1,070,033	813,037	823,412
Production by region					
Asia	8,917,046	7,423,096	7,104,015	5,660,335	5,356,885
America	7,020,893	6,445,688	5,384,047	4,786,713	4,373,231
Africa	3,251,328	2,470,905	2,013,058	1,587,199	1,126,779
Europe	473,992	556,145	750,431	822,097	995,433
Subtotal	19,663,259	16,895,833	15,251,551	12,856,343	11,852,328
World	19,710,451	16,927,935	15,266,803	12,860,200	11,864,180
World ranking country					
Brazil	1	2	2	2	1
India	2	1	1	1	3
Myanmar	3	7	9	13	12
China	4	3	3	3	2
Mexico	5	5	5	4	4
United States of America	6	4	4	5	5

Common beans were introduced to Africa in the 16th Century and today are grown at 6.4 million ha mainly by smallholders but the crop shows low grain yields ranging from 0.35 to 0.75 t ha^{-1} (Katungi *et al*., 2009; Wortmann *et al*., 1998). Wortmann *et al*. (1998) indicated that the annual per capita consumption in Africa ranged from 12 to 58 kg.

The developing world (Africa, Asia and Latin America) increased common bean production, whereas the production of the crop has decreased in Europe throughout the last decade. Brazil and Myanmar have continued expanding common bean production in the last decade.

1.4 Botanical characteristics of common beans

Common bean is an annual, self-pollinated crop, mostly grown in tropical and subtropical areas of the world. Four growth habits characterize common bean cultivars: determinate bush, indeterminate bush, indeterminate prostrate and indeterminate climbers. Common bean cultivars are also classified into two classes by its growth height: dwarf (20-60 cm) and climbing (≥ 2 m). Common bean is a short-day plant, but some cultivars may adapt to different photoperiod length (White & Laing, 1989). The biological cycle of common beans plant development includes vegetative and reproductive phases. The phases have 10 plant developing stages (i.e., five stages in each phase) that are described in a manual of the Centro Internacional de Agricultura Tropical (1986b).

Debouck (1991) used plant descriptors of stem, branches, flowers and bract development for classifying the genus *Phaseolus*. Classification of widely-grown common beans helps to distinguish among cultivars and between other edible legume species (Voysest & Dessert, 1991). Seed size, color, shape are important morphological characters for describing common bean cultivars. Bean markets, traders, consumers, farmers, researchers and breeding programs use these descriptors worldwide. There are 11 groups according to seed color, while there are 3 defined clusters for seed size or 100 seed weight (small < 25 g, medium 25-40 g, and large > 40 g).

1.5 Pathogens and pests

Common bean production affected by several pathogens considered of economic importance since they reduce significantly both grain yield and quality. Major pathogens are usually transmitted by contaminated seed into bean growing areas. They include anthracnose (caused by *Colletotrichum*

lindemunthianum), angular leaf spot (*Phaeoisariopsis griseola*), halo blight (*Pseudomonas syringae pv. phaseolicola*), common bacterial blight (*Xanthomonas axonopodis pv. phaseoli*), *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV) and rust (*Uromyces appendiculatus*). These pathogens cause a grain yield loss that ranges between 5 and 100% in Latin America, depending on races of the respective pathogen and cultivars grown by farmers. The pathogens also caused serious grain yield loss in the past both in Europe and North America (Miklas *et al.*, 2006; CIAT, 1989; Schwartz *et al.*, 1982). The fungi *Phaeoisariopsis griseola*, *Colletotrichum lindemunthianum* and *Uromyces appendiculatus* cause 1.8 million t annual grain yield loss while halo blight and common bacterial blight add other 0.8 million annual grain yield loss. *Bean common mosaic virus* and insect pests (bruchids and aphids) also account for 0.4 million and 0.8 million of annual grain yield loss, respectively (Wortmann *et al.*, 1998).

1.5.1 Bean common mosaic virus (BCMV)

Bean common mosaic virus (BCMV) belongs to the *Potyvirus* genus in the *Potyviridae* family (Bos, 1971). It was named bean virus–1 (synonym *Common bean mosaic virus*) and differentiated from other viruses through their symptoms affecting common beans (Pierce, 1935). BCMV is usually transmitted through seeds (i.e., through infected embryo), but not all infected embryos will develop into an infected seedling (Ekpo & Saettler, 1974). Aphids are an important vector for spreading the virus in bean production areas and causing BCMV epidemics (Kelly, 1992). BCMV infects not only common beans but also other *Phaseolus* species and various wild legumes (Spence, 1992; Provvidenti & Braverman, 1976). BCMV seems to be widely distributed where common beans are grown and it can reduce grain yield between 35 and 98% (Wortmann *et al.*, 1998; Galves & Morales, 1989; Hampton, 1975). These viruses have been reported in common beans in Europe (Drijfhout, 1978; Drijfhout & Bos, 1977), Africa (Sengooba *et al.*, 1997; Spence & Walkey, 1995; Silbernagel *et al.*, 1986), Asia (Kapil *et al.*, 2011; Naderpour *et al.*, 2010; Ha *et al.*, 2008), America (Flores-Estevez *et al.*, 2003; Guzman *et al.*, 1997; Kelly *et al.*, 1983; Tu, 1986).

The virus movement in a plant can be local (virus moves slow from cell-to-cell) and systemic (virus moves fast from infected surface to the different plant parts). A single dominant *I* gene, which is associated with hypersensitivity, provides host plant resistance to BCMV. The recessive resistance *bc*-genes control the virus multiplication in the infected tissue. Common bean cultivars can be grouped into two clusters based on the presence

and absence of *I* resistance gene. The susceptible cultivars often show foliar symptoms with green mosaic, leaf malformation and rolling (Fig. 3).

Figure 3. BCMV / BCMNV mosaic symptoms lacking dominant *I* gene (i.e., plant bears recessive *i* alleles) and *bc*-genes.

Figure 4. Plant reactions to the infection of BCMNV NL3 strain in a greenhouse. Symptoms include red-brown pinpoint lesions on the infected primary leaves; top necrosis (synonym "black root") and later death of the infected plant.

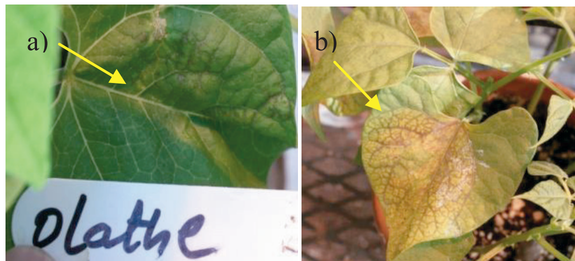


Figure 5. Mild mosaic symptoms on a primary leaf infected with a BCMNV NL3 strain. The plant lacks the dominant *I* gene but has recessive *bc-1*^{2} gene plus *bc-u* gene. a) Early symptoms of local necrosis (left); b) Infected leaf showing mild mosaic symptoms (right).

Figure 6. Local lesions on the BCMNV infected primary leaf.

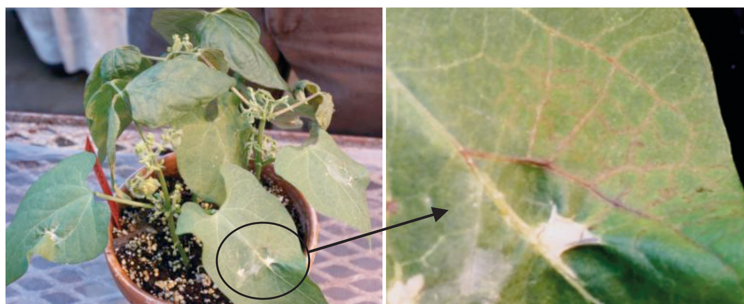


Figure 7. Vein-necrosis on the primary leaf infected by BCMNV NL3 strain. The plant has *I* and *bc-1*^{2} genes.

Resistant plants that possess *I* gene can have different symptoms depending of the presence or absence of specific *bc*-genes. It can be systemic necrosis and later plant death (Fig. 4), or mild mosaic, vein necrosis, local lesion (Fig. 5, Fig. 6, Fig. 7), or immunity (Kelly *et al.*, 1995; Drijfhout *et al.*, 1978).

1.5.2 Anthracnose in common beans

Anthracnose is one of the most destructive seed borne diseases infecting common beans (Balardin *et al.*, 1997). This fungus can attack other *Phaseolus* species and leave for up to 5 years in stored infected pods and seeds (Pastor-Corrales & Tu, 1989). It can reduce grain yield by up to 90% in susceptible plants of common bean cultivars in environments favoring this pathogen (Tu, 1981). Environmental factors (e.g. rainfall, temperature < 28°C, and high humidity) as well as infected seeds play important role for the pathogen's spore development and distribution in farms (Tu, 1982). Anthracnose symptoms are usually associated with dark brown or black lesions on the leaves, pods and stems (Fig. 8).

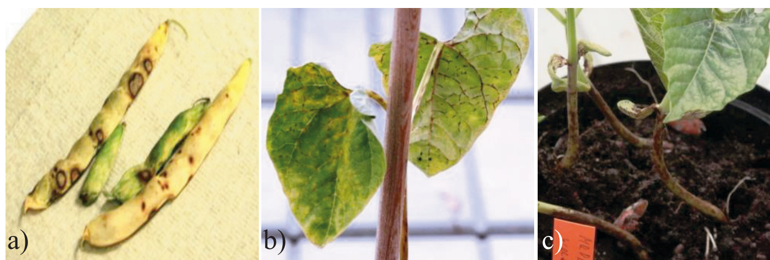


Figure 8. Anthracnose symptoms (i.e. brown dark lesion necrosis) on the different parts of common bean plant: a) in pods; b) on the primary leaf; c) on the stem.

1.6 Common bean genetic resources and genetic diversity

Gene banks have only partially characterized and evaluated the agro-morphological, physiological and pathological traits of preserved accessions (Ortiz & Engels, 2004). An appropriate characterization and evaluation of bean genetic resources facilitate their use in genetic enhancement. Bean breeding can have positive impact on increasing average grain yield in production areas. FAO (2011) data shows a yield increase from 660 kg ha^{-1} in 1990 to 760 kg ha^{-1} in 2011, which has been ascribed to the use of elite cultivars showing resistance to pathogen, pests and enhanced adaptation to abiotic stresses (Beebe *et al.*, 2011).

Several national and international gene banks were established in the last century. In 1967, CIAT (Centro Internacional de Agricultura Tropical) established its international gene bank at its headquarters in Cali (Colombia), which today holds 37,064 accessions of *Phaseolus* species, of which 26,500 are *Phaseolus vulgaris*.

Phenotypic and genotypic diversity assessments have been made in common beans and other *Phaseolus* species based on morphology and isozymes (Bassiri & Adams, 1978). A high variation was found within major common bean gene pools based on morphological characters, such as growth habit, shapes of bracteole and seed size (Singh *et al.*, 1991a); allozymes (Singh *et al.*, 1991c); phaseolin type (Koenig *et al.*, 1990); and the combination of morphology, phaseolin and isozymes (Singh *et al.*, 1991b). PCR technology developed in 1983 by Kary Mullis, rapidly found application in studying crops genetic diversity including that of common bean. The genetic diversity of common beans has been studied using different PCR-based techniques, such as random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphisms (AFLP) (Beebe *et al.*, 2001; Beebe *et al.*, 2000).

Simple sequence repeats (SSR) or microsatellites are typical co-dominant markers consisting of 2 to 6 tandem sequence repeat base pairs (Goldstein *et al.*, 1995). This marker can be used in genome analysis and for identifying a specific region (locus) in the genome. Microsatellites are highly informative markers and have been used for detecting genetic variation in many higher eukaryotic organisms including higher plants (Powell *et al.*, 1996). SSR have been also widely used for gemplasm evaluation and characterization, and to detect variation within gene pools and among races (Zhang *et al.*, 2008; Diaz & Blair, 2006). Several microsatellites have been developed and are available for

common bean characterization, and genetic diversity assessments (Blair *et al.*, 2006; Masi *et al.*, 2003; Gaitan-Solis *et al.*, 2002; Yu *et al.*, 1999).

Microsatellites have been used to assess various common bean core collections (Gill-Langarica *et al.*, 2011; Blair *et al.*, 2009), which could allow the identification of sources of variation for adaptation to the diverse climate condition (Ortiz, 2012). For example, Asfaw *et al.* (2009) and Zhang *et al.* (2008) used microsatellites with different numbers of primers (30-38) to assess diversity of landraces from Ethiopia and Kenya, and from 14 provinces of China, respectively. They were able to identify various divergent landraces for further preservation and utilization. Both *ex situ* (in gene banks) and *in situ* (on-farm) conservation types are important for preserving crop genetic resources, which can be further used as sources of variation by breeding programs (Maxted *et al.*, 2008).

Common beans from Latin America were brought together with their seed borne pathogens to Africa, Europe, and Asia. Today a wide diversity of *C. lindemuthianum* races in all regions where common beans are grown is evident. However, the largest variations of the fungus as well as the source for resistances are found in Mesoamerica (Balardin *et al.*, 1997; Pastor-Corrales *et al.*, 1995). Similarly, various strains of BCMV as well as BCMNV are known (Mckern *et al.*, 1992; Drijfhout *et al.*, 1978). Resistance source for both BCMV and BCMNV are available in common bean cultivars belonging to both Andean and Mesoamerican gene pools, which have the dominant *I* gene and six *bc*-genes. DNA marker-aided breeding is widely used for breeding BCMV resistance with the dominant *I* gene (Melotto *et al.*, 1996; Haley *et al.*, 1994; Hegay *et al.,* 2013), and recessive genes *bc-1*^{2} (Vandemark & Miklas, 2002; Miklas *et al.*, 2000), *bc-3* (Mukeshimana *et al.*, 2005; Johnson *et al.*, 1997) and *bc-u* (Strausbaugh *et al.*, 1999).

1.7 Breeding strategy for resistance to biotic stress, BCMV and anthracnose

Various breeding schemes, selection methods, screening protocols and sources of host plant resistance have been used to breed common bean cultivars to BCMV and anthracnose (Drijfhout, 1978; Mastenbroek, 1960; Kruger *et al.*, 1977). Pyramiding distinct sources in single genotypes can lead to durable host plant resistance. This task can be facilitated by the use of DNA markers tightly linked to a desired gene or quantitative trait locus (QTL) of interest. This

breeding method is known as marker-aided selection and includes the use of DNA markers in the backcrossing; i.e., marker-aided back crossing (MABC).

Plant breeding has been successful for developing cultivars with host plant resistance to bacteria, fungi and viruses (Drijfhout, 1978; Coyne & Schuster, 1974; Mastenbroek, 1960). Irrespective of the breeding strategy, the first step will be always to define the goal of the endeavor, the materials to improve (cultivars, lines, pre-breeding germplasm), the target environments for testing and release, the sources of variation for the desired trait(s) and the genetics involved. One dominant *I* gene and six recessive genes (*bc-1*, $bc\text{-}1^2$, *bc-2*, $bc\text{-}2^2$, *bc-3* and *bc-u* located at four loci ($bc\text{-}1/bc\text{-}1^2$ are allelic and $bc\text{-}2/bc\text{-}2^2$ are allelic) provide host plant resistance to BCMV. The non-strain specific *bc-u* gene is required in the absence of *I* gene for the expression of recessive *bc*-genes (Drijfhout, 1978).

One of the advantages of relying on DNA markers as selection aid is that the environment does not affect them. Likewise, DNA markers can assist when epitasis occurs; i.e., one gene masking the effects of another gene. This could be the case of *I* gene that affects the expression of *bc-3* gene in a plant after the infection with a BCMNV strain (Mukeshimana *et al.*, 2005; Drijfhout, 1978).

Choosing and introducing host plant resistance gene(s) into a susceptible cultivar depend on pathogen strains and the environments where the strain(s) thrives. Drijfhout *et al.* (1978) selected 22 BCMV strains to define, strain groups according to reaction of host group (i.e. differential cultivars). There are seven virus strain groups (I, II, III, IV, V, VI, VII) and eight isolates (i.e. NL1, NL2, NL3, NL4, NL5, NL6, NL7 and NL8). Mckern *et al.* (1992) did a further molecular analysis and found that BCMV consists of two distinct potyviruses, which are associated with serotype groups A and B. The A group includes NL3, NL5, NL8 and TN1 of BCMNV strains, while group B comprises NL1, NL2, NL4, NL6 and NL7 of BCMV strains. BCMNV is associated with non-temperature sensitive necrosis symptoms.

The use of MABC can lead to pyramiding resistant genes against various strains or races of a pathogen. In this regard, germplasm screening against various strains or races must be part of a breeding program. For example, BCMV/BCMNV strains are very diverse. Isolates from Europe are different compared to those from Africa (Gibbs *et al.*, 2008). Hence host plant resistance to all strains of the virus can be achieved by combining *I* and *bc-3* genes.

Nonetheless, screening against BCMNV strain will be required to confirm it (Larsen *et al.*, 2005).

Test infection is necessary to confirm the presence of *I* gene. Infected offspring seedlings should have 'black root' symptoms. Alternatively, the presence of sequence characterized amplified region (SCAR) SW13 marker, which is tightly linked to the *I*-locus, indicates the presence of *I* gene. Host plant resistance breeding for *bc-3* gene should still consider infection with both BCMV and BCMNV strains (Larsen *et al.*, 2005; Miklas *et al.*, 1998).

In the case of anthracnose, use clean seeds with at least 2-year crop rotations are recommended to limit its epidemics (Pastor-Corrales & Tu, 1989). Fungicide treatment can reduce the fungus activity, but is costly and limited success has been achieved when comparing with the use of resistant cultivars. There are various anthracnose strains (Kelly & Vallejo, 2004; Bent, 1996). Greek letters as well as binary numbers are used for naming the anthracnose races (Kelly & Vallejo, 2004; Menezes & Dianese, 1988).

Various resistance sources to anthracnose are available from both Andean and Mesoamerican common bean gene pools. Nine major independent genes (*Co-1* to *Co-10* being *Co-3* and *Co-9* allelic), are known sources of resistance to anthracnose in common bean (Kelly & Vallejo, 2004). These dominant resistance genes are involved in different plant mechanisms (by producing chemical and protein compounds) that deactivate the cell-to-cell infection movement in the plant tissue.

The *Co*-genes, independently or combined, provide durable resistance to anthracnose (Kelly & Vallejo, 2004; Pastor-Corrales *et al.*, 1994). Although DNA polymorphisms were not noted among anthracnose isolates from various bean-producing areas, plant resistance to known races can be broken-down by the new race(s) due to the evolution of the pathogen (Schwartz *et al.*, 1982; Kruger *et al.*, 1977; Mastenbroek, 1960).

Guzman *et al.* (1995) and Balardin & Kelly (1998) noted a parallel evolution between pathogens (for angular leaf spot and for anthracnose) and the origin of common bean according to their gene pool. It has been also noted a co-evolution of common bean diversity and pathogen virulence in both American gene pools (Ansari *et al.*, 2004; Melotto & Kelly, 2000). The diversity of Mesoamerican common beans was higher than in the Andean gene pool (Hegay *et al.*, 2012b; Papa & Gepts, 2003), while a high level of diversity

of anthracnose isolates (races) occurred in Mesoamerica (Mahuku & Riascos, 2004). The *Co* resistance genes are mainly found in Mesoamerican beans: only one dominant resistant *Co-1* gene has an Andean origin, while *Co-2* to *Co-9* resistance genes belong to Mesoamerican gene pool (Kelly & Vallejo, 2004). The spectrum of diversity among anthracnose isolates from Mesoamerica and the Andes remains unknown. The search for host plant resistance sources based on the infection of in excess of 20,000 accession held at CIAT gene bank revealed that some Mesoamerican accessions were resistant to all known anthracnose races, where the Andean accessions were on average highly susceptible (Pastor-Corrales *et al.*, 1995).

The Mexican cultivar G2333 (Mesoamerican gene pool), was previously selected as a source for host plant resistance to anthracnose because it carries three dominant independent resistance genes *Co-4*^{2}, *Co-5* and *Co-7*, was resistant to all known anthracnose isolates (Young *et al.*, 1998; Pastor-Corrales *et al.*, 1994). Resistance was however broken-down in G2333 by new anthracnose isolates, thereby leading to further search for new host plant resistance sources in the secondary gene pool, i.e., *P. coccineus* and *P. polyanthus* (Mahuku *et al.*, 2002).

Due to small genetic diversity in anthracnose pathogenicity in common beans of Andean origin, resistant genes should be incorporated from Mesoamerican into Andean germplasm. The well-known *Co-1* resistance gene from the Andean gene pool can be used in breeding of Mesoamerican common beans to achieve long-term host plant resistance. Indeed, combining *Co-1* and *Co-2* genes from both bean gene pools has been already suggested for breeding durable resistance to anthracnose in common beans (Balardin & Kelly, 1998). The durability of host plant resistance to anthracnose infection of an improved cultivar bearing *Co*-gene(s) may also depend on the Andean or Mesoamerican source (Balardin & Kelly, 1998). Such finding confirms that selecting the *Co*-genes to introduce into the breeding materials depends on germplasm source and on the pathogen strain(s) affecting the crop in a particular growing area.

1.8 Common beans in Kyrgyzstan

When Kyrgyzstan achieved its independence in 1991, the agricultural land belonged to the State but this changed after the privatization process. At the beginning of the 1990s collective farms were transformed into private farms. More than 300,000 smallholders are registered today in Kyrgyzstan and

together they practice agriculture in 1.28 million ha (0.64% of the country area) of total arable land (STATCOM, 2011).

Common beans were introduced to Kyrgyzstan during the ruling of the Soviet Union in the 20th century (Hegay *et al.*, 2012b). Common beans are mostly grown in small farmer's field (0.5 to 5 ha) at Talas and Chui oblasts in northern Kyrgyzstan (Fig. 9). Farmers prefer to grow dry beans types (Hegay, 2012). After harvesting, farmers manually separate their produce according to bean market classes that are defined by seed shape and color. Farmers also put aside some grains during the harvest, to use in the next growing season. In 2010, 71,400 t of beans were produced (Fig. 9), and 90% of the harvest was exported mainly to Turkey, Bulgaria and Russia (STATCOM, 2011). Kyrgyzstan has a moderate bean production compared with other bean-producing countries (Beebe *et al.*, 2011), but ranks however among the top 20 bean exporters worldwide (Akibode & Maredia, 2011). The income from common bean export about USD 20 million (FAOSTAT, 2009), which provides about 43% of income for a family growing common beans (STATCOM, 2011). Farmers in the Talas oblast strongly depend on common bean production that provides job for 162,000 people or 76% of the total population (Hegay *et al.*, 2012a).

In Kyrgyzstan, the common bean market is in its infancy and the seed chain scheme (breeder ↔ seed grower ↔ farmer ↔ farmer) does not work well. Consequently, it is not possible to secure clean seeds to be sown by farmers each year (Asanaliev & Nurgaziev, 2012). The reduction of grain yield in locally grown common bean cultivars has been observed due to the spread of pathogens (Hegay *et al.*, 2012a; Hegay *et al.,* 2013). If this spread continues, it could pose a significant threat to both food security and earnings from export in the country.

Introduced cultivars from Russia, France or Turkey are poorly adapted to Kyrgyz climates or for meeting preferences of bean markets. The identification of the main pathogens affecting common beans in the country and screening potential genotypes against these pathogens was one of the tasks for the Kyrgyz breeding program (Hegay *et al.*, 2008). This PhD research was therefore undertaken to assess the diversity of common beans grown in Kyrgyzstan (Chapter 1), identify to which breeding pool they belong (Chapter 2) and to appraise if MABC can be a sound breeding method for introducing host plant resistance genes to BCMV (Chapter 3) and anthracnose (Chapter 4) in locally grown cultivars.

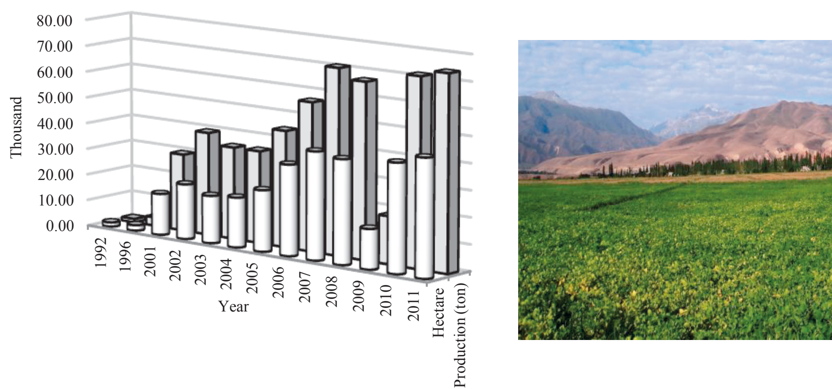


Figure 9. Production and farming of common bean in Kyrgyzstan (Graph data after FAO (2013)).

2 Objectives of the study

The objectives of this thesis were to: select potential common bean types for creating primary breeding material; determine the level of genetic diversity in main grown market types of common beans; determine BCMV strains found in Kyrgyzstan; evaluate effectiveness of DNA markers in combination with inoculation tests for host plant resistance breeding.

The specific objectives in this research were to:

1. Determine the level of diversity and population organization of the main grown Kyrgyz beans types and the gene pool they belong
2. Preliminary evaluation and comparison using both morphological qualitative descriptors and microsatellite markers among main grown beans in Kyrgyzstan
3. Identify BCMV strains and introduce resistance genes with MABC to main grown bean market types in Kyrgyzstan
4. Introduce resistance to anthracnose with MABC to main grown bean market types in Kyrgyzstan

3 Material and methods

3.1 Plant material

Three main grown bean market types in Kyrgyzstan (Lopatka-kidney, Kytayanka-navy and Ryabaya-cranberry) were included in this research (Papers I-IV). Twenty-eight accessions including five Kyrgyz cultivars were used for the genetic diversity study (Paper I), while 27 accessions were included for the research described in paper II (Fig. 10). Foreign bean accessions were kindly provided by Limagrain, International Agricultural Co-operative (France), Michigan State University (USA) and United State Department of Agriculture (Pullman) for further use in bean breeding (Papers III & IV).

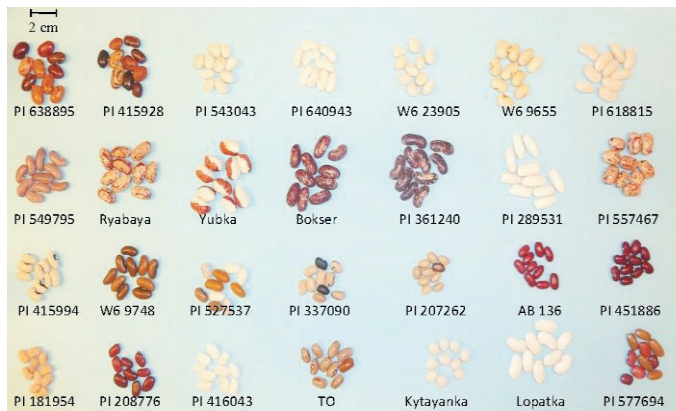


Figure 10. Foreign and Kyrgyz common bean accessions evaluated within this thesis.

3.2 DNA markers

SCAR and STS markers (SW13, SBD5, ROC11, SG6, SQ4 and SCAreoli) were used to determine the presence or absence of resistance genes for BCMV and anthracnose in papers III and IV, while SSR primer pairs were used for the genetic diversity study papers I and II (Table 2).

Table 2. *The nucleotide sequences of the forward and reverse primers and the PCR amplification profile for the 15 DNA loci studied.*

Locus	Forward (top) and reverse (bottom) primers nucleotide sequences	PCR amplification profile	Cycles (#)	Reference
SW13[a]	F:CACAGCGACATTAATTTTCCTTTC R:CACAGCGACAGGAGGAGCTTATTA	94°C, 10s 67°C, 40s 72°C, 120s 72°C, 5 min	34	Melotto *et al.,* 1996
SBD5[a]	F:GTGCGGAGAGGCCATCCATTGGTG R:GTGCGGAGAGTTTCAGTGTTGACA	94°C, 10s 65°C, 40s 72°C, 120s 72°C, 5 min	34	Miklas *et al*., 2000
ROC11[a]	F:CCAATTCTCTTTCACTTGTAACC R:GCATGTTCCAGCAAACC	94°C, 10s 55°C, 40s 72°C, 120s 72°C, 5 min	34	Johnson *et al.*, 1997
SG6[a]	F:GTGCCTAACCGAGTTATCTAGAGT R:GTGCCTAAC CCTCCTAAATGACCT	94°C, 10s 55°C, 60s 72°C, 60s 72°C, 5 min	30	Mukeshimana *et al.*, 2005
SQ4[b]	F:CCTTAGGTATGGTGGGAAACGA R:TGAGGGCGAGGATTTCAGCAAGTT	94ºC, 10s 59ºC, 40s 72ºC, 2 min 72ºC, 5min	34	Awale *et al.*, 2008
SCAreoli[b]	F:GGGAGACATCCATCAGACAACTCC R:GTATCCATTTGAAGGAGCT	94ºC, 3 min 94ºC, 60s 58ºC, 60s 72ºC, 60s 72ºC, 5 min	35	Geffroy *et al.*, 1998
BMd9[c]	F:TATGACACCACTGGCCATACA R:CACTGCGACATGAGAGAAAGA	95°C, 3 min touchdown	40	Blair *et al.*, 2003
BMd17[c]	F:GTTAGATCCCGCCCAATAGTC R:AGATAGGAAGGGCGTGGTTT	profile (94°C, 30s		Blair *et al.*, 2003
BMd18[c]	F:AAAGTTGGACGCACTGTGATT	70°C, 30s		Blair *et al.*,

	R:TCGTGAGGTAGGAGTTTGGTG	annealing	2003
BMd33[c]	F:TACGCTGTGATGCATGGTTT	reduced by	Blair *et al.*,
	R:CCTGAAAGTGCAGAGTGGTG	1°C every	2003
BMd53[c]	F:TGCTGACCAAGGAAATTCAG	cycle,	Blair *et al.*,
	R:GGAGGAGGCTTAAGCACAAA	72°C, 45s)	2003
BMd54[c]	F:GGCTCCACCATCGACTACTG	72°C, 20 min	Blair *et al.*,
	R:GAATGAGGGCGCTAAGATCA		2003
PVM075[c]	F:ATTGGAAGGGGGATGAACCT		Hanai *et al.*,
	R:TAGGAGAGTGCCCAGTGCTT		2010
PVM148[c]	F:ACCTCAAAACCCACCACAAA		Hanai *et al.*,
	R:GAAGTGCTCCCAGATGAAGG		2010
PVM152[c]	F:ATTTGGAGCGAAACAGCAT		Hanai *et al.*,
	R:GAGAACCTCGTCGTCGTCTT		2010

The superscripts "[a-c]" refer to the studies: a= paper III; b= paper IV; c= papers I and II.

3.3 Phenotyping and screening common beans for resistance to BCMV and anthracnose

Common bean accessions were sown in a greenhouse, nine microsatellites and 13 qualitative morphological descriptors were recorded on 10 randomly chosen individual plants per accession (Table 3).

Table 3. *Microsatellite loci and morphology traits.*

SSR			Morphology		
Primer	Ng	H_i	Trait name	# of traits	Trait characteristics
BMd9[a]	4	0.140	Seed color	11	Seed color; white, brown, cream-beige, black, green, purple, yellow, red, cream-beige blue, light pink, or purple-striped
BMd17[a]	4	0.248	Seed shape	4	Round (oval), kidney, rhomboid, or elongate (cylindrical)
BMd18[a]	2	0.444	Flower color	4	White, pink, violet (purple), or red
BMd33[a]	3	0.322	Photoperiod	2	Day light14 hours; neutral or sensitive
BMd53[a]	3	0.295	Seed size/weight of 100 seeds, gram	4	Small (< 25 g), medium (25–40 g), large (40–60 g), or very large (>60 g)
BMd54[a]	3	0.235	Hypocotyl color	3	Green, red or pink
PVM075[b]	2	0.439	Stem color	3	Green, red or pink
PVM148[b]	4	0.226	Bract shape and size	9	Large cordate, medium cordate, small cordate, large lanceolate, medium lanceolate, small lanceolate,

					medium ovate, small ovate, or small triangular
PVM152[b]	2	0.340	Pod string	2	Measurement at half dry stage; present or absent
			Fiber	2	Measurement at half dry stage; present or absent
			Pod beak position	2	Placental or central
			Straight leaf hairs	2	Present or absent
Mean	3	0.299	Plant habit	4	Determinate bush, indeterminate bush, indeterminate prostrate or indeterminate climbing

References for SSR primers [a]Blair *et al.* (2003); [b]Hanai *et al.* (2010) (Papers I & II)

N_g = observed genotypic number

H_i = average gene diversity (estimated as expected for dominant locus)

Identification of BCMV strains found in Kyrgyzstan were made through evaluation of the Kyrgyz and differential cultivars for four years (2007 to 2010) using four replications in a field in Kyrgyzstan and two years (2009 and 2010) in a greenhouse at the Swedish University of Agricultural Sciences (Alnarp, Sweden). Plants of the three Kyrgyz cultivars that were not attacked by pathogens in the field were selected as candidates for resistance, and their seeds were collected for further research (Papers I-IV). The necrotic NL3 strain was used for greenhouse screening of the offspring of Lopatka, Ryabaya and Kytayanka for their resistance to this virus strain, which was kindly provided by Plant Research International, Wageningen, the Netherlands and by Michigan State University, USA (Paper III). Plants were infected manually using a mixture of the inoculum, carborundum and 13 mM phosphate buffer (10 mM $(Na_2HPO_4)_2H_2O$, 3 mM K_2PO_4; pH 7.7). Primary leaves of seedlings were rub-inoculated and thereafter the plants were grown in the greenhouse at 25 ±1ºC under 16-hr light per day. Inoculated plants were examined and symptoms were recorded 7 to 14 days after inoculation. Detached leaf assay was also applied on BC_4F_2 generation derived from Lopatka-1 × Vaillant crosses (Paper III).

Dr. Elie Marx (Limagrain, France) kindly provided the gamma and delta anthracnose races. Growing medium used for spore cultivation of both races included 0.28% glucose, 0.12% $MgSO_47H_20$, 0.27% KH_2PO_4, 0.2% bacto peptone and 2% agar (Mathur *et al.*, 1950). The medium was autoclaved for 20 minutes at 110°C and cooled down for 30 minutes at room temperature before it was poured into Petri dishes for spore cultivation. The optimum spore concentration (1.2×10^6 ml^{-1}) was determined using a hemocytometer and used

in all infection tests in which a disease control plants (20 plants of each parent) also was included. The dipping inoculation method (Kruger *et al.*, 1977) was used to score disease development, and to select resistant offspring for further backcrossing. Diseases symptoms were evaluated after 7 to 10 days and visually scored. Healthy plants and plants which showed small brown necrosis or lesions were considered as resistant while plants which showed symptoms were rated as susceptible (Paper IV).

3.4 Transferring host plant resistance to Kyrgyz cultivars

Pollination was done manually with and without emasculation during the initial crossing and backcrossing steps. Crosses were made between BCMV and anthracnose resistant plants from Vaillant and Flagrano cultivars that carry *I*, $bc\text{-}1^2$ and *Co-2* genes and plants derived from Lopatka, Ryabaya and Kytayanka to transfer the *I*, $bc\text{-}1^2$ and *Co-2* genes to the Kyrgyz cultivars. This experiment was conducted in the greenhouse at temperature range of 18ºC-27ºC as recommended by Temple and Smithson (1989). The F_1 hybrids were confirmed visually through their growth habit. Four backcrosses were made (BC_1F_1 to BC_4F_1) for Lopatka-1 whereas three backcrosses (BC_1F_1 to BC_3F_1) were conducted for Ryabaya-125 to regain desirable traits of the recurrent parents. In Kytayanka-5 × Vaillant crosses only two backcross generations were generated whereas three backcross generations were made in the case of Kytayanka-5 × Flagrano crosses (Papers III & IV). Marker-aided selection, with SW13, SBD5 and SCAreoli gene was used from BC_1F_1 until BC_4F_1.

3.5 DNA extraction, polymerase chain reaction (PCR) and electrophoresis

DNA was extracted from fresh leaves using the CTAB method, as described in Bekele *et al*. (2007). DNA concentration was determined using a Nanodrop® ND-1000 spectrophotometer (Saveen Werner, Sweden). Gene Amp PCR system 9700 (Applied Biosystems Inc, USA) and a Master cycler Ep gradient S (Eppendorf, VWR International) were used for DNA amplification (Table 2). Obtained PCR products for all DNA markers were separated by 1.5% agarose gel electrophoresis and visualized using ethidium bromide. In addition, PCR products for the SCAreoli marker were digested with the restriction enzyme *Dra I* (Fermentas) and STS and SSR markers were separated in the polyacrylamide gel electrophoresis (PAGE). For the estimation of PCR fragment sizes, a 50 bp ladder was used. Furthermore, the selected SSR forward primers were fluorescently 5′ labeled with fluorescent dyes, 6FAM™, VIC™, HEX™,

NED™. Then the PCR amplified products were multiplexed into panels as indicated by Geleta *et al.* (2012) and were sent to the DNA fragment analyses.

3.6 Data analysis

Allele sizing was performed using GeneMarker® V2.2.0 software (SoftGenetics, LLS, State College, Pennsylvania) based on the internal Genescan-500 LIZ size standard. Observed and expected heterozygosities, percent polymorphism, Shannon's index and Nei's gene diversity were calculated using POPGENE version 1.31 (Yeh & Boyle, 1997). Data analyses with qualitative morphological traits were based on the methods: discriminant analysis, principal component analysis and best subset regression that were performed in Minitab 15 statistical software (State College, Pennsylvania) (Paper II). NTSYS-pc (Rohlf, 2000) was used for calculating of genetic similarity and distance coefficients used for the cluster analysis in unweighted pair group method with arithmetic average (UPGMA) using the sequential agglomerative hierarchical nested clustering (SAHN) and in principal coordinate analysis (PCoA) (Papers I & II). A two-way Mantel test (Mantel, 1967) was used to estimate the correlation between the morphological characters and the microsatellite based matrices. The goodness of fit for UPGMA tree and PCoA matrices were performed in NTSYS-pc with 10,000 random permutations. The bootstrap values for the UPGMA dendrogram were obtained via 1,000 resampling procedure using the FreeTree program (Pavlicek *et al.*, 1999). The TreeView program (Page, 1996) was used to display the tree. The overall genetic diversity of common bean accessions was estimated through the analysis of molecular variance (AMOVA) using Arlequin 3.5 (Excoffier & Lischer, 2010). The software STRUCTURE (Pritchard *et al.*, 2000) was used to find the number of clusters among genotypes. The admix model with 5000 burning periods and 50 000 replicates was used to estimate each K value, with ten independent runs from K = 1 to 10. Delta K (population number) was estimated as described by Evanno *et al.* (2005), and population clusters were produced using the DISTRUCT software (Rosenberg, 2004). The chi-square test (χ^2) was used to test the goodness of fit for the segregation of the offspring at BC_nF_1 (1:1) and BC_nF_2 (1:2:1) generations (Paper IV).

4 Summary of results and discussion

4.1 Genetic diversity and characterization common bean accessions (Papers I & II)

4.1.1 Genetic diversity in common beans estimated by microsatellites

Nine microsatellites were used for assessing diversity among 28 accessions. A total of 24 polymorphic alleles were observed. The number of alleles per locus ranged from 2 to 4 with an average of 2.67. The total allelic diversity and allelic richness observed in the 28 accessions was smaller than the diversity noted by Diaz *et al*. (2011) among 92 landraces from Colombia when using 45 microsatellites. Yu *et al*. (1999) noticed seven microsatellites (with 2-10 alleles per locus, and an average of 4.4 per locus) in 12 common bean breeding lines. The variation of alleles per locus can be explained, by the number of repeats, the frequency distribution within chromosomes, and microsatellite mutation rates per locus, which can vary in the same species and as per the genotype (Schlötterer, 2000). The observed heterozygosity at each polymorphic locus ranged from 0 (BMd18, PVM075, PVM152) to 0.0087 (BMd9). The observed heterozygosity in our study was higher in the Andean gene pool (0.076) than in the Mesoamerican gene pool (0.006). Duarte *et al*. (1999) and Blair *et al*. (2010) also noted higher polymorphism for Mesoamerican gene pool vis-à-vis the Andean gene pool using microsatellites. The observed heterozygosity in our research was overall low (0.05), because many of the common bean accessions were from gene banks, and grown in a greenhouse. We know that *Phaseolus vulgaris* is a strong self-pollinated crop (Temple & Smithson, 1989; Bliss, 1980) but outcrossing occurs at various rates (Ferreira *et al.*, 2007; Ibarra-Perez *et al.*, 1997; Wells *et al.*, 1988). The average percent polymorphism, number of allele per locus, Shannon's diversity index and Nei's gene diversity were higher for bean accessions from African countries while

the lowest diversity was observed for accessions from former Soviet Union countries (Table 4).

Table 4. *Percentage of polymorphism (%P), heterozygosity and diversity measurements of common bean accessions.*

Accession groups	%P	Mean number of allele	Observed hetero-zygosity	Expected hetero-zygosity	Shannon index	Nei´s gene index
Origin						
South America	16.65	1.15	0.01	0.037	0.065	0.040
Central and North America	13.32	1.12	0.006	0.042	0.063	0.040
Europe	19.42	1.17	0.28	0.312	0.066	0.038
Asia	16.65	1.15	0	0.05	0.077	0.049
Africa and Australia	25.93	1.26	0	0.116	0.171	0.116
Former Soviet Union	6.93	1.06	0.012	0.031	0.031	0.019
Gene pool						
Andean	7.63	1.06	0.076	0.091	0.028	0.017
Mesoamerican	24.05	1.22	0.006	0.079	0.121	0.078

Accessions were grouped into Andean and Mesoamerican gene pools and gene diversity was estimated among groups. The variance component estimated by SSR for the Mesoamerican gene pool (0.747) was higher than the Andean gene pool (0.363) as per the AMOVA. The observed genetic variation was higher among accessions than within accessions (Table 5). This result could ensue from the inbred nature of common beans, effective barriers for gene flow among populations (in spite of seed exchange between farmers), and human selection of pure lines for use as cultivars. The genetic variation observed in the American centers of diversity was higher than elsewhere, which was also noticed by Blair *et al.* (2009).

Table 5. *Analysis of molecular variance (AMOVA) for common bean accessions based on microsatellite polymorphism: (A) for all 28 accessions, (B) only for the 12 Mesoamerican accessions, (C) only for the 16 Andean accessions, (D) grouping the accessions according to two known gene pools, (E) grouping the accessions according to known races, (F) grouping the accessions according to primary and secondary centers of diversity as per Blair et al. (2009).*

Groups	Sources of variation	Degrees of freedom	Variance components	Variation (%)
(A) All accessions	Among accessions	27	Va=1.62	94.71***
	Within accessions	532	Vb=0.09	5.29***
	Total	559	1.71	
(B) Mesoamerican gene	Among accessions	11	Va=0.75	78.97***

pool	Within accessions	228	Vb=0.19	21.03***
	Total	239	0.94	
(C) Andean gene pool	Among accessions	15	Va=0.36	83.15***
	Within accessions	304	Vb=0.07	16.85***
	Total	319	0.43	
(D) Accessions as per gene pools	Among gene pools	1	Va= 2.15	76.71***
	Among accessions within gene pools	26	Vb=0.52	18.76***
	Within accessions	532	Vc= 0.13	4.54***
	Total	559	2.80	
(E) Accessions as per races	Among races	4	Va =1.53	73.48***
	Among accessions within races	23	Vb=0.42	20.40***
	Within accessions	532	Vc=0.13	6.12***
	Total	559	2.08	
(F) Primary center versus secondary center of diversity	Among groups	1	Va=0.08	4.72***
	Among accessions within groups	26	Vb=1.58	88.17***
	Within accessions	532	Vc=0.13	7.11ns
	Total	559	1.79	

4.1.2 Comparative advantages of using molecular and morphological data in common beans

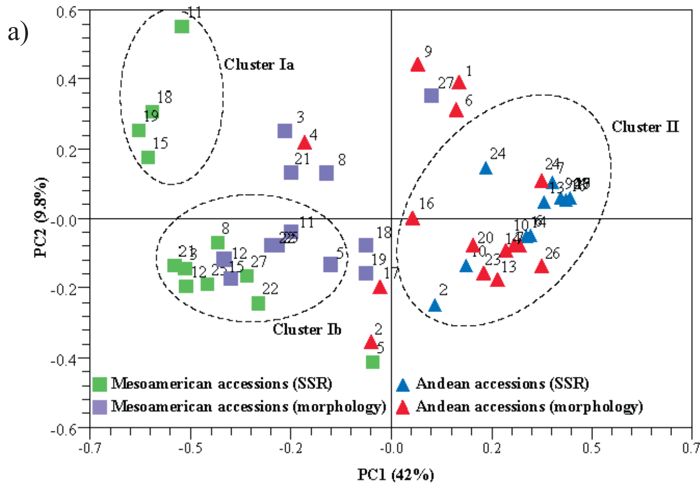
Common bean accessions were clearly separated into two groups corresponding to the Mesoamerican and Andean gene pools with using as well morphological qualitative traits as by SSR markers. The average genetic diversity (H_i) among 27 accessions estimated by microsatellite was 0.299 across nine primers (Ng). It ranged from 2 (BMd18, PVM075 and PVM152) to 4 (BMd9, BMd17 and PVM148), and the average was 3 (Table 3). The genetic diversity within common bean accessions estimated with microsatellites was higher than that observed using morphological qualitative descriptors (Shannon index 0.08 and 0.05, respectively). The assessment of morphological qualitative traits of common bean accessions included in our study did not show large morphological variation because many accessions were from gene bank and may have reduced some morphological variation in the past. Discriminant analysis (DA), best subset regression analysis and principal component analysis (PCA) identified there most important morphological traits: seed size, pod beak positions, size and shape of the bract for grouping common bean accessions into gene pools (Table 3, Table 4, Fig.1, Paper II). Together, the first three principal components accounted for 46% of the total variance in PCA. Individuals (99%) were assigned to the correct group with using 13 morphological descriptors generated by DA. Best subset regression

showed 79% of the total variation with three predictors included into model. Variation in seed size, pod beak positions, growth habit for grouping accessions into gene pools, were previously reported by Singh *et al*. (1991a) and Burle *et al*. (2011). Overall, qualitative morphological descriptors and appropriate statistical methods differentiate common bean accessions and assign them into respective gene pools.

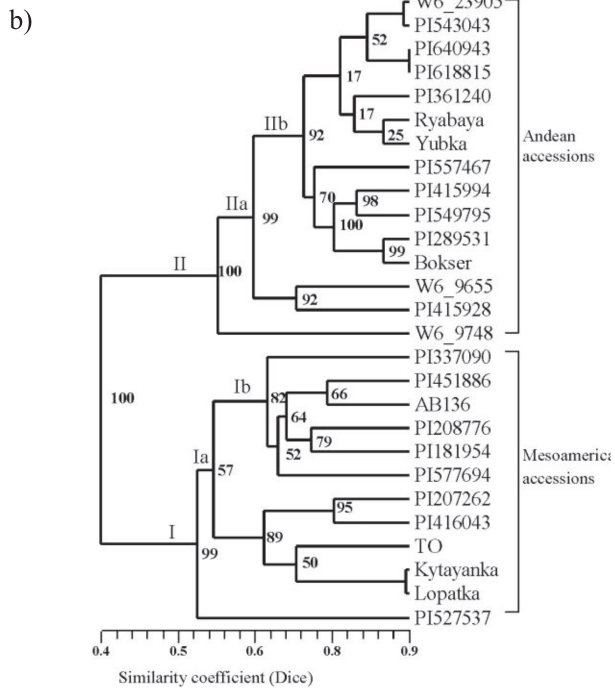
Common bean may have similar phenotypes such as seed color, color flower and molecular markers (e.g., microsatellites) can be used to differentiate genotypes in such morphologically homogenous germplasm. Our results confirmed previous research by Singh *et al.* (1991b), who found that variation on the growth habit in common beans could be independent. The cluster analyses and PCoA defined two main groups, which correspond to accessions of Mesoamerican and Andean gene pools (Fig. 11). Moreover, STRUCTURE based on the allele frequencies at individual level also supports two clusters of common bean gene pools and additionally identified heterogeneous accessions. The grouping of common beans at STRUCTURE K=3 further divided the Mesoamerican gene pool in two sub-groups: Mesoamerica race and together the Durango plus Jalisco races, which are from Mexico. Similarly Diaz & Blair (2006) and Beebe *et al.* (2000) also found that accessions from races Durango and Jalisco were grouped together because of their geographic origin. Singh *et al.* (1991a) and Beebe *et al*. (2000) were able to differentiate Durango and Jalisco races using morphology and RAPD markers, respectively.

Common beans characterized both with morphological traits and microsatellites were grouped into clusters corresponding to their gene pools of origin. Kyrgyz cultivars originated from both Andean and Mesoamerican gene pools. Classification and divergence between common bean accessions analyzed in this study may help to preserve plant material *in situ* and *ex situ.* Furthermore, our study provides information to the Kyrgyz breeders that help them to optimize the selection of plant material in improving this very important grain legume crop. The diversity indicators used in our research depend on many factors including the method of sampling used, number and size ranges of loci characterized, and marker distribution on the genome from gene coding or non-gene coding regions. Nonetheless, our study shows the ability of microsatellites to discriminate among common bean accessions, even using a small sample size, and to putatively assign modern cultivars to their gene pools or races.

a)



b)



c)

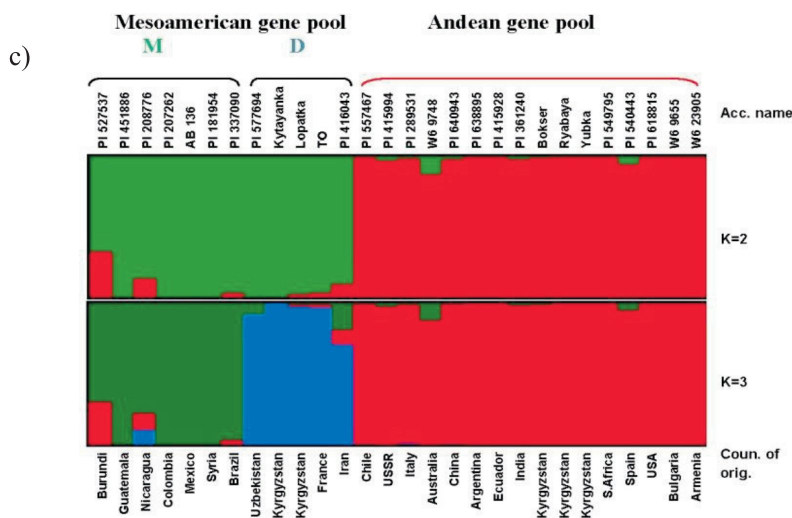


Figure 11. Principal coordinate and cluster analyses (STRUCTURE and UPGMA): a) based on SSR and morphology, clusters Ia and Ib – groups of Mesoamerican accessions; cluster II – grouping Andean accessions. (Mantel, 1967) test matrix comparison of the two matrices was significant *r* = 0.49** according (Lapointe & Legendre, 1992); b) dendrogram based on both SSR and morphology data, showing two main groups of Mesoamerican and Andean accessions. The bootstrap value from 1,000 resampling indicated in between two branches. Mantel test comparison of two (UPGMA) cophenetic matrices was *r* = 0.95**; c) The colors at K=2 indicate Andean and Mesoamerican gene pools, the sub-groups (races) based on within gene pools at STRUCTURE analysis K=3. The letters M and D are for the Mesoamerica and Durango-Jalisco race grouping, respectively. Accessions country of origin indicated at the bottom of the chart.

4.2 Marker-aided breeding for resistance to *Bean common mosaic virus* (Paper III)

4.2.1 Determination of BCMV strains found in Kyrgyzstan

The field experiments conducted in northern Kyrgyzstan to determine BCMV strains found in the country resulted in the identification of only one strain. The phenotypic reaction of the differential cultivars to the Kyrgyz strain were compared to previously reported responses of the cultivars to eight known differential BCMV strains. The phenotypic reactions of the cultivars to the Kyrgyz strain match their previous responses to NL6, which suggests that the Kyrgyz strain is most likely NL6 (Table 2, Paper III). The fact that all individual plants analyzed from the three Kyrgyz cultivars were susceptible to this strain, suggests the lack of genetic diversity in common bean gene pool for resistance to BCMV in the country. Strain NL6 is non-necrosis inducing strain

that belongs to the BCMV serotype B (Mckern *et al.*, 1992; Vetten *et al.*, 1992). This strain has a wide distribution in the world and have been reported in USA (Drijfhout *et al.*, 1978), Africa (Spence & Walkey, 1995) and Europe (Schmidt *et al.*, 1987). Mosaic symptoms during this initial cultivar screening indicated that non-resistant genotypes were found after the screening Kyrgyz cultivars used in this study because the plants were susceptible to the necrosis inducing NL3 strain of BCMNV. The analysis of the offspring derived from Kyrgyz cultivars with SW13 marker revealed that none of them carry the marker, suggesting the absence of the dominant resistance gene at the *I*-locus (Table 2, Paper III). Similar, SBD5 marker linked to $bc\text{-}1^2$ gene was found in offspring derived from Ryabaya-125 and Lopatka-1, suggesting the presence of the resistance gene in these cultivars. However, the SBD5 marker was not amplified in the progenies of Kytayanka-5, and hence they lack the $bc\text{-}1^2$ gene. The presence of SG6 and the absence of ROC11 markers suggest the presence of *bc-3* gene. The application of these markers on the progenies of Kytayanka-5, Lopatka-1 and Ryabaya-125 revealed that the gene is present in Kytayanka-5, Lopatka-1 and absent in Ryabaya-125 (Table 2, Paper III).

4.2.2 Host plant resistance to BCMV in Kyrgyz cultivars

The best strategy will be to prevent the introduction of new BCMV strains into the country while simultaneously developing resistant cultivars to NL6 and other strains. Crosses were made to transfer the *I* gene to all three Kyrgyz cultivars, and the $bc\text{-}1^2$ gene to Kytayanka-5 from Vaillant and Flagrano (Table 3, Paper III). In the case of Kytayanka-5 × Vaillant derived offspring, two backcrosses were conducted to generate BC_2F_1 plants, of which three plants were positive for both SW13 and SBD5 markers, suggesting the presence of *I* and $bc\text{-}1^2$ genes. In Kytayanka-5 × Flagrano derived offspring, three backcrosses were made and BC_3F_1 generation was analyzed for the presence of SW13 and SBD5 markers. In Ryabaya-125 × Vaillant derived offspring, three backcrosses were made to obtain BC_3F_1 seeds. Seeds harvested from plants bearing markers were safely kept in cold storage for future research and breeding. In the case of Lopatka-1 × Vaillant derived offspring (Table 3, Fig. 1, Paper III), four backcrosses were conducted to generate (BC_4F_1). The BC_4F_2 plants that produced the BC_4F_3 seeds were similar to the recurrent parents in most phenotypic traits. Some of the BC_4F_3 seeds are expected to be homozygous for SW13 marker and hence resistant to BCMV.

Cultivars that combine the *I* and *bc-3* genes are resistant to all strains of BCMV and BCMNV (Mukeshimana *et al.*, 2005). The analysis with ROC11 and SG6 suggests that cultivars Kytayanka-5 and Lopatka-1 could be homozygous

for the *bc-3* gene but the Kyrgyz cultivars were susceptible to NL3 strain (Table 2, Paper III). Previous research indicated that cultivars with *bc-3* gene are resistant to BCMV and BCMNV strains (including NL3) in the presence of *bc-u* gene or *I* gene (Mukeshimana *et al.*, 2005; Drijfhout *et al.*, 1978). Morales & Castano (1987) noted that NL3 strain can be transmitted at a high rate trough infected seeds from generation to generation. Interestingly, DNA marker analysis suggested that Lopatka-1 bears *bc-3* gene. Hence, it is likely that the resistant BC_4F_3 lines bearing SW13 marker also bear *bc-3* gene, which makes the line immune to all BCMV and BCMNV strains. This can be further proven through inoculating the lines with the viral strains. Necrotic BCMNV strain(s) have not been found in Kyrgyzstan, where BCMV NL6 predominates. Introduced in the local cultivars the *I* gene can prevent the virus spread. The combined use of DNA markers and inoculation test provides an important tool to identify sound markers for aided breeding for host plant resistance to BCMV across common bean germplasm.

4.3 Marker-aided breeding for resistance to anthracnose (Paper IV)

Three Kyrgyz cultivars to which *Co-2* gene was introduced are very important genetic resources to prevent the spreading of anthracnose in Kyrgyzstan. That well-known gene gives resistance to different anthracnose isolates in the major common bean producing countries such as India (Pathania *et al.*, 2006). In developed countries, the single *Co-2* gene has been successfully used as main source of resistance to the anthracnose by bean breeding programs (Ghaderi *et al.*, 1990; Park *et al.*, 1987; Mastenbroek, 1960). The segregation ratio in backcross generations fit the expected 1 resistant: 1 susceptible, thereby confiming that SCAreoli was tightly linked to the *Co-2* resistance gene (Table 2, Table 3, Paper IV). SCAreoli was a useful marker for introgressing host plant resistance to anthracnose into Kyrgyz susceptible cultivars (Fig. 1, Paper IV). The advanced Lopatka × Vaillant BC_4F_2 generation combines the seed size, shape and color of the local Kyrgyz cultivar Lopatka with the host plant resistance to races delta and gamma of the *C. lindemuthianum* as per its resistant foreign ancestor Vaillant. The BC_4F_3 plants that are homozygous for the SCAreoli marker may also be homozygous for *Co-2* gene and hence should be used for further breeding. Overall, improving Kyrgyz cultivars belonging to the two bean gene pools (Papers I & II) and introgression the *Co-2* gene through MABC breeding will lead to an accelerated development of new bean cultivars suitable for the local market.

5 Conclusions

1. Kyrgyz cultivars belong to both gene pools. Some of those from Mesoamerican gene pool are in the sub-cluster of Durango race. These accessions can be further used in the country's breeding program.

2. Common beans of Mesoamerica origin were more diverse than accessions belonging to Andean gene pool, as revealed by both morphological descriptors and genotyping.

3. BCMV NL6 strain was found in most common bean fields of northern Kyrgyzstan.

4. MABC and inoculation-based backcrossing were successfully used to breed host plant resistance to BCMV and anthracnose into popular common beans of Kyrgyzstan. Their derived resistant offspring carry both the DNA markers tightly linked to host plant resistance gene, and showed resistance after infection with pathogens. They will be kept in the seed storage and will be further used for field trials across locations in Kyrgyzstan.

6 Recommendations and future prospects

1. The screening of germplasm with traits of interest (such as seed color, shape, plant habit), DNA fingerprinting, and the classification of common beans as per their gene pools (Mesoamerican and Andean) will help plant breeders to improve common beans that meet growers' needs and consumers' demands. The Kyrgyz cultivars are in the two gene pools. Important traits can be improved through back cross breeding particularly if parents are from the same gene pool and same market class.

2. MABC provides an opportunity to pyramiding host plant resistance genes to BCMV and anthracnose. It can also accelerate breeding of cultivars with multiple resistances to several pathogens.

References

Akibode, S. & Maredia, M. (2011). *Global and regional trends in production, trade and consumption of food legume crops*: Department of Agricultural, Food and Resource Economics, Michigan State University, MI, USA.

Andradf-Aguilar, J.A. & Jackson, M.T. (1988). Attempts at interspecific hybridization between *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray-using embryo rescue. *Plant Breeding* 101(3), 173-180.

Ansari, K.I., Palacios, N., Araya, C., Langin, T., Egan, D. & Doohan, F.M. (2004). Pathogenic and genetic variability among *Colletotrichum lindemuthianum* isolates of different geographic origins. *Plant Pathology* 53(5), 635-642.

Aragao, F.J.L., Brondani, R.P.V. & Burle, M.L. (2011). *Phaseolus*. In: Kole, C. (Ed.) *Wild crop relatives: Genomic and breeding resources*. pp. 223-236. Heidelberg, Germany: Springer. ISBN 978-3-642-14386-1.

Asanaliev, A.J. & Nurgaziev, R.Z. (2012). Food chain of agriculture of Kyrgyzstan - gained experience, learned lessons and development perspectives. In: Alpas, H., *et al.* (Eds.) *Strategies for achieving food security in Central Asia*. pp. 21-30. The Netherlands: Springer. ISBN 978-94-007-2502-7.

Asfaw, A., Blair, M.W. & Almekinders, C. (2009). Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from the East African highlands. *Theoretical and Applied Genetics* 120(1), 1-12.

Awale, H.E., Ismail, S.M., Vallejo, V.A. & Kelly, J.D. (2008). SQ4 SCAR marker linked to the *Co-2* gene on B11 appears to be linked to the *UR-11* gene. *annual report of the Bean Improvement Cooperative* 51, 174-175.

Balardin, R.S. & Kelly, J.D. (1998). Interaction between *Colletotrichum lindemuthianum* races and gene pool diversity in *Phaseolus vulgaris*. *Journal of the American Society for Horticultural Science* 123(6), 1038-1047.

Balardin, R.S., Jarosz, A.M. & Kelly, J.D. (1997). Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central, and North America. *Phytopathology* 87(12), 1184-1191.

Bakker, H. (2011). *Food security in Africa and Asia: Strategies for small-scale agricultural development*. CAB International. ISBN 9781845938420.

Bassiri, A. & Adams, M.W. (1978). Electrophoretic survey of seedling isozymes in several *Phaseolus* species. *Euphytica* 27(2), 447-459.

Beebe, S., Ramirez, J., Jarvis, A., Rao, I.M., Mosquera, G., Bueno, J.M. & Blair, M.W. (2011). Genetic improvement of common beans and the challenges of climate change. In: Yadav, S.S., *et al.* (Eds.) *Crop adaptation to climate change*. pp. 356-369. Wiley-Blackwell. ISBN 9780470960929.

Beebe, S., Rengifo, J., Gaitan, E., Duque, M.C. & Tohme, J. (2001). Diversity and origin of Andean landraces of common bean. *Crop Science* 41(3), 854-862.

Beebe, S., Skroch, P.W., Tohme, J., Duque, M.C., Pedraza, F. & Nienhuis, J. (2000). Structure of genetic diversity among common bean landraces of Middle American origin based on correspondence analysis of RAPD. *Crop Science* 40(1), 264-273.

Bent, A.F. (1996). Plant disease resistance genes: function meets structure. *The Plant Cell* 8(10), 1757-1771.

Bekele, E., Geleta, M., Dagne, K., Jones, A.L., Barnes, I., Bradman, N. & Thomas, M.G. (2007). Molecular phylogeny of genus *Guizotia* (Asteraceae) using DNA sequences derived from ITS. *Genetic Resources and Crop Evolution* 54(7), 1419-1427.

Berglund-Brücher, O. & Brücher, H. (1976). The South American wild bean (*Phaseolus aborigineus* Burk.) as ancestor of the common bean. *Economic Botany* 30(3), 257-272.

Bitocchi, E., Nanni, L., Bellucci, E., Rossi, M., Giardini, A., Zeuli, P.S., Logozzo, G., Stougaard, J., McClean, P., Attene, G. & Papa, R. (2012). Mesoamerican origin of the common bean (*Phaseolus vulgaris* L.) is revealed by sequence data. *Proceedings of the National Academy of Sciences* 109(14), 788-796.

Blair, M.W., Chaves, A., Tofino, A., Calderon, J.F. & Palacio, J.D. (2010). Extensive diversity and inter-genepool introgression in a world-wide collection of indeterminate snap bean accessions. *Theoretical and Applied Genetics* 120(7), 1381-1391.

Blair, M.W., Diaz, L.M., Buendia, H.F. & Duque, M.C. (2009). Genetic diversity, seed size associations and population structure of a core collection of common beans (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 119(6), 955-972.

Blair, M.W., Giraldo, M.C., Buendia, H.F., Tovar, E., Duque, M.C. & Beebe, S.E. (2006). Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 113(1), 100-109.

Blair, M.W., Pedraza, F., Buendia, H.F., Gaitan-Solis, E., Beebe, S.E., Gepts, P. & Tohme, J. (2003). Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 107(8), 1362-1374.

Bliss, F.A. (1980). Common bean. In: Fehr, W.R., *et al.* (Eds.) *Hybridization of crop plants*. pp. 273-284. Madison, Wisconsin, USA: American Society of Agronomy and Crop Science Society of America. ISBN 9780891180340.

Bliss, F.A. (1990). Genetic alteration of legume seed proteins. *Hortscience* 25(12), 1517-1520.

Bos, L. (1971) *Bean common mosaic virus*. [online] (2013-02-20) Available from: http://www.dpvweb.net/dpv/showdpv.php?dpvno=73. [Accessed 2013-02-20].

Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P. & Vanderleyden, J. (2003). Beans (*Phaseolus* spp.) - model food legumes. *Plant and Soil* 252(1), 55-128.

Brücher, H. (1988). The wild ancestor of *Phaseolus vulgaris* in South America. In: Gepts, P. (Ed.) *Genetic resources of Phaseolus beans*. pp. 185-214. The Netherlands: Springer (Current plant science and biotechnology in agriculture; vol. 6). ISBN 978-94-010-7753-8.

Burle, M.L., Fonseca, J.R., Jose del Peloso, M., Melo, L.C., Temple, S.R. & Gepts, P. (2011). Integrating phenotypic evaluations with a molecular diversity assessment of a Brazilian collection of common bean landraces. *Crop Science* 51(6), 2668-2680.

Burkart, A. & Brücher, H. (1953). *Phaseolus aborigineus* Burkart, die mutmassliche andine Stammform der Kulturbohne. *Theoretical and Applied Genetics* 23(3), 65-72.

CIAT (1986a). CIAT study guide. In: Song, L.*, et al.* (Eds.) *The cultivated species of Phaseolus*. Cali, Colombia: Centro Internacional de Agricultura Tropical. Series 04EB-09.02.

CIAT (1986b). CIAT study guide. In: Gepts, P.*, et al.* (Eds.) *Stage of development of the common bean plant*. Cali, Colombia: Centro Internacional de Agricultura Tropical. Series 04EB-09.03.

CIAT (1989). Bean production problem in the tropics. In: Schwartz, H.F.*, et al.* (Eds.). 2. ed. Cali, Colombia, Centro Internacional de Agricultura Tropical. ISBN 958-9183-04-2.

Coyne, D.P. & Schuster, M.L. (1974). Breeding and genetic studies of tolerance to several bean (*Phaseolus vulgaris* L.) bacterial pathogens. *Euphytica* 23(3), 651-656.

Dapice, D.O., Vallely, T.J., Wilkinson, B., McPherson, M., Kennedy, J.F. & Montesano, M.J. (2011). Myanmar agriculture in 2011: Old problems and new challenges. *Ash Center for Democratic Governance and Innovation Harvard Kennedy School*.

Debouck, D. (1991). Systematic and morphology. In: van Schoonhaven, A.*, et al.* (Eds.) *Common beans: research for crop improvement*. pp. 55-117. CAB International and CIAT, Wallingford, UK. ISBN 085198679X.

Debouck, D.G. (1986). Primary diversification of *Phaseolus* in the Americas: three centers? *Plant Genetic Resources Newsletter* 67, 2-8.

Debouck, D.G. (1994). Beans (*Phaseolus* spp.). In: Bermejo, J.E.H.*, et al.* (Eds.) *Neglected crops:1492 from a different perspective.* pp. 47-62. Rome, Italy: FAO. (Plant production and protection series; vol. 26).

Delgado-Salinas, A., Bibler, R. & Lavin, M. (2006). Phylogeny of the genus *Phaseolus* (Leguminosae): a recent diversification in an ancient landscape. *Systematic Botany* 31(4), 779-791.

Diaz, L.M. & Blair, M.W. (2006). Race structure within the Mesoamerican gene pool of common bean (*Phaseolus vulgaris* L.) as determined by microsatellite markers. *Theoretical and Applied Genetics* 114(1), 143-154.

Diaz, L.M., Buendia, H.F., Duque, M.C. & Blair, M.W. (2011). Genetic diversity of Colombian landraces of common bean as detected through the use of silver-stained and fluorescently labelled microsatellites. *Plant Genetic Resources Characterization and Utilization* 9(1), 86-96.

Ditmer, E.E., Ivanov, N.R. & Popova, G.M. (1937). *Phaseolus* (Transl. mss. in Dept. Geog. Univ. Cal., Berkeley). *Kulturnaya flora USSR* 4, 457-620.

Drijfhout, E. (1978). *Genetic interaction between* Phaseolus vulgaris *and bean common mosaic virus with implications for strain identification and breeding for resistance*. Wageningen, The Netherlands: Center for Agricultural Publishing and Documentation. ISBN 90-220-0671-9.

Drijfhout, E. & Bos, L. (1977). Identification of two new strains of bean common mosaic virus. *Netherlands Journal of Plant Pathology* 83(1), 13-25.

Drijfhout, E., Silbernagel, M.J. & Burke, D.W. (1978). Differentiation of strains of bean common mosaic virus. *Netherlands Journal of Plant Pathology* 84(1), 13-26.

Duarte, J.M., dos Santos, J.B. & Melo, L.C. (1999). Genetic divergence among common bean cultivars from different races based on RAPD markers. *Genetics and Molecular Biology* 22(3), 419-426.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14, 2611-2620.

Ekpo, E.J.A. & Saettler, A.W. (1974). Distribution pattern of bean common mosaic virus in developing bean seed. *Phytopathology* 64, 269-270.

Excoffier, L. & Lischer, H.E.L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10, 564-567.

FAO (2011) United Nations Food and Agriculture Organization. [online] (2013-02-20) Available from: http://www.fao.org/ [Accessed 2013-02-25].

FAO (2013) United Nations Food and Agriculture Organization. [online] (2013-03-26) Available from: http://www.fao.org/ [Accessed 2013-02-25].

FAOSTAT (2009). United Nations Food and Agriculture Organization. Statistical Database [online] (2012-10-10) Available from: http://faostat.fao.org/site/567/default.aspx#ancor. [Accessed 2012-10-10].

FAOSTAT (2013) United Nations Food and Agriculture Organization. Statistical Database. [online] (2013-03-25) Available from: http://faostat.fao.org/site/567/default.aspx#ancor. [Accessed 2013-03-25].

Ferreira, J.L., Carneiro, J.E.D., Teixeira, A.L., de Lanes, F.F., Cecon, P.R. & Borem, A. (2007). Gene flow in common bean (*Phaseolus vulgaris* L.). *Euphytica* 153(1-2), 165-170.

Flores-Estevez, N., Acosta-Gallegos, J.A. & Silva-Rosales, L. (2003). Bean common mosaic virus and Bean common mosaic necrosis virus in Mexico. *Plant Disease* 87(1), 21-25.

Freyre, R., Rios, R., Guzman, L., Debouck, D.G. & Gepts, P. (1996). Ecogeographic distribution of *Phaseolus* spp. (Fabaceae) in Bolivia. *Economic Botany* 50(2), 195-215.

Gaitan-Solis, E., Duque, M.C., Edwards, K.J. & Tohme, J. (2002). Microsatellite repeats in common bean (*Phaseolus vulgaris*): Isolation, characterization, and cross-species amplification in *Phaseolus* ssp. *Crop Science* 42(6), 2128-2136.

Galves, G.E. & Morales, F.J. (1989). Aphid-transmitted viruses. In: Schwartz, H.F*., et al.* (Eds.) *Bean production problems in the Tropics*. 2. ed. pp. 333-342. Cali, Colombia: CIAT, Centro Internacional de Agricultura Tropical. ISBN 958-9183-04-2.

Geffroy, V., Creusot, F., Falquet, J., Sevignac, M., Adam-Blondon, A.F., Bannerot, H., Gepts, P. & Dron, M. (1998). A family of LRR sequences in the vicinity of the *Co-2* locus for anthracnose resistance in *Phaseolus vulgaris* and its potential use in marker-assisted selection. *Theoretical and Applied Genetics* 96(3), 494-502.

Geleta, M., Herrera, I., Monzón, A. & Bryngelsson, T. (2012). Genetic diversity of arabica coffee (*Coffea arabica* L.) in Nicaragua as estimated by simple sequence repeat markers. *The Scientific World Journal* 2012, 1-11.

Gentry, H.S. (1969). Origin of the common bean, *Phaseolus vulgaris*. *Economic Botany* 23(1), 55-69.

Gepts, P. & Bliss, F.A. (1985). F1-hybrid weakness in the common bean - differential geographic origin suggests two gene pools in cultivated bean germplasm. *Journal of Heredity* 76(6), 447-450.

Gepts, P. & Bliss, F.A. (1986). Phaseolin variability among wild and cultivated common beans (*Phaseolus vulgaris*) from Colombia. *Economic Botany* 40(4), 469-478.

Gepts, P., Moore, P., Ming, R., Aragao, F.J.L., Barros, E., Blair, M.W., Brondani, R., Broughton, W., Galasso, I., Hernandez, G., Kami, J., Lariguet, P., McClean, P., Melotto, M., Miklas, P., Pauls, P., Pedrosa-Harand, A., Porch, T., Sanchez, F., Sparvoli, F. & Yu, K. (2008). Genomics of *Phaseolus* beans, a major source of dietary protein and micronutrients in the Tropics. In: Moore, P.H.*, et al.* (Eds.) *Genomics of tropical crop plants*. pp. 113-143. Springer (Plant genetics and genomics: Crops and models; vol.1). ISBN 978-0-387-71218-5.

Ghaderi, A., Kelly, J.D., Adams, M.W., Saettler, A.W., Hosfield, G.L., Varner, G.V., Uebersax, M.A. & Taylor, J. (1990). Registration of 'Blackhawk' tropical black bean. *Crop Science* 30(3), 744-745.

Gibbs, A., Trueman, J. & Gibbs, M. (2008). The bean common mosaic virus lineage of potyviruses: where did it arise and when? *Archives of Virology* 153(12), 2177-2187.

Gill-Langarica, H.R., Muruaga-Martínez, J.S., Vargas-Vázquez, M.L., Rosales-Serna, R. & Mayek-Pérez, N. (2011). Genetic diversity analysis of common beans based on molecular markers. *Genetics and Molecular Biology* 34(4), 595-605.

Goldstein, D.B., Linares, A.R., Cavalli-Sforza, L.L. & Feldman, M.W. (1995). An evaluation of genetic distances for use with microsatellite loci. *Genetics* 139(1), 463-471.

Guzman, P., Gilbertson, R.L., Nodari, R., Johnson, W.C., Temple, S.R., Mandala, D., Mkandawire, A.B.C. & Gepts, P. (1995). Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests coevolution with the common bean (*Phaseolus vulgaris*). *Phytopathology* 85(5), 600-607.

Guzman, P., Rojas, M.R., Davis, R.M., Kimble, K., Stewart, R., Sundstrom, F.J. & Gilbertson, R.L. (1997). First report of bean common mosaic necrosis potyvirus (BCMNV) infecting common bean in California. *Plant Disease* 81(7), 831-831.

Ha, C., Revill, P., Harding, R.M., Vu, M. & Dale, J.L. (2008). Identification and sequence analysis of potyviruses infecting crops in Vietnam. *Archives of Virology* 153(1), 45-60.

Haley, S.D., Afanador, L. & Kelly, J.D. (1994). Identification and application of a random amplified polymorphic DNA marker for the *I* gene (potyvirus resistance) in common bean. *Phytopathology* 84 (2), 157-160.

Hampton, R.O. (1975). The nature of bean yield reduction by bean yellow and bean common mosaic viruses. *Phytopathology* 65, 1342-1346.

Hanai, L.R., Santini, L., Camargo, L.E.A., Fungaro, M.H.P., Gepts, P., Tsai, S.M. & Vieira, M.L.C. (2010). Extension of the core map of common bean with EST-SSR, RGA, AFLP, and putative functional markers. *Molecular Breeding* 25(1), 25-45.

Hegay, S. (2012). Handbook: Breeding and seed production of common beans in Kyrgyzstan. In: Kyrgyz National Agrarian University, Bishkek, Kyrgyz Republic.

Hegay, S., Asanaliev, A., Islamov, A., Baltabaev, E., Bolokbaev, J., Hede, A., Marx, E., Merker, A., Gustavsson, L., Persson, H. & Bryngelsson, T. (2012a). DNA markers aided breeding in Kyrgyz common bean (*Phaseolus vulgaris* L.) populations for BCMV resistance. *Vestnik Kyrgyz National Agrarian University* 1(23), 37-40.

Hegay, S., Asanaliev, A., Torutaeva, E., Hede, A., Islamov, A., Baltabaev, E. & Marx, E. (2008). Selection from local populations *Phaseolus vulgaris* for breeding perspective. *Vestnik Kyrgyz National Agrarian University* 3(11), 159-163.

Hegay, S., Geleta, M., Bryngelsson, T., Gustavsson, L., Persson Hovmalm, H. & Ortiz, R. (2012b). Comparing genetic diversity and population structure of common beans grown in Kyrgyzstan using microsatellites. *Scientific Journal of Crop Science* 1(4), 63-75.

Hegay, S., Ortiz, R., Garkava-Gustavsson, L., Persson Hovmalm, H. & Geleta, M. (2013). Marker-aided breeding for resistance to bean common mosaic virus in Kyrgyz bean cultivars. *Euphytica*. Doi 10.1007/s10681-013-0928-9.

Ibarra-Perez, F.J., Ehdaie, B. & Waines, J.G. (1997). Estimation of outcrossing rate in common bean. *Crop Science* 37(1), 60-65.

Johnson, W.C., Guzman, P., Mandala, D., Mkandawire, A.B.C., Temple, S., Gilbertson, R.L. & Gepts, P. (1997). Molecular tagging of the *bc-3* gene for introgression into Andean common bean. *Crop Science* 37(1), 248-254.

Kapil, R., Sharma, P., Sharma, S.K., Sharma, O.P., Dhar, J.B. & Sharma, P.N. (2011). Pathogenic and molecular variability in bean common mosaic virus infecting common bean in India. *Archives of Phytopathology and Plant Protection* 44(11), 1081-1092.

Kaplan, L. (1956). The cultivated beans of the prehistoric Southwest. *Annals of the Missouri Botanical Garden* 43(2), 189-251.

Kaplan, L. (1981). What is the origin of the common bean? *Economic Botany* 35(2), 240-254.

Kaplan, L. & Lynch, T.F. (1999). *Phaseolus* (Fabaceae) in archaeology: AMS radiocarbon dates and their significance for pre-Colombian agriculture. *Economic Botany* 53(3), 261-272.

Kaplan, L., Lynch, T.F. & Smith, C.E. (1973). Early cultivated beans (*Phaseolus vulgaris*) from an intermontane Peruvian valley. *Science* 179(4068), 76-77.

Kaplan, L. & MacNeish, R.S. (1960). Prehistoric bean remains from Caves in the Ocampo region of Tamaulipas, Mexico. *Botanical Museum leaflets Harvard University, Camdridge, Massachusetts* 19(2), 33-56.

Katungi, E., Farrow, A., Chianu, J., Sperling, L. & Beebe, S. (2009). *Common bean in Eastern and Southern Africa: a situation and outlook analysis*. International Center for Tropical Agriculture; vol.61.

Kelly, J.D. (1992). Breeding strategies for genetic control of BCMV. *annual report of the Bean Improvement Cooperative* 35, 60-61.

Kelly, J.D., Afanador, L. & Haley, S.D. (1995). Pyramiding genes for resistance to bean common mosaic virus. *Euphytica* 82(3), 207-212.

Kelly, J.D., Saettler, A.W. & Morales, M. (1983). New necrotic strain of bean common mosaic virus in Michigan. *annual report of the Bean Improvement Cooperative* 26, 49-50.

Kelly, J.D. & Vallejo, V.A. (2004). A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. *Hortscience* 39(6), 1196-1207.

Koenig, R. & Gepts, P. (1989). Allozyme diversity in wild *Phaseolus vulgaris*: further evidence for two major centers of genetic diversity. *Theoretical and Applied Genetics* 78(6), 809-817.

Koenig, R.L., Singh, S.P. & Gepts, P. (1990). Novel phaseolin types in wild and cultivated common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany* 44(1), 50-60.

Kruger, J., Hoffmann, G.M. & Hubbeling, N. (1977). The Kappa race of *Colletotrichum lindemuthianum* and sources of resistance to anthracnose in *Phaseolus* beans. *Euphytica* 26, 23-25.

Lapointe, F.-J. & Legendre, P. (1992). Statistical significance of the matrix correlation coefficient for comparing independent phylogenetic trees. *Systematic Biology* 41(3), 378-384.

Larsen, R.C., Miklas, P.N., Druffel, K.L. & Wyatt, S.D. (2005). NL-3 K strain is a stable and naturally occurring interspecific recombinant derived from bean common mosaic necrosis virus and bean common mosaic virus. *Phytopathology* 95(9), 1037-1042.

Mahuku, G.S., Jara, C.E., Cajiao, C. & Beebe, S. (2002). Sources of resistance to *Colletotrichum lindemuthianum* in the secondary gene pool of *Phaseolus vulgaris* and in crosses of primary and secondary gene pools. *Plant Disease* 86(12), 1383-1387.

Mahuku, G.S. & Riascos, J.J. (2004). Virulence and molecular diversity within *Colletotrichum lindemuthianum* isolates from Andean and Mesoamerican bean varieties and regions. *European Journal of Plant Pathology* 110(3), 253-263.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research* 27(2), 209-220.

Masi, P., Zeuli, P.L.S. & Donini, P. (2003). Development and analysis of multiplex microsatellite markers sets in common bean (*Phaseolus vulgaris* L.). *Molecular Breeding* 11(4), 303-313.

Mastenbroek, C. (1960). A breeding programme for resistance to anthracnose in dry shell haricot beans, based on a new gene. *Euphytica* 9(2), 177-184.

Mathur, R.S., Barnett, H.L. & Lilly, V.G. (1950). Sporulation of *Colletotrichum lindemuthianum* in culture. *Phytopathology* 40(1), 104-114.

Maxted, N., Dulloo, E., Ford-Lloyd, B.V., Iriondo, J.M. & Jarvis, A. (2008). Gap analysis: a tool for complementary genetic conservation assessment. *Diversity and Distributions* 14(6), 1018-1030.

McClean, P., Gepts, P. & Kami, J. (2004). Genomics and genetic diversity in common bean. In: Wilson, R.F.*, et al.* (Eds.) *Legume crop genomics*. pp. 60-82. Champaign, IL: AOCS Press. ISBN 1-893997-48-0.

Mckern, N.M., Mink, G.I., Barnett, O.W., Mishra, A., Whittaker, L.A., Silbernagel, M.J., Ward, C.W. & Shukla, D.D. (1992). Isolates of bean common mosaic virus comprising two distinct potyviruses. *Phytopathology* 82(9), 923-929.

Melotto, M., Afanador, L. & Kelly, J.D. (1996). Development of a SCAR marker linked to the *I* gene in common bean. *Genome* 39(6), 1216-1219.

Melotto, M. & Kelly, J.D. (2000). An allelic series at the *Co-1* locus conditioning resistance to anthracnose in common bean of Andean origin. *Euphytica* 116(2), 143-149.

Menezes, J.R. & Dianese, J.C. (1988). Race characterization of Brazilian isolates of *Colletotrichum lindemuthianum* and detection of resistance to anthracnose in *Phaseolus vulgaris*. *Phytopathology* 78(6), 650-655.

Mercado-Ruaro, P. & Delgado-Salinas, A. (1998). Karyotypic studies on species of *Phaseolus* (Fabaceae: Phaseolinae). *American Journal of Botany* 85(1), 1-9.

Miklas, P., Lambert, S., Mink, G. & Silbernagel, M. (1998). Many beans with *bc-3* resistance to BCMNV are susceptible to BCMV. *annual report of the Bean Improvement Cooperative* 41, 33-34.

Miklas, P.N., Kelly, J.D., Beebe, S.E. & Blair, M.W. (2006). Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. *Euphytica* 147(1-2), 105-131.

Miklas, P.N., Larsen, R.C., Riley, R. & Kelly, J.D. (2000). Potential marker-assisted selection for *bc-1*(*2*) resistance to bean common mosaic potyvirus in common bean. *Euphytica* 116(3), 211-219.

Morales, F.J. & Castano, M. (1987). Seed transmission characteristics of selected *bean common mosaic virus* strains in differential bean cultivars. *Plant Disease* 71(1), 51-53.

Mukeshimana, G., Paneda, A., Rodriguez-Suarez, C., Ferreira, J.J., Giraldez, R. & Kelly, J.D. (2005). Markers linked to the *bc-3* gene conditioning resistance to bean common mosaic potyviruses in common bean. *Euphytica* 144(3), 291-299.

Naderpour, M., Mohammadi, M., Mossahebi, G.H. & Koohi-Habibi, M. (2010). Identification of three strains of *Bean common mosaic necrosis virus* in common bean from Iran. *Plant Disease* 94(1), 127.

Ortiz, R. (2012). *Climate change and agricultural production. Technical notes. Inter-American Development Bank*. Washington D.C.

