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I. Cattan-Toupance · Y. Michalakis · C. Neema

Genetic structure of wild bean populations in their South-Andean centre of origin

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Abstract The genetic structure of wild common bean populations was studied in the South-Andean centre of origin of the species. Plants were collected from 21 populations in Argentina and genetic variability was assessed for molecular and resistance markers. Polymorphism was weak for phaseolin, the major seed-storage protein, and for RAPD markers, while a high level of polymorphism was observed for resistance to anthracnose, one of the most important diseases of common bean. For the three traits, within-population variability was important and represented between 43.6% and 67.5% of the total variation. Although among-population differentiation was significant for all the traits, no correlation was found between the population distances calculated from RAPDs and resistance. These results indicate that pathogen selection pressure may be an important factor influencing the distribution of variability within and among host plant populations.

Key words Common bean · Anthracnose resistance · RAPD · Genetic structure · Centre of origin

Introduction

Genetic variability is not uniformly distributed in plant populations. Some regions are particularly polymorphic, especially the centres of origin of cultivated species. In these areas, wild populations are known to be a source of genes of interest, including those responsible for adaptation to different climatic conditions, resistance to stresses (e.g. drought, salinity) and resistance to pathogens (Harlan 1976). Wild relatives of crops have been used in plant breeding and many research programs have been developed to estimate the genetic and agronomic potential of natural plant populations.

Different traits have been used for the evaluation of plant diversity. Morphological and agronomic traits were used first. Biochemical markers (particularly isozymes) were used later and facilitated the detection of genetic variability independent of environmental factors. More recently, molecular markers (restriction fragment length polymorphism or random amplified polymorphic DNA) (Welsh and McClelland 1990; Williams et al. 1990) have allowed the study of polymorphism at the DNA level. These molecular markers are now prevalent for the study of diversity, though agronomic traits remain essential for detecting economically useful variability. One such agronomic trait is resistance to pathogens which may be subject to strong selection in both natural and cultivated plant populations.

The model studied in the present paper is the interaction between the common bean, *Phaseolus vulgaris*, and the anthracnose agent, *Colletotrichum lindemuthianum*. The common bean is a widely distributed crop of considerable importance in tropical countries where it represents a main protein source for human nutrition. Natural common bean populations are distributed in the highlands of Latin America between Northern Mexico and Northern Argentina (Gentry 1969; Berglund-Brücher and Brücher 1976). Wild common beans consist of two major gene pools which have been distinguished by their morphology (Evans 1976), phaseolin protein type (Gepts et al. 1986; Gepts 1990), isozymes (Koenig and Gepts 1989; Singh et al. 1991),

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I. Cattan-Toupance · C. Neema (✉)
Laboratoire de Phytopathologie Moléculaire,
Institut de Biotechnologie des Plantes, Université Paris-Sud,
91 405 Orsay Cedex, France
Fax: +33-169336424
E-mail: neema@ibp.u-psud.fr

Y. Michalakis
Laboratoire d'Ecologie, Université P. and M. Curie CNRS, CC 237,
7 quai St. Bernard, 75252 Paris Cedex 05, France

RFLPs of mitochondrial (Khairallah et al. 1992) or genomic DNA (Becerra-Velasquez and Gepts 1994). The Middle-American gene pool extends from Northern Mexico to Colombia and the Andean gene pool ranges from Southern Peru to Northern Argentina. Two domestication events occurred leading to two parallel gene pools in the cultivated common bean. Wild and cultivated beans are considered to belong to the same annual, selfing, and diploid species ($2n = 22$). Many studies have been carried out on cultivated and wild common beans using different kinds of molecular markers. Some of them showed that diversity was greater in wild populations than in cultivated beans (Gepts 1990; Sonnante et al. 1994) while other studies did not reveal any difference (Singh et al. 1991; Becerra-Velasquez and Gepts 1994). To-date, no study has been undertaken to estimate the variability for resistance to disease in cultivated and wild bean populations.

Anthracnose is one of the most important diseases of common bean. This disease is present in every country where the bean is cultivated as well as in natural populations. It is caused by the fungus, *C. lindemuthianum*, and is responsible for severe losses in bean yield (Pastor-Corrales and Tu 1989). The interaction between common bean and *C. lindemuthianum* has been shown to fit the gene-for-gene model of Flor (1955) in which each resistance gene in the plant corresponds to an avirulence gene in the pathogen. Five specific resistance genes known to be involved in the interaction (Pastor-Corrales and Tu 1989) are used in common bean breeding.

The goal of the present study was to compare the genetic structure of wild common bean populations for neutral and potentially selective markers. It is generally much easier to characterize differences between populations for molecular markers than for agronomically important traits. Because molecular markers are, *a priori*, neutral while agronomic traits are most likely to be under selection even in natural populations, it is interesting to compare the inferences one can make from observations on these two kinds of traits. Wild common bean populations were sampled in Argentina, which is part of the Andean centre of origin of the common bean. Variability was assessed for phaseolin type, the major storage protein of the seed, RAPD markers and resistance to anthracnose.

Materials and methods

Plant material

Plants were collected in north-west Argentina where common bean distribution extends between 22° and 33°36′ latitude south on the eastern slopes of the Andes cordillera. Collections were carried out in May (1992) when bean pods are mature. Both infected and healthy plants were collected. The distance between two sampled plants was at least 1 m.

One hundred and twenty eight plants were collected in 21 populations along a 500-km south-north distribution (Fig. 1) crossing three provinces: Tucuman, Salta, and Jujuy (see Table 2). Most of the plants were infected with anthracnose except in the province of Jujuy where the dry weather probably did not allow good development of the fungus. Wild common bean is a climbing plant and is associated with other plant species (*Lamphrotiosus*, *Rