GENETIC IMPROVEMENT OF THE COMMON BEAN (*Phaseolus vulgaris* L.) USING LOCAL GERMPLASM ASSISTED BY MOLECULAR MARKERS

Doctoral thesis

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Academic dissertation

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Contributions

The following table presents the contributions of the authors to the original publications or manuscripts of this thesis:

Contribution	**I**	**II**	**III**	**IV**
Original ideas	OJ	OJ	OJ	OJ
Research planning	OJ, HK	OJ, HK	OJ, HK	OJ, HK
Conducting the experiments	OJ	OJ	OJ	OJ
Data analyses	OJ	OJ	OJ	OJ, HK
Manuscript preparation	OJ, HK	OJ, HK	OJ, HK	OJ, HK, AR, PE, JV

OJ=Oswalt R. Jiménez, HK= Helena Korpelainen, AR= Aldo Rojas, PE= Paula Elomaa, JV= Jari P.T Valkonen.

Abbreviations

AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
ANT	Anthracnose
BCMV	Bean Common Mosaic Virus
BGYMV	Bean Golden Yellow Mosaic Virus
CIAT	International Center for Tropical Agriculture
CIPRES	Center for Promotion, Research, and Rural and Social Development
CNDF y Semillas	National Center of Phytosanitary Diagnostic and Seeds
CNIA	National Center of Agricultural Research (known formerly as CNIAB)
DNA	Deoxyribonucleic acid
FAM	6-carboxyfluorescein
HEX	Hexachlorofluorescein
IICA	Inter-American Center of Agricultural Cooperation
INTA	Nicaraguan Institute of Agricultural Technology
MAGFOR	Ministry of Agriculture and Forestry (now name is changing to Ministry of Agriculture and Livestock, MAG)
NIFAPRO	Nicaragua-Finland Agrobiotechnology Program
PCCMCA	Central American Cooperative Program for the improvement of Crops and Animals
PCR	Polymerase Chain Reaction
PP	Pods per Plant
SCAR	Sequence Characterized Amplified Region
SP	Seeds per Pods
SW	100-Seed Weight
TET	Tetrachlorofluorescein
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
YP	Yield per Plant

Keywords: *Phaseolus vulgaris*, landraces, genetic diversity, genetic purity, marker-assisted selection, mixed model analysis, microsatellite and SCAR markers, plant breeding.

Contents

Abstract

The common bean (*Phaseolus vulgaris* L.) is an important component of food security programs aiming to provide better human nutrition in developing countries. However, low yields, climate change affecting production and variable local demands of specific types of cultivars suggest that we should consider the utilization of local bean genetic resources with high market acceptance in local breeding programs. Those efforts would complement regional breeding agendas. In this study, the genetic diversity of a collection of landraces was found to be considerably higher (mean 8.9 alleles per microsatellite locus) than previously reported, with a population structure of three main clusters grouped according to seed weights.

Of these landraces, two promising divergent sources of genetic variation, accessions PV0006 and PV0023, were chosen for single crosses. The hybridity of F_1 generation was tested using polymorphic microsatellite markers. Computer simulations demonstrated that the selection of F_1 individuals possessing the highest degree of allele recombination following the pedigree method, instead of using the whole set of F_1 individuals as is usually done, could improve the selection gains for yield. Between 128 and 1024 pure lines could be obtained after a reasonable number of generations.

Subsequently, 420 F_2 plants originating from 15 marker-selected F_1 plants were established in three augmented blocks together with both parents and check cultivar 'INTA ROJO'. Variables PP, SP, SW and YP were measured for each plant. PP and YP were considered the most appropriate traits for selection based on ANOVAs, high heritability values, and predicted genetic gains obtained for both traits. After conducting plant selection using mixed model analyses, 81 and 74 F_2 plants (with 61 plants in common for both groups) were selected based on their superior yield potential compared with both parents and check cultivar. Resistance to BCMV, BGYMV, ANT and rust was confirmed in the segregating population, and their higher potential over 40 bean landraces was validated by computer simulations constructed using genotype frequencies. The level of resistance was unexpectedly found to be similar to that of current cultivars in use. However, further experiments to confirm resistance should be conducted. Moreover, resistance genes for BCMV (*bc-3* and *bc-1*^{2}) absent in our segregating plants were detected in bean landraces PV0015, PV0016, PV0017, PV0026, PV0031, PA0001, PA0002, and PA0003. It would be beneficial to pyramid broader resistance to this seed-borne virus in our populations by means of new crosses.

All these findings emphasize the feasibility of utilizing local landraces for genetic improvement using efficient statistical methods aided by molecular markers. Additionally, molecular markers were efficiently used to test the genetic purity of cultivar 'INTA ROJO'. The detected significant changes in genotype frequencies in four seed categories were probably caused by inadequate roguing procedures and isolation distances during seed production. Similarly, molecular markers were able to discriminate off-type seeds and plants from 'INTA ROJO', opening the possibility to complement current phenotypic methods employed in the genetic quality testing of seed lots.

1. Introduction

1.1 Importance of the common bean in the developing world

The common bean (*Phaseolus vulgaris* L.) is considered one of the most important protein sources in developing countries (Broughton et al. 2003; Mora-Avilés et al. 2007). In such countries, the production of this legume is usually carried out by a high number of small-scale farmers with low incomes and facing many production problems. In addition to its importance for subsistence in rural families, common bean also represents an important part of people's nutrition in urban regions. For instance in Mexico, being second only to maize (*Zea mays* L.), common bean is reported as indispensable in any food security and sovereignty program aiming to provide adequate nutrition to deprived people (Sangerman-Jarquín et al. 2010). The same situation can be found in other Latin American countries sharing the same cultural roots and food preferences.

According to FAO (2013), during 2012, African bean production was calculated to be 4,740,016 tons and Latin American production about 11,293,922 tons, both combined representing around 49% of global bean production. However, after viewing these international statistics, it can be found out that despite the social importance of beans and international efforts, the average yields for Latin American countries have not improved significantly in the last ten years. The average yields for 2002 and 2012 were calculated to be 708.3 and 744.5 kg/ha, respectively, with an improvement equaling 5%. Curiously, for the Central American region, average yields were 753.4 and 724 kg/ha, respectively, with a decrease equaling -3.9%. On the contrary, South American countries seemed to have the greatest progress in common bean yields, increasing from 770.3 to 1048.2 kg/ha, i.e. 36% during the same period of time. It can be observed that for the Central American region, the improvement in bean production is low in comparison with another crop of similar importance, maize, with yields increasing from 2,515.4 to 2,889.5 kg/ha over the same period of time, i.e. 15% between the years 2002 and 2012.

The importance of common bean as a legume crop has also been discussed in many studies regarding the possible negative effects of climate change in an agricultural context. The increasing global temperature and atmospheric CO_2 concentration, in combination with more frequent droughts, could reduce the productivity of most crop species and their current cultivars. Nonetheless, these predicted environmental conditions could be less catastrophic for legume crops, which seem to take an advantage of the higher CO_2 concentrations for increasing yields, improving nitrogen fixation and carbon sequestration at the same time (Wurr et al. 2000; Serraj 2003; Jensen et al. 2012; Vadez et al. 2012). Wurr et al. (2000) found that higher temperatures could shorten the growth cycle of cultivars. This physiological change could benefit plants' avoidance of terminal drought periods. However, phenological changes should be carefully interpreted considering all yield components and other climatic and plant variables in order to maximize yield potential under predicted scenarios (Hay and Porter 2006). In response to this, more research aiming to improve common bean physiology and to identify new sources of genetic variation to cope adverse environmental conditions and the occurrence of new pests and diseases appears to be justified during future years.

1.2 Common bean genetic resources from Nicaragua

The common bean is a self-pollinated species belonging to the Fabaceae family, which includes also other important crop species, such as soybean [*Glycine max* (L.) Mer.], pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), alfalfa (*Medicago sativa* L.) and peanut (*Arachis hypogaea* L.). There are many theories about common bean domestication, but most of them indicate that this species was probably domesticated in two main events that occurred in Mesoamerica and the Andes (Gepts and Debouck 1991; Chacón et al. 2005). However, recent studies that focused on nucleotide diversity in five important genes suggest that the Andean gene pool could have been originated from Mesoamerican germplasm introductions, emphasizing the importance of Mesoamerican gene pool as source of genetic variation (Bitocchi et al. 2012). After domestication, human activity spread common bean to African, Asian and European countries, developing novel and adapted genetic diversity along the years in those continents. The high genetic diversity found in African, Asian and European germplasm has suggested the possibility that those continents could be considered as other centers of genetic diversity for common bean (Ocampo et al. 2005; Zhang et al. 2008, Asfaw at al. 2009; Blair et al. 2010, Santalla et al. 2010; Maras et al. 2013; Mercati et al. 2013; Sharma et al. 2013).

Nicaragua is a tropical country located in the Mesoamerican center of genetic diversity for *Phaseolus* species. The scarce available reports indicate that there is a high phenotypic diversity but moderate genetic diversity in local landraces conserved *ex situ* and *in situ* (Gómez et al. 2004, 2005). Unfortunately, there are no reports about the number of common bean landraces exploited, their current situation and their contribution to the Nicaraguan economy. Nonetheless, a high level of genetic diversity could be assumed by looking at the availability of diverse common bean landraces in national markets. They show a wide phenotypic variability, expressed as many different seed colorations, sizes and shapes as products of farmers' selection and the diverse agro-ecological niches where common bean production takes place.

The lacking national policy regarding the conservation of plant genetic resources, the obsolete seed legislation and the low sustainability of many projects could be among the possible causes of failure of any initiative to promote the conservation of bean genetic resources in Nicaragua. Furthermore, the low research activity and rare use of local bean germplasm in breeding programs could affect the sustainability of any project aiming to conserve bean genetic resources, because there is no justification to invest funds in non-active gene banks or collections. In consequence, most bean genetic resources have had to be conserved *in situ* on farms. Under those conditions, common bean landraces and old cultivars evolve continuously according to farmers' preferences and environmental conditions. The varied agricultural practices, consumer preferences and environmental conditions have produced a good amount of different genetic materials. Most of them have never been studied before, but some could be very promising sources of genetic variation for breeding purposes aiming to satisfy local and regional demands.

1.3 Common bean breeding activities in Nicaragua

1.3.1 Classical breeding experiences

The historical references of common bean breeding programs conducted in Nicaragua were described by Voysest (2000) as two main stages, before 1976 and between 1976 and 1999. A summary of that valuable piece of information is provided in the next three paragraphs.

In the 1950s, there were some reports that point out the possible preference to black-seeded cultivars by Nicaraguan consumers. This is based on the cultivar offer during that time. Until 1953, all common bean production relied on landraces. However, since that year, a collection of landraces was established and many cultivars began to be imported from neighboring countries. One of the most successful introductions was the black-seeded cultivar 'RICO', released by IICA, Costa Rica. There were also other notable cultivar introductions, such as 'TURRIALBA 1', 'JALAPA' and 'PORRILLO N^{o} 1'. Mass selection conducted on the genetically highly variable cultivar 'JALAPA' originated the cultivar 'VERANIC 2'.

Through PCCMCA, cultivar introductions and evaluations continued during following years. In 1970, the first red-seeded cultivar, 'HONDURAS 46', was released. The acceptable seed coloration of that cultivar contributed to the replacement of all previously introduced black-seeded materials. In 1972, a black-seeded cultivar, 'TURRIALBA 4', was introduced. At that time, the red-seeded cultivar 'ORGULLOSO', brown- seeded material "café mono" and many white-seeded genetic materials were among the widespread traditional cultivars. To select for a better vegetative cycle and growth habit, 'HONDURAS 46' was used by bean breeders Humberto Tapia and José Angel Ponce resulting in new cultivars 'C-5R', 'C-7R' and 'C-13R'. Similarly, selection on 'TURRIALBA 4' resulted in new black-seeded cultivars 'C-7N' and 'C-11N', and selection on 'ORGULLOSO' produced cultivars 'ORGULLOSO 1' and 'ORGULLOSO 2'.

In the same way as other Central American countries, Nicaragua was incorporated into the international network of cultivar experiments promoted by CIAT and later to the Central American regional network PROFRIJOL in 1976. After that, through those projects, many red-seeded advanced lines were released in Nicaragua between 1979 and 1984. Some successful examples were cultivars 'REVOLUCION 79', 'REVOLUCION 79A', 'REVOLUCION 81', 'REVOLUCION 83', 'REVOLUCION 83A', 'REVOLUCION 84' and 'REVOLUCION 84A'. However, at the end of the 1980s, the tragic death of Humberto Tapia marked a slowdown in the genetic improvement of common bean in Nicaragua. Definitely, this notable bean breeder contributed significantly to the development of this crop during those years. From that date, almost all local bean breeding activities were simplified to the evaluation and validation of advanced genetic materials provided by regional programs without conducting complete breeding agenda at the local level.

Since 1990, with the enforcement of the regional project and the devastating advance of BGYMV, common bean nurseries from PROFRIJOL (VIDAC-ECAR) provided a source of promising advanced lines for the evaluation and release of the new red-seeded cultivars 'ESTELI 90A', 'ESTELI 90B' 'DOR 364', 'COMPAÑIA' and 'CNIGB 93'. Rosas et al. (2004a) described that during the 1990s, scientists from the University of Puerto Rico and the Pan-American Agricultural School, ZAMORANO,

with the support of the program Bean/Cowpea CRSP, were incorporated into PROFRIJOL and they were involved in bean breeding activities in the Central American and Caribbean region. In 1993, INTA was created through Presidential decree 22-93 and it was given the responsibility of plant breeding activities in Nicaragua. However, INTA continued following the model described before.

Between 2000 and 2013 many red and black-seeded advanced lines were introduced, evaluated, validated and released as new cultivars. Cultivars 'INTA CANELA', 'INTA MASATEPE', 'INTA CARDENAS', 'INTA ROJO', 'INTA NUEVA GUINEA', 'INTA PRECOZ', INTA MATAGALPA', 'INTA FUERTE SEQUIA', 'INTA NUTRITIVO', 'INTA CENTRO SUR' and 'INTA CENTRO NORTE' are examples of releases (INTA 2013). All these cultivars have the dominant gene *I* for resistance to BCMV, and genes *bgm-1*, *bgp-1* and major *QTL* (from 'DOR 364') for resistance to BGYMV. Growth habits are of type II, with a physiological maturity between 68 and 74 days after sowing. Seeds are ovoid in shape with weights varying between 0.21 and 0.25g/100 seeds. Nonetheless, in the national and regional markets, landraces are still preferred because of their seed coloration and culinary quality. On the other hand, all released cultivars have counterparts in other countries, because each country names the received advanced lines following a different nomenclature (Rosas et al. 2004a). This behavior creates the wrong perception that many different cultivars are in use in Central American and Caribbean countries, while there are only relatively very few genotypes.

1.3.2 Participatory breeding experiences

In 1999, CIPRES and INTA started a breeding project in Pueblo Nuevo, Estelí under a participatory approach. Almekinders et al. (2006) and Almekinders (2011) have provided a description of the main activities and results obtained from this project. I summarize the main achievements in this section.

The high incidence and severity of BGYMV motivated farmers to join this project with commitment, even though there were not enough funds to cover all the expenses. Common bean nurseries from CIAT, 15 families in total ($F_{3:4}$ generations), were distributed among 50 bean farmers. After first meetings and discussions, five farms were selected to conduct replicated experiments. Crop management, selection methods and target traits were according to farmers' criteria, but the final decision was a consensus of the whole group with the assistance of a bean breeder from INTA. The main traits to improve during the selection were resistance to BGYMV, seed coloration, yield, tolerance to drought and culinary quality (soup quality). The performance of selected families was compared with check cultivar 'INTA MASATEPE' (very popular during that time).

After some cycles of within and between family selections, the best five genetic materials were evaluated and eventually the best one was formally released as the cultivar 'PUEBLO NUEVO JM'. This was a good example of how a participatory breeding approach can produce a new cultivar with local relevance. Nevertheless, the current seed legislation makes it difficult to register this kind of cultivars and limits their benefits in the formal seed market in the country.

1.3.3 Current activities and justification for further actions

To date, traditional breeding activities are conducted adopting the same model as used during the last 20 years. However, there is a small number of breeding projects conducted using the participatory approach. Those initiatives have been subsidized by funds from the European Union, and they aim to increase the production of common bean landraces and to provide high quality seeds at the local level. Also, there is an on-going initiative to improve seed legislation to open a place for genetic materials obtained using this approach. However, there is still no solid outcome from those actions.

Finally, it can be concluded that current approach could have limited the development of common bean. Here, I do not want to downplay the important role of regional breeding programs, because they have performed a great work. Nonetheless, the Nicaraguan breeding agenda for common bean should be complemented with local actions in order to handle the effects of climate change on bean production and to discover new cultivars with a better acceptance in local and regional markets. This kind of approach will also promote the conservation of active germplasm collections, and it will enhance the research activity in this area by justifying the investment of funds in upcoming years.

2. Aims of this study

The general objective of this study was to conduct genetic improvement of the common bean using local germplasm with high potential aided by molecular markers. The specific objectives were as follows:

1. To identify promising sources of genetic variation in a representative collection of Nicaraguan common bean landraces.

2. To incorporate the genetic diversity of two promising landraces into a segregating population and evaluate its genetic characteristics.

3. To select F_2 plants with the highest yield potential, confirming their resistance to common diseases.

4. To test the genetic purity of the cultivar 'INTA ROJO' using molecular markers.

3 Materials and methods

3.1 Common bean populations

For the genetic diversity study, 37 common bean landraces and three tepary bean (*Phaseolus acutifolius* A. Gray) populations, never studied before, were chosen from a bean germplasm collection that was sampled from different agro-ecological regions in Nicaragua. The selection of the landraces was based on their origin, seed coloration and the level of acceptance by farmers. Details of the seed sampling and population passport data can be found in sub-study I.

The segregating population (F_1 generation) described in sub-study II was created by single crossing landraces PV0006 and PV0023. Those populations were selected based on their high genetic diversity, different genetic structure, high adaptation to local environmental conditions and good market acceptance because of their seed coloration [similar to colors 2.5R 4/10, 5R 3/8 and 5R 3/10 in the Munsell color charts for plant tissue (1977)] and culinary quality. Computer simulations were conducted using two base populations, F_1 selected individuals and the whole set of F_1 plants.

For sub-study III, the F_2 generation composed of 420 plants was obtained by selfing 15 F_1 plants selected using microsatellite markers. Thereafter, 61 F_2 plants were selected to originate the F_3 generation. Plant selection was based on mixed model analyses and their resistance to common diseases confirmed using SCAR markers. For computer simulations, two base populations were used, one built using genotype frequencies of F_2 generation (calculated assuming Mendelian inheritance for each locus from F_1 to F_2), and another using genotype frequencies of 100 random individuals from 40 bean landraces. Detailed information of these genotype frequencies is showed in Method S1 in sub-study III.

The seed lots, breeder's, foundation and registered seeds, were sampled from the cold room for seeds at CNIA. Certified seeds were produced in Matagalpa, Nicaragua and sampled from a seed farmer in collaboration with MAGFOR. Seed samples were obtained following the methodology described by ISTA (2004). Off-type seeds, "frijol viterra" and "frijol rojo oscuro" were obtained by screening samples from all stages of the bean seed production between September and November 2008 in collaboration with CNDF y Semillas. Finally, off-type plants were sampled at the research station "La Compañía" located in Carazo, Nicaragua in cooperation with seed inspectors from MAGFOR. More information about those populations can be found in sub-study IV.

3.2 Methods

3.2.1 Laboratory experiments

For all experiments described in sub-studies I, II, III and IV, PCR methods and molecular markers were used to genotype populations. The PCR reactions were designed as suggested in literature regarding each marker, and the procedures are showed in detail in each publication. Genomic DNA extractions were carried out following a method described in sub-studies I and IV. In those sub-studies, highly polymorphic microsatellite markers were employed to evaluate genetic diversity and cultivar identity. However, in

sub-study II, only microsatellite markers polymorphic in both parents were used to assess the hybridity of each F_1 individual. For all above mentioned markers, forward primers were fluorescently labeled with FAM, TET and HEX dyes, and the fragment sizes were analyzed using the capillary electrophoresis system 3730 DNA Analyzer (Applied Biosystems) in the Sequencing Laboratory of the Institute of Biotechnology, University of Helsinki, Finland. DNA fragment sizes were measured using the software PEAK SCANNER version 1.0 (Applied Biosystems).

For sub-study III, SCAR markers tightly linked to alleles for resistance to common diseases were used, and DNA fragment sizes were scored in gel systems. Details about two screenings are described in the manuscript.

3.2.2 Breeding methods

Landraces used as parents for crosses were chosen considering the highest level of genetic diversity discovered in sub-study I. Plant crosses were conducted in a greenhouse using the methodology available as Method S1 in sub-study II. The selection at the F_1 generation was performed using microsatellite markers to discriminate hybrid and non-hybrid plants, and to select the highest level of allele recombinants. In sub-study III, the F_2 generation was evaluated and selected based on the highest PP and YP values through mixed models analyses and also based on resistance to common diseases, as confirmed by SCAR markers. Negative selection for plants exhibiting complete prostrate growth habits was applied. Selected plants produced seeds for the F_3 generation for further selection processes. All selection methods followed a pedigree scheme assisted by molecular markers. Records of each evaluated and selected plant were kept in detail to track the origin of any plant at any stage. A flowchart of the breeding scheme adopted is presented in Figure 1.

3.2.3 Field and greenhouse experiments

Greenhouse experiments were described in sub-study II to make crosses and to evaluate the hybridity of the F_1 generation. During this experiment, traits PP, SP, SW and YP were recorded for each individual. Those traits were useful when simulating the selection of the most recombinant individuals. On the other hand, for sub-study III, the field experiment was designed as three augmented blocks containing both parents and the check cultivar ‘INTA ROJO’ (three common treatments) and 420 non-common treatments (F_2 plants). Experimental conditions of both experiments are showed in corresponding papers.

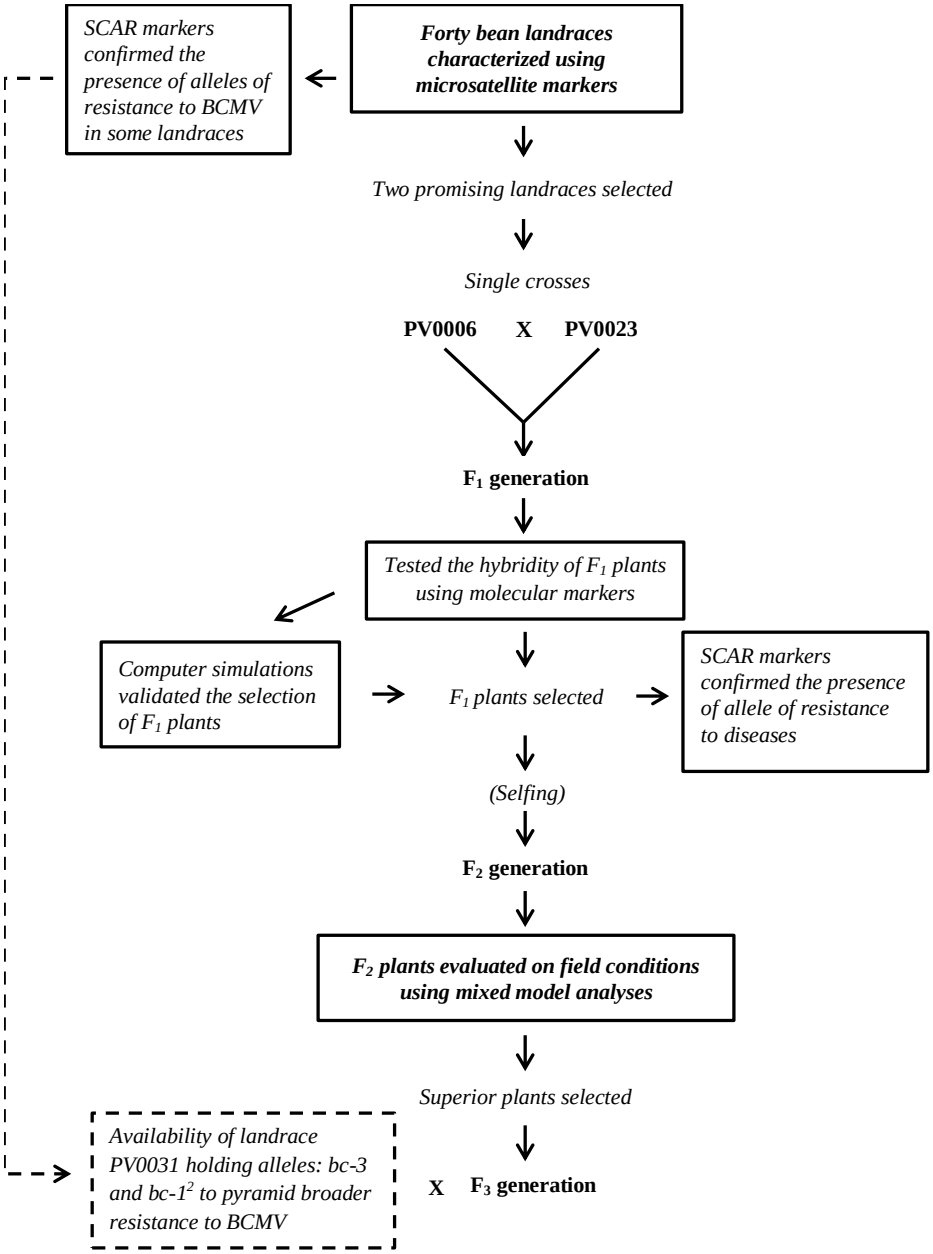

Figure 1. *Flowchart of the modified pedigree scheme of selection adopted during this study. Note that the dotted arrow shows possible further actions.*

3.2.4 Statistical methods, modeling and simulations

Statistical methods used in four sub-studies are described in detail in each corresponding publication. In brief, for calculating genetic parameters, discriminating hybridity, and testing cultivar identity, I used the following software: ARLEQUIN versions 3.1 and 3.5.1.2 (Excoffier et al. 2006; Excoffier and Lischer 2010), R versions 2.6.2 and 2.15.0 (Venables and Smith 2008; Venables and Smith 2012), and GENEPOP version 4.1 (Rousset 2008) (sub-studies I, II, and IV). For inferring phylogenetic relationships and Bayesian population structure, I employed software MEGA versions 4.1 and 5.1 (Tamura et al. 2007, 2011) and STRUCTURE version 2.3.3 (Pritchard et al. 2000) (sub-studies I and IV).

For conducting germplasm-regression-combined marker–trait association analyses (Ruan et al. 2009; Ruan 2010), I used software SPSS versions 16.0 and 20.0 (SPSS, Chicago, IL, USA; http://www.spss.com) (sub-studies I and II). For computer simulations, the software QuGENE engine version 2.3.01 (Podlich and Cooper 1998, Wang et al. 2006) and QuLINE version 2.0 (Wang et al. 2006) were employed (sub-studies II and III). For ANOVA and comparisons of adjusted means obtained from field experiments, I used statistical package 'agricolae' version 1.1-3 (De Mendiburu 2013) run under software R version 2.15.0 (Venables and Smith 2012) (sub-study III). For modeling plant selection, I applied models 74 and 102 from the software SELEGEN-REML/BLUP (Resende 2007) (sub-study III).

4. Results

4.1 Genetic diversity and population structure in Nicaraguan common bean landraces (sub-study I)

The genetic diversity of 37 common bean landraces and three tepary bean accessions was assessed using 14 polymorphic microsatellite markers distributed in different linkage groups. The results indicated that those populations have a high genetic diversity, possessing 115 different alleles in total. The average allelic diversity was 8.9 alleles per microsatellite locus. A total of 134 genotypes were identified in all populations. When each population was evaluated individually, landraces PV0006, PV0013, PV0023, PV0024 and PV0028 were found to possess highest levels of allelic variation; excluding tepary bean populations and accession PV0037, allele numbers ranging from 27 to 31 (mean 2.4 alleles per polymorphic locus). Allelic diversity for each locus is presented in more detail in Table 2 in sub-study I.

The UPGMA tree constructed using F_{ST} values was in correspondence with the Bayesian structure analysis. The latter analysis inferred the genetic structure of common bean landraces into three main clusters (K=3), showing genetic differentiation of common bean from tepary bean landraces (cluster 1) and an apparent tendency to group the populations according to their seed weights (Figure 2a). Considering only the populations with the highest genetic diversity, landraces PV0006 and PV0024 were included in cluster 2. On the contrary, landraces PV0013, PV0023 and PV0028 belonged to cluster 3. Landraces PV0006 and PV0023, despite of possessing high genetic diversity, they also exhibit market-acceptable seed colorations, which attracts a special attention.

Three stepwise multiple regression runs followed by curve-fitting testing were performed to outline the correlations of 107 genotypes (excluding tepary bean accessions) with quantitative seed traits (seed length, width and weight). For seed length, genotypes $BM156_{224}$, $AG1_{138}$ and $PVag001_{278}$ showed positive (the first one) and negative (the last two) correlations. Genotype $PVag001_{278}$ showed the highest (R^2= 0.214) significant (P = 0.003, t = -3.133) correlation with a high standardized β value of -0.463. The addition of the genotypes $BM156_{224}$ and $AG1_{138}$ to the model increased considerably the correlation index (R^2 = 0.339 and 0.423, respectively). For seed width, only the genotype $BM156_{222}$ showed a positive (R^2 = 0.175) significant (P = 0.009, t = 2.767) correlation with a standardized β value of 0.459. Finally, two genotypes, $BM205_{131}$ and $BM205_{133}$, showed significant correlations with seed weight. Genotype $BM205_{131}$ showed the greatest (R^2 = 0.340) significant (P = 0.000, t = 4.304) positive correlation. The standardized β value was also high (0.583). When both genotypes were added to the model, the correlation increased significantly (R^2 = 0.429). More statistical details can be found in Table 3 in sub-study I.

4.2 Reshuffling the genetic diversity of two promising landraces (sub-study II)

Two adapted landraces, PV0006 and PV0023, were chosen as parents based on phenotypic and molecular characteristics, and they were single crossed (Figure 2b). Segregating F_1 generation was obtained and plants' level of hybridity was tested using 14

microsatellite markers polymorphic in both parents. Ten out of 14 markers efficiently discriminated hybrids from non-hybrid individuals. About 43% of the F_1 individuals were heterozygous for at least seven out of ten loci. AMOVA and Fisher's exact test showed that most alleles and their frequencies present in parents were kept in the segregating population with minor variations (Table 1 in sub-study II). The level of allele recombination suggested that theoretically between 128 and 1024 pure lines could be obtained after a reasonable number of generations. Fifteen F_1 individuals were selected because they showed highest levels of allele recombination.

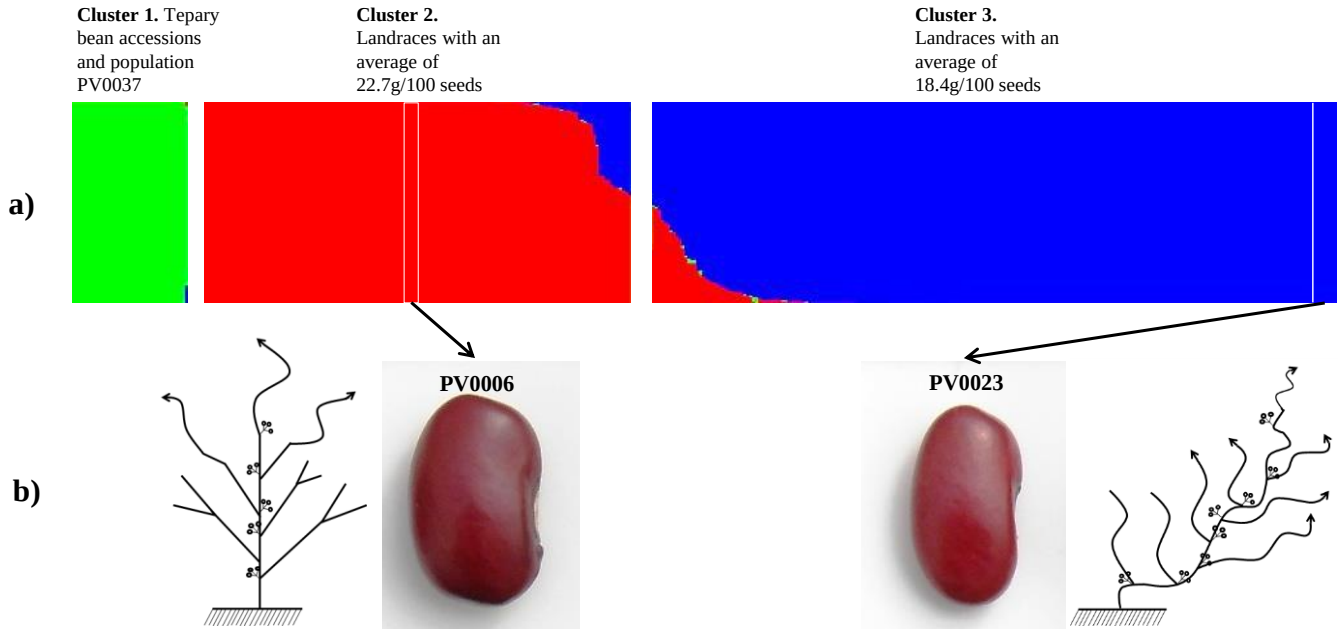

Figure 2. *(a) Genetic population structure (K=3) sorted by membership coefficient (Q value). Cluster 1 contains tepary bean populations and landrace PV0037, cluster 2 contains populations with relatively large seeds and cluster 3 contains populations with relatively small seeds. (b) Selected parents, PV0006 and PV0023. Note the phenotypic differences between parents and the disadvantageous growth habit of parent PV0023 for cultivation.*

4.3 Computer simulations of breeding strategies using marker-selected F_1 individuals (sub-study II)

Two stepwise multiple regression runs and curve-fitting testing were implemented to detect significant correlations between 37 microsatellite alleles and PP, SP, SW, and YP values at the greenhouse level. Six out of 37 alleles showed correlations with these traits. For SW, alleles $AG1_{138}$ and $BM184_{159}$ showed negative and positive correlations, respectively. However, allele $AG1_{138}$ exhibited the greatest correlation (R^2 = 0.695, P = 0.000, t = -10.789) with a high standardized β value (-0.834). Both alleles together increased significantly the correlation index (R^2 = 0.749). For YP, alleles $BM143_{153}$ and $BM199_{276}$ showed positive and negative correlations, respectively. Allele $BM143_{153}$ showed the highest significant correlation (R^2 = 0.387, P = 0.006, t = 5.675) with a standardized β value of 0.622. When both alleles were added to the model the correlation increased significantly (R^2 = 0.543). More detailed information about other marker-trait correlations can be found in Table 2 in sub-study II.

All significant correlations at loci BM143, BM199, AG1, BM184, and BM205 were used to construct a breeding simulation in order to compare two base populations, the most segregating individuals and all F_1 generation individuals (marker BM205 was included based on results obtained in sub-study I). The results demonstrated that the marker selection of the most segregating individuals at F_1 generation could improve the genetic gains of yield under the pedigree method. Using this method, the most segregating individuals achieved the complete target genotype during the third cycle of selection. On the contrary, using all F_1 individuals without any testing, complete target genotype was obtained during the fifth cycle of selection. Mass and bulk selection strategies were not efficient for either population (Table 3 in sub-study II). Fitness adjusted by target genotype values and Hamming distances for each population selected under pedigree method are plotted in Figure 1 in sub-study II.

4.4 Identification and selection of superior plants at F_2 generation (sub-study III)

In total 420 F_2 plants produced from 15 marker-selected F_1 plants, both parents, and the check cultivar 'INTA ROJO' were evaluated under field conditions. ANOVAs and mixed model analyses indicated that PP and YP were adequate traits for single plant selection at the F_2 generation, because of their significant differences among plants and treatments, and their higher relative heritabilities (0.43 and 0.27, respectively). For both traits, there were significant differences between F_2 plants and parents and the check cultivar. Tukey's means comparisons (P < 0.050) clustered all individuals for PP and YP into 54 and 29 groups, respectively. In contrast, no clusters were found for SW and only three clusters for SP.

After discarding completely prostrate individuals (as showed for PV0023 in Figure1b) and considering the general mean as the lower limit for selecting the best individuals, 81 and 74 individuals were selected, which represent 46.3 and 42.3% of individuals. Due to some individuals were present in both groups of selected traits, and considering individuals selected for both traits as superior, 61 individuals (35%) were selected at the end. Among selected individuals, the predicted genetic gains for PP were from 4.33 to 12.14 and for YP from 2.14 to 7.59g. For SP and SW the general average

predicted genetic gains among all individuals were 0.13 and 0.68g, respectively. The Method S2 in sub-study III showed more detailed information about predicted genetic parameters. All selected plants exhibited phenotypic superiority in comparison with the check cultivar 'INTA ROJO'. There were also a marked flowering and maturity earliness among the selected plants. Figure 3 shows how the selected F_2 plant coded as PV23/PV6-HERY achieved physiological maturity earlier than the cultivar 'INTA ROJO' with apparent superior yield.

***Figure 3**. Phenotypic comparison between selected F_2 plant PV23/PV6-HERY (left) and a plant from cultivar 'INTA ROJO' (right) under the same environmental conditions.*

4.5 Confirmation of resistance to common diseases and breeding potential at F_2 generation (sub-study III)

The level of disease resistance of the selected F_2 individuals was tested by screening founder F_1 individuals and comparing with the whole collection of 40 landraces (used previously on sub-study I) and three cultivars with known resistances to BCMV, BGYMV, ANT and rust. The screening was conducted using 14 SCAR markers tightly linked to genes of resistance to those diseases.

During the first round of screening, only primers for $SW13_{690}$, $SR2_{533}$, $SW12_{700,}$ $SY20_{830}$, $SAZ20_{845}$ and $SF10_{1072}$ clearly produced the target DNA fragments in at least one parent. The presence of these disease resistance alleles was successfully confirmed at the F_1 generation. However, cultivar 'DOR 364' did not possess the dominant resistance to BCMV (marker $SW13_{690}$), as the other cultivars did.

In a second round of screening, using 40 landraces, markers $SAS13_{950}$, $SAB3_{400}$ and $SB12_{350}$ did not produce any target DNA fragment in the whole set of landraces. The marker $ROC11_{420}$ produced the target DNA fragment in landraces PV0017, PV0026 and PV0031. The marker $SBD5_{1250}$ produced the target fragment in landraces PV0015, PV0016, PV0031, PA0001, PA0002 and PA0003 (the last three originating from tepary bean). None of the above mentioned markers produced any target bands on either parents or cultivars. Additionally, primers for $SW13_{690}$, $SR2_{533}$, $SW12_{700,}$ $SY20_{830}$, $SAZ20_{845}$ and $SF10_{1072}$ produced DNA fragments in some landraces as showed in Method S3 in sub study III.