Ortiz, R. & Engels, J. (2004). Genebank management and the potential role of molecular genetics to improve the use of conserved genetic diversity. In: de Vicente, C.M. (Ed.) *The evolving role of genebanks in the fast-developing field of molecular genetics. Issues in genetic resources*. Rome, Italy: IPGRI; vol.11. ISBN 92-9043-623-9.

Page, R.D.M. (1996). TreeView: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12(4), 357-358.

Papa, R. & Gepts, P. (2003). Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. *Theoretical and Applied Genetics* 106(2), 239-250.

Park, S.J., Tu, J.C. & Aylesworth, J.W. (1987). Dresden field bean. *Canadian Journal of Plant Science* 67(3), 821-822.

Pastor-Corrales, M.A., Erazo, O.A. & Singh, S.P. (1994). Inheritance of anthracnose resistance in common bean accession G2333. *Plant Disease* 78(10), 959-962.

Pastor-Corrales, M.A., Otoya, M.M., Molina, A. & Singh, S.P. (1995). Resistance to *Colletotrichum lindemuthianum* isolates from Middle America and Andean South America in different common bean races. *Plant Disease* 79(1), 63-67.

Pastor-Corrales, M.A. & Tu, J.C. (1989). Anthracnose. In: Schwartz, H.F., *et al.* (Eds.) *Bean production problems in the Tropics*. pp. 77-104. Cali, Colombia: CIAT. ISBN 958-9183-04-2.

Pathania, A., Sharma, P.N., Sharma, O.P., Chahota, R.K., Ahmad, B. & Sharma, P. (2006). Evaluation of resistance sources and inheritance of resistance in kidney bean to Indian virulences of *Colletotrichum lindemuthianum*. *Euphytica* 149(1), 97-103.

Pavlicek, A., Hrda, S. & Flegr, J. (1999). FreeTree-freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap jackknife analysis of the tree robustness. Application in the RAPD analysis of genus *Frenkelia*. *Folia Biologica* 45(3), 97-99.

Pierce, W.H. (1935). The identification of certain viruses affecting leguminous plants. *Journal of Agricultural Research, Washington, D. C.* 51(11), 1017-1039.

Powell, W., Machray, G.C. & Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Science* 1(7), 215-222.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155(2), 945-959.

Provvidenti, R. & Braverman, S.W. (1976). Seed transmission of bean common mosaic virus in phasemy bean. *Phytopathology* 66, 1274-1275.

Rohlf, F.J. (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system. Setanket, New York.

Rosenberg, N.A. (2004). DISTRACT: a program for the graphical display of population structure. *Molecular Ecology Notes* 4(1), 137-138.

Schlötterer, C. (2000). Evolutionary dynamics of microsatellite DNA. *Chromosoma* 109(6), 365-371.

Schmidt, H.E., Reichnbacher, D., Lehmann, C., Verderevskaja, T.D., Basky, Z. & Horvath, J. (1987). Identification of pathotypes of bean common mosaic virus occurring in the German Democratic Republic, in the Soviet Union and in the Hungarian Peoples Republic. *Archives of Phytopathology and Plant Protection* 23(2), 105-115.

Schwartz, H.F., Corrales, M.A.P. & Singh, S.P. (1982). New sources of resistance to anthracnose and angular leaf spot of beans (*Phaseolus vulgaris* L.). *Euphytica* 31(3), 741-754.

Sengooba, T.N., Spence, N.J., Walkey, D.G.A., Allen, D.J. & Lana, A.F. (1997). The occurrence of bean common mosaic necrosis virus in wild and forage legumes in Uganda. *Plant Pathology* 46(1), 95-103.

Silbernagel, M.J., Mills, L.J. & Wang, W.Y. (1986). Tanzanian strain of bean common mosaic virus. *Plant Disease* 70(9), 839-841.

Singh, S.P. (1989). Patterns of variation in cultivated common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany* 43(1), 39-57.

Singh, S.P. (2001). Broadening the genetic base of common bean cultivars. *Crop Science* 41(6), 1659-1675.

Singh, S.P., Gepts, P. & Debouck, D.G. (1991a). Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany* 45(3), 379-396.

Singh, S.P., Gutierrez, J.A., Molina, A., Urrea, C. & Gepts, P. (1991b). Genetic diversity in cultivated common bean. 2. Marker-based analysis of morphological and agronomic traits. *Crop Science* 31(1), 23-29.

Singh, S.P., Molina, A. & Gepts, P. (1995). Potential of wild common bean for seed yield improvement of cultivars in the tropics. *Canadian Journal of Plant Science* 75(4), 807-813.

Singh, S.P., Nodari, R. & Gepts, P. (1991c). Genetic diversity in cultivated common bean .1. Allozymes. *Crop Science* 31(1), 19-23.

Spence, N.J. & Walkey, D.G.A. (1995). Variation for pathogenicity among isolates of bean common mosaic virus in Africa and a reinterpretation of the genetic relationship between cultivars of *Phaseolus vulgaris* and pathotypes of BCMV. *Plant Pathology* 44(3), 527-546.

Spence, N.J., Walkey, D.G.A. (1992). The susceptibility of wild legume species to bean common mosaic virus (BCMV). *annual report of the Bean Improvement Cooperative* 35, 140-141.

STATCOM (2011) *National Statistical Committee of the Kyrgyz Republic*. [online] (2013-02-05) Available from: http://stat.kg. [Accessed 2013-02-05].

Strausbaugh, C.A., Myers, J.R., Forster, R.L. & McClean, P.E. (1999). *Bc-1* and *Bc-u*—two loci controlling bean common mosaic virus resistance in common bean are linked. *Journal of the American Society for Horticultural Science* 124(6), 644-648.

Temple, S. & Smithson, J.B. (1989). The crossing of beans. In: Arregoces, O., *et al.* (Eds.) *Study guide CIAT*. pp. 1-50. Cali, Colombia: Centro Internacional de Agricultura Tropical. Series 04EB-08.02.

Torres, R.I.G., Villalobos, R.A., Solís, E.G. & Debouck, D.G. (2004). Wild common bean in the Central valley of Costa Rica: ecological distribution and molecular characterization. *Agronomía Mesoamericana* 15 (2), 145-153.

Tu, J.C. (1981). Anthracnose (*Colletotrichum lindemuthianum*) on white bean (*Phaseolus vulgaris* L.) in southern Ontario - spread of the disease from an infection focus. *Plant Disease* 65(6), 477-480.

Tu, J.C. (1982). Effect of temperature on incidence and severity of anthracnose on white bean. *Plant Disease* 66, 781-783.

Tu, J.C. (1986). Isolation and characterization of a new necrotic strain (NL-8) of bean common mosaic virus in southwestern Ontario. *Canadian Plant Disease Survey* 66(1), 13-14.

USDA (2013) *United States Department of Agriculture, Economic Research Service - vegetables & pulses: dry beans*. [online] (2013-02-28) Available from: http://www.ers.usda.gov/. [Accessed 2013-02-28].

Vandemark, G.J. & Miklas, P.N. (2002). A fluorescent PCR assay for the codominant interpretation of a dominant SCAR marker linked to the virus resistance allele *bc-1(2)* in common bean. *Molecular Breeding* 10(4), 193-201.

Vavilov, N.I. (1931). The problem of the origin of the world's agriculture in the light of the latest investigations. *Science at the cross roads: papers presented to the International congress of the history of science and technology held in London from June 20th to July 3rd, 1931 by the delegates of the U.S.S.R.* [online] (2013-03-28) Available from: http://www.marxists.org. [Accessed 2013-03-28].

Vetten, H.J., Lesemann, D.E. & Maiss, E. (1992). Serotype A and B strains of bean common mosaic virus are two distinct potyviruses. *Archives of virology. Supplementum* 5, 415-431.

Voysest, O. & Dessert, M. (1991). Bean cultivars: classes and commercial seed types. In: van Schoonhoven, A., *et al.* (Eds.) *Common beans: research for crop improvement*. pp. 119-162. CAB International and CIAT, Wallingford, UK.

Wells, W.C., Isom, W.H. & Waines, J.G. (1988). Outcrossing rates of 6 common bean lines. *Crop Science* 28(1), 177-178.

White, J.W. & Laing, D.R. (1989). Photoperiod response of flowering in diverse genotypes of common bean (*Phaseolus vulgaris*). *Field Crops Research* 22(2), 113-128.

Wortmann, C.S., Kirkby, R.A., Eledu, C.A. & Allen, D.J. (1998). *Atlas of common bean* (Phaseolus vulgaris *L.*) *production in Africa*. Cali, Colombia: CIAT, Centro Internacional de Agricultura Tropical. ISBN 958-9439-94-2.

Yeh, F.C. & Boyle, T.J.B. (1997). Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian Journal of Botany* 129, 157.

Young, R.A., Melotto, M., Nodari, R.O. & Kelly, J.D. (1998). Marker-assisted dissection of the oligogenic anthracnose resistance in the common bean cultivar, 'G 2333'. *Theoretical and Applied Genetics* 96(1), 87-94.

Yu, K.F., Park, S.J. & Poysa, V. (1999). Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus* and *Vigna*). *Genome* 42(1), 27-34.

Zhang, X.Y., Blair, M.W. & Wang, S.M. (2008). Genetic diversity of Chinese common bean (*Phaseolus vulgaris* L.) landraces assessed with simple sequence repeat markers. *Theoretical and Applied Genetics* 117(4), 629-640.

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Генетическое разнообразие фасоли в Кыргызстане и использование молекулярных маркеров в селекции на устойчивость к вирусу обыкновенной мозаики и антракнозу

Резюме

Фасоль (*Phaseolus vulgaris* L.), начиная с конца 20-го века - важная экспортная культура в Кыргызстане. Производство фасоли в Кыргызстане обеспечивает работой 76% населения Таласской области, производимый объем составляет около 70 000 тонн в год. Изучение генетического разнообразия фасоли помогает выбрать подходящий материал для будущей селекционной работы. Образцы из двух генетических центров Мезоамериканского и Андийского, включая основные типы фасоли, возделываемые в Кыргызстане, исследовались с помощью микросателлитов (SSR-маркеров) и морфологических качественных признаков. Установлена положительная корреляция (r = 0.49**) с матрицами коэффициентов сходства между молекулярными и морфологическими данными. Кластерный анализ и тех, и других данных определил образцы фасоли в соответствии с их принадлежностью к генетическому центру происхождения. Образцы Мезоамериканского центра были разнообразнее, чем образцы, относящиеся к Андийскому центру происхождения. Оба типа маркеров, как морфологические, так и микросателлиты, использовались в нашем исследовании для определения сортов фасоли, их принадлежности к центру происхождения, а также в создании первичного материала с признаками, значимыми для кыргызских фермеров. Мы обнаружили болезни, которые значительно снижают урожай зерна данной культуры в Кыргызстане - это штамм NL6 вируса обыкновенной мозаики (BCMV), а также антракноз. Восприимчивые кыргызские сорта - Рябая, Китаянка и Лопатка использовались как рекуррентные родители в возвратных скрещиваниях, при получении устойчивости от сортов-доноров. После 4-го беккросса семена (цвет, форма и размер) и бобы (форма) сохранили признаки, характерные рекуррентным родителям. Мы успешно использовали SCAR-маркеры (SW13, SBD5 и SCAreoli) при создании многоступенчатой устойчивости *I*, $bc\text{-}1^2$ и *Co-2* генов, соответственно. Данная устойчивость растений дает иммунитет к вирусу обыкновенной мозаики (BCMV) и антракнозу. Инфекционный тест (биологические расы антракноза - delta и gamma, а также штамм NL3 вируса BCMNV) и ДНК-маркеры подтвердили устойчивость в полученных беккросс-поколениях.

Ключевые слова: беккросс-программа, ДНК-маркеры, генетическое разнообразие, микросателлиты, *Phaseolus vulgaris*

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Bean common mosaic virus strain NL6 and anthracnose affect common bean crop and reduces significantly its grain yield in Kyrgyzstan. Sequence characterized amplified region (SCAR) markers were used in marker-aided backcross breeding for pyramiding the *I*, *bc-1*^{2} and *Co-2* genes, which provide host plant resistance to BCMV and anthracnose, respectively. Inoculation tests with anthracnose races delta and gamma, and virus strain NL3, and DNA marker aided-analysis provided means for confirming host plant resistance in the offspring.

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