Figure 4a shows the allele diversity found in different landraces with homozygous genotypes at the co-dominant locus SR2. On the other hand, Figure 4b shows the heterozygous genotype confirmed in each F_1 selected plant. Here, the allele associated with resistance to BGYMV (*533-bp* fragment) was undoubtedly inherited from the parent PV0006.

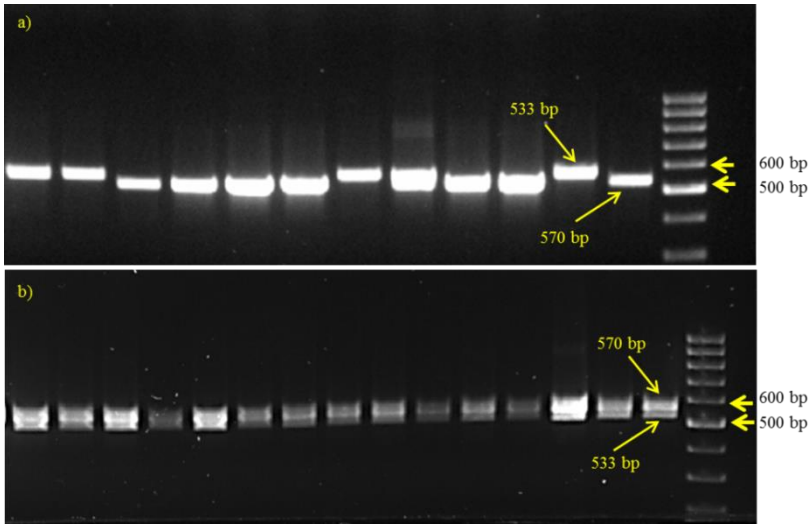

Figure 4. *Screening for resistance to BGYMV using the codominant SCAR marker SR2. The 533-bp DNA fragment indicates linkage to gene bgm-1(resistance) and the 570-bp fragment to susceptibility (Blair et al. 2007a). Common bean landraces (a), and F_1 selected individuals (b) possessing both alleles.*

Due to the presence of resistance alleles for markers $SW13_{690}$, $SR2_{533}$, $SW12_{700}$, $SY20_{830}$, $SAZ20_{845}$ and $SF10_{1072}$ in the bean landraces in at least one of the parents and in most F_1 individuals, breeding simulations were conducted using genotype frequencies of selected individuals and a base population composed of 40 landraces. Computer simulations indicated that the pedigree method could be a more effective strategy for obtaining complete resistance in a relative shorter period of time. Mass and bulk methods were not efficient to attain the target genotype by the end of the fifth cycle of selection.

When 40 landraces together and selected F_2 plants were simulated using the pedigree method, the target genotype was achieved after the second cycle of selection for all resistance traits using F_2 selected plants. On the contrary, using the population composed of 40 landraces the target genotype was not achieved even by the end of the fifth cycle of selection. Fitness adjusted by target genotype values for both populations are plotted in Figure 1 in sub-study III.

4.6 Genetic purity of the cultivar 'INTA ROJO' tested using microsatellite markers (sub-study IV)

The usefulness of microsatellite markers was tested to determine the genetic purity of the cultivar 'INTA ROJO' during seed production. The genotype frequencies of four seed generations (breeder's, foundation, registered and certified seed) were estimated using ten polymorphic microsatellite markers. The Fisher's exact test conducted to compare genotype frequencies of the reference population, breeder's seed, with its subsequent generations showed that there were significant changes in genotype frequencies in foundation seed at locus GATS091 (P = 0.000). Similarly, registered seed also showed changes in genotype frequencies in foundation seed at locus GATS091 (P = 0.002). Finally, certified seed exhibited changes in genotype frequencies at loci BM172 (P = 0.002), BM137 (P = 0.000), and GATS091 (P = 0.000). Ten new genotypes (not present in breeder's seed) were identified in the other three seed categories.

Additionally, the allelic composition of breeder's seed was compared with off-type seeds ("frijol viterra" and "frijol rojo oscuro") and plants. In all, 27 alleles were found in the four groups. Four alleles were exclusively detected in off-type individuals. The "frijol viterra" population possessed nine monomorphic loci, while off-type plants and "frijol rojo oscuro" all had four monomorphic loci. The pairwise F_{ST} values ranged from 0.0768 to 0.4307. The average F_{ST} value equaled 0.2213. The results showed that only "frijol viterra" was genetically similar to breeder's seed. Four out of ten microsatellites (loci BM175, GATS091, BM137 and BM188) proved to be useful in discriminating off-type plants and seeds from 'INTA ROJO', because each of those loci possessed one different allele not found in any individual in the four seed categories. Allelic composition of 'INTA ROJO' and off-type individuals is showed in Table 4 in sub-study IV.

5. Discussion

Several studies point out that the plant genetic resources could play a crucial role in agricultural sustainability for next decades (FAO 2010; Hodgkin and Bordoni 2012). In many cases, the utilization of local germplasm in breeding programs aiming to increase the productivity and resistance to diseases should be a viable approach. So far, most common bean breeding activities are carried out by regional breeding programs, and only little work is conducted at the local level. In this study, the feasibility of the utilization of local common bean germplasm in breeding programs aided by molecular markers was assessed through three independent sub-studies. These sub-studies covered aspects related to germplasm assessment, parent selection, the creation of segregating populations and plant selection in F_2 generation. In the fourth sub-study, the genetic purity of a released cultivar was successfully tested using marker information. The most important findings and achievements are discussed for each sub-study in the following paragraphs.

The evaluation of genetic diversity conducted for 37 common bean landraces and three tepary bean populations, both groups never studied before, detected a significantly higher genetic diversity (average 8.9 alleles per microsatellite locus) when compared with previous references, which found a maximum of 5.7 alleles per locus while analyzing similar Nicaraguan populations (Gómez et al. 2004, 2005). This level of genetic diversity was also higher when compared with other studies involving other common bean genetic materials in other countries (Yu et al. 2000; Gaitán-Solís et al. 2002; Blair et al. 2006a; Díaz and Blair, 2006; Benchimol et al. 2007; Zhang et al. 2008; Díaz et al. 2010; Santalla et al. 2010; Cabral et al. 2011; García et al. 2011; Avila et al. 2012). Blair et al. (2009) found allelic variation averaging 18.4 alleles per locus, but the result was obtained from a worldwide core collection of 604 accessions. Thus, I can suggest that the level of genetic diversity found in this study is exceptionally high and should call our attention.

The population structure was inferred into three main clusters (K=3), of which two contained common bean populations. In these two clusters, landraces PV0006, PV0013, PV0023, PV0024 and PV0028 showed the highest allelic variation. Of these, PV0006 and PV0023 were found promising for germplasm improvement because they were in two different clusters, both had good seed colorations and showed phenotypic differences that suggest that a highly variable segregating population could be achieved from them (personal observation, Oswalt R. Jiménez).

The significant marker-trait associations in seed traits suggested that the identification of promising allele diversity could have significant implications for breeding purposes. There is no documented evidence that parents used in crosses, in those Nicaraguan common bean cultivars currently under use, were chosen using marker information (Rosas et al. 2004a, 2004b). Frequently, germplasm assessing, hybrid testing, plant selection, and cultivar identification are among the possible uses of molecular markers (Ashraf and Foolad 2013; Sivolap 2013). At this stage, I used molecular information to identify and choose promising landraces and parents for genetic improvement, assuming a high potential in populations possessing the highest level of allelic diversity.

Accordingly, landraces PV0006 and PV0023 were selected as parents for a breeding project, and 60 random individuals were single crossed. The hybridity of F_1

individuals was tested using microsatellite markers polymorphic for both parents, and the most allele recombinant individuals (15 F_1 individuals in total) were selected for the following generation. The use of molecular markers to test heterozygosity has been only employed to check the genetic quality of hybrid cultivars in different crop species, such as maize (*Zea mays* L.), broccoli (*Brassica oleraceae*), rice (*Oryza sativa* L) and pepper (*Capsicum spp*) (Smith and Register, 1998; Crockett et al. 2002; Yashitola et al. 2002; Mongkolporn et al. 2004; Sundaram et al. 2008; Sivolap 2013; Ye et al. 2013). There are no reports about the use of molecular markers to test hybridity and select individuals at the F_1 generation in common bean, as performed in this study. All these methods used in common bean account to the novelty of this study.

The most common method to evaluate the hybridity of F_1 individuals is to use morphological markers (differences in the seed coloration of parents, for instance), something not possible here because PV0006 and PV0023 have similar seed colorations (Figure 2b). Also the high genetic diversity makes the exact phenotypic discrimination of F_1 plants difficult. For crossings, it is common to trust the skills and experience of the person who performs the flower emasculation and pollination. However, even though not documented, some degree of selfing is accidently retained when conducting crossings and this should be avoided. The presence of non-hybrids and individuals with low level of segregation could significantly reduce genetic gains in breeding programs when the success relies on new allele combinations. Therefore, as proposed by Acquaah (2007, 2012), the use of an efficient hybridity testing method to rogue out non-hybrid individuals may be needed, and molecular marker techniques seem to be more efficient than morphological methods in most cases.

The significance of marker-selection conducted for F_1 individuals was confirmed when population of selected individuals achieved the target genotype in a significantly reduced period of time in comparison with the whole set of F_1 individuals under simulated experiments using three breeding methods (pedigree, mass and bulk methods). It was obvious that the selection process is more efficient when we concentrate on the most segregating individuals at the F_1 generation. Again, there are no available reports about this kind of selection being used in common bean breeding, but the statistical software used here has previously proven to be a powerful tool for conducting *in silico* testing of different breeding strategies in wheat (*Triticum spp*), barley (*Hordeum vulgare* L.) and rice (*Oryza sativa* L.) (Wang et al. 2003, 2005, 2006, 2007; Wang and Pfeiffer 2007; Ye et al. 2007; Qi et al. 2011).

Computer simulations are very important when planning and conducting breeding programs, because with an adequate amount of information we are able to choose the most potential populations and efficient methods, thus reducing considerably costs and time otherwise needed for field experiments. Of course, it is important to have enough information in order to improve the precision of the estimations. This study suggests that the pedigree method is the most efficient method for selection, similarly as Brazilian breeding programs have reported in a survey carried out for bean cultivars released between 2001 and 2008 (Moreira et al. 2010).

The F_2 generation obtained from 15 F_1 marker-selected individuals (420 individuals in total) was evaluated in field conditions with the purpose of selecting superior individuals using 'INTA ROJO' and both parents as check treatments. Plant selection was based on PP and YP simultaneously, because of the significant differences among individuals in ANOVAs, high heritability values, and predicted genetic gains obtained for both traits. Heritability values, predicted genetic gains, coefficients of variance, and accuracy of selection for PP and YP were moderately appropriate to

discriminate superior common bean plants in the F_2 generation, and their values were in a similar range as previously reported in other studies, thus confirming the results obtained in those analyses (Nienhuis and Singh 1988; Gonçalves-Vidigal et al. 2008; Bertoldo et al. 2010; Borel et al. 2013). PP is a quantitative trait that has been found correlated with yield in common beans (White and Izquierdo 1991) and its higher relative heritability suggests that it is an appropriate complement to yield selection (Singh 1991). Nonetheless, higher PP values must be combined with more compact growth habits to maximize the seed yields. For instance, parent PV0023 has higher PP values than PV0006, but it has prostrate growth habits. That means that most pods are in contact with soil surface and then potential pathogens. If this landrace is produced on slopes, excess of water is drained. But on plain fields, yield losses caused by pathogen infections on pods often occur (Oswalt R. Jiménez, personal observation).

After conducting mixed model analyses for YP and PP, 81 and 74 individuals were selected, respectively, with 61 individuals being selected for both traits. Classical methodology suggests low selection intensity at that stage, because of the high level of heterozygous loci present in segregating population. However, the selection of genetically superior individuals is still possible. The augmented block design and mixed models analyses have a robust statistical base to discriminate F_2 plants in the presence of suitable check treatments. These methods have been applied to select individual plants in different crops with acceptable results (Aruna and Audilakshmi 2008; Piepho et al. 2008; Upadhyaya et al. 2009; Oliveira et al. 2012; Gonçalves-Vidigal et al. 2008; Balestre et al. 2013).

Our field observations point out that there was a significant phenotypic diversity among F_2 individuals. Consequently, it is reasonable to expect that the 61 selected individuals will split into a high number of families or pure lines in further generations. Parents PV0006 and PV0023 exhibited many positive and negative characteristics that were segregated into different phenotypic variants. That includes F_2 plants showing both positive attributes such as higher yield, upright growth habit and good seed coloration. The F_2 plant PV23/PV6-HERY showed in Figure 3 was an example of that. Singh (1991) reviewed possible causes of failure when increasing yield in common bean cultivars. Some of the factors involve poor general combinatory ability of parents in crosses caused by genetic similarities between them, something that was overcome in this study. The same author continues stressing that the removal of poorly-performing crosses at early generations should be carried out “without any hesitation”, justifying our selection strategy. Perhaps that conclusion has also been realized by other researchers who have adopted selection at early generations in common beans (Atuahene-Amankwa et al. 1998; Singh and Terán 1998; Terán and Singh 2002; Terán et al. 2013). Nonetheless, caution must be paid to plants initially selected based on one trait independently (PP or YP) because their performance could be better in next testing.

The average predicted genetic gains calculated for PP and YP (7.6 and 4.11, respectively) were comparable with those values found in other studies aiming to increase the yield of the common bean (Nienhuis and Singh 1988; Singh et al. 1999; Ramalho et al. 2005; Chiorato et al. 2010). The identification of superior F_2 plants is encouraging if we consider that the improvement of common bean yield has been moderate in last years (Beaver and Osorno 2009). Thus, the creation and evaluation of novel genetic variation seem to be reasonable. However, other important characteristics, such as more compact growth habits, earliness and more efficient photosynthates partitioning among other traits, should be considered for future evaluations.

It was unexpected that according to the SCAR markers used here, F_2 plants showed similar levels of resistance to BCMV, BGYMV, ANT and rust as cultivars released from regional breeding programs. This finding enforces our hypothesis that adapted Nicaraguan landraces are a good reservoir of useful genetic variation fixed by natural and human selection. Yet, this genetic variation should be improved through efficient selection methods if we intend to improve local landraces. SCAR markers linked to diseases have been suggested as an important tool for conducting selection for disease resistance at any stage of breeding programs (Melotto et al. 1996; Correa et al. 2000; Miklas et al. 2000b; Alzate-Marin et al. 2003; Kelly et al. 2003; Vandermark and Miklas 2005; Blair et al. 2007b; Sharma et al. 2008; Rocha et al. 2012; Gonçalves-Vidigal et al. 2013; Hegay et al. 2013). In addition to numerous references previously available, Blair et al. (2007b) showed successful examples of the application of marker assisted selection for resistance to viral diseases in common bean breeding programs conducted by CIAT in Latin America. In practical terms, it is advantageous and less expensive to test the resistance to diseases by means of markers instead of the complex and time consuming greenhouse screening. However, caution must be paid to the fact that resistance confirmation using local strains of viruses should be conducted at some stage of the breeding program in order to confirm the resistance of the genetic material. Also, more efficient laboratory methods (for instance, DNA extraction, PCR, DNA fragment visualization, etc.) should be developed and optimized to test higher numbers of individuals at lowest costs.

Most local researchers have the general consensus that Nicaraguan common bean landraces are very susceptible to common diseases and have a low potential for yield improvement. However, landraces reported here have not been previously screened for resistance to diseases and most opinions were based on field observations on very heterogeneous populations. In the light of the present results, there could be three possible explanations to the observed characteristics. First, there could be a constant gene flow from cultivars to landraces in farm conditions that transfers alleles for disease resistance. Different rates of gene flow have been reported for common bean in literature (Papa and Gepts 2003; Ferreira et al. 2007; Chaves-Berrantes et al. 2009) and that could support the suggestion of gene flow. Second, some landraces could really be "old cultivars" or "old intermediate genetic materials" with some resistance to diseases. They could have been abandoned by researchers during experimental validation carried out by research institutions. After validation, they could have been welcomed by farmers (because of their promising characteristics), even though they did not succeed for cultivar registration. Third, landraces could have evolved under a high disease pressure for a long period of time, fixing some natural genetic variation and incorporating resistance to common diseases.

All three hypotheses seem to be feasible and they could be verified in future research. However, the high level of genetic diversity and allele identity found in landraces do not support the second possible explanation. In relation to this, some studies mentioned that adequate levels of resistance to diseases can be found in landraces from different origins (Gonçalves-Vidigal et al. 2009; Rodiño et al. 2009; Larsen et al. 2010; Singh and Schwartz 2010).

It was observed that some landraces possess important alleles of resistance to BCMV (*bc-3 and bc-1*^{2}) not present in our segregating individuals (Method S3 in sub study III). Thus, in concordance to the strategy proposed by Mukeshimana et al. (2005) and Hegay et al. (2013), I am considering to plan new crosses with landrace PV0031 in order to pyramid a broad spectrum of resistance to BCMV in our selected individuals,

which possess allele *I*. The addition of broad resistance to the important potyvirus could increase the yield potential of susceptible genetic materials (Hampton 1975; Singh and Schwartz 2010).

The superiority of selected F_2 plants was inferred also by simulating the improvement of two base populations, selected plants and a hypothetical population composed of 40 landraces. The results indicated that our selected plants have more potential to fix all desired alleles into a target genotype than the whole collection of landraces in a reduced period of time. This was evident because of the presence of many alleles of resistance in both parents, which increases significantly their frequency. The feasibility of using computer simulations was previously discussed in this section.

The genetic purity of the cultivar 'INTA ROJO' was tested using 12 microsatellite markers. The results demonstrated that there were significant genetic changes in genotype frequencies at three microsatellite loci, which reflects the genetic deterioration of the cultivar during seed production. The identity of off-type plants and seeds were also confirmed by the same methods demonstrating that there was genetic contamination in that cultivar. The genetic purity testing of a released cultivar is important at any seed system, because the quality control guarantees the expression of the genetic potential of the genotype. There is abundant information and references of genetic purity testing using molecular markers in economically important crops (Smith and Register, 1998; Crockett et al. 2002; Yashitola et al. 2002; Ilbi 2003; Mongkolporn et al. 2004; Schuster et al. 2004; Veteläinen et al. 2005; Sundaram et al. 2008; Korir et al. 2013; Kwon 2013; Ye et al. 2013). However, the genetic purity of common bean cultivars has never been tested before using microsatellite markers.

The most common method for maintaining the genetic purity through different seed generations is by roguing out off-type seeds and plants from the seed lots (MAGFOR 2002). Nonetheless, without considering proper information on the phenotype of the cultivar (morphological descriptor) it is easy to perform selection instead of roguing. This is the possible explanation for the significant changes in genotype frequencies between breeder's seed and the other seed categories. During field expeditions, I observed, for instance, that plants possessing red pigmentation on pods at physiological maturity were rogued out from seed fields by seed inspectors, even thought that phenotypic characteristic was already described during cultivar registration (Figure 5) (Rosas et al. 2004b).

Figure 5. *Common bean plant from cultivar 'INTA ROJO' rogued out from the seed field, because of the scarce red pigmentation on pods at physiological maturity. Note that this trait was reported during cultivar registration.*

Undoubtedly, inappropriate roguing procedures could be one of the main causes of cultivar deterioration. It has serious consequences for bean seed farmers, because it could increase the rate of seed rejections caused by the high occurrence of off-types during the seed certification process. Also, the continued selection (instead of roguing) modifies the genotype and phenotype frequencies causing confusion when the cultivar is contrasted using its varietal descriptors. In addition, I have presented that the reference seed sample of cultivar 'DOR 364' used in field experiments does not possess the dominant resistance allele to BCMV (sub study III), even thought it was supposed to possess that allele (Rosas et al. 2004a). This could be an additional piece of evidence of the cultivar deterioration that demands attention and further actions. A similar case was reported for resistance to stripe rust in the wheat cultivar 'SUNVALE' grown in Australia (Simpfendorfer et al. 2013). Probably, when important genes are located in the proximity to other genes that express an undesirable phenotype, they are at risk to be lost during roguing.

Molecular markers were efficient in demonstrating genetic similarities of 'INTA ROJO' and "frijol viterra", and confirming the genetic differentiation with "frijol rojo oscuro". Some of the alleles detected as new genotypes and present in off-types individuals have also been observed in landraces during the molecular characterization discussed in this paper (Oswalt R. Jiménez, personal observation). Thus, it is possible that these results also account as proof for gene flow between cultivars and landraces, something previously proposed as a possible explanation for resistance to diseases in landraces. In practical terms, the minimum isolation distances between a seed lot and next bean field stated as five meters by MAGFOR (2002) must be revised, discussed and adjusted in the light of these results.

The use of molecular markers to test the genetic purity is still not a routine practice during seed production in Nicaragua. Also, there are no available related reports

in the Central American region. Nonetheless, due to the high costs for genotyping every certified seed bag, the monitoring of the genetic quality of smaller but relevant amounts of seeds, for example, breeder's seed, seems to be feasible to maintain the genetic quality of subsequent seed categories. Yet, seed inspectors and seed farmers should be educated in "good roguing practices" to avoid eroding the genetic quality of cultivars.

6. Conclusions and final thoughts

All the results described in this study successfully proved the high potential of local bean germplasm in breeding programs, emphasized by the use of molecular markers and modern statistical and computer methods. The main conclusions can be summarized as follows:

First, we can conclude that two promising sources of genetic variation were identified and selected using marker information from a collection composed of 40 bean landraces.

Second, the genetic diversity of two promising sources of genetic variation was incorporated into a segregating population, followed by the selection of the most allele recombinant individuals using polymorphic markers for both parents. This selection was supported by computer simulations.

Third, preliminarily, 61 F_2 plants were selected based on the highest PP and YP values by means of mixed model analyses. Additionally, molecular markers confirmed the presence of alleles of resistance to common diseases in selected plants, and their breeding potential was confirmed by computer simulations. All those plants were chosen to create families in the following generation or be parental material in the next round of crosses. Nonetheless, predicted genetic gains must be confirmed in following field evaluations. Alleles of resistance to BCMV missing in our segregating plants but present in some landraces could be incorporated by means of further crosses.

Fourth, microsatellite markers were able to quantify the genetic purity of cultivar 'INTA ROJO' during seed production, opening the possibility to complement current phenotypic methods.

It was evident that local common bean germplasm has a great potential for genetic improvement for two of the most disadvantageous characteristics ascribed to landraces, low yield and susceptibility to common diseases. Also, molecular markers are a valuable tool to assist selection and for keeping the genetic purity of cultivars optimal. The common bean is an orphan crop. That situation is obvious when comparing the limited available molecular tools for common bean improvement with other crops with a closely similar economic importance, such as wheat, maize and rice. However, using efficiently the available technology and breeding methods, and balancing research costs with potential benefits could make local common bean breeding programs profitable, something of profound relevance in the developing world.

Finally, the active utilization of local bean germplasm in breeding programs could indirectly promote its conservation, because it will justify the funds designated to maintain local gene banks. This does not mean that we will be reluctant to continue working with regional breeding programs. But, it means that we are committed to local onward solutions to food and seed deficits by expanding our research agendas in response to local farmers' needs, food demand and future climate change challenges.

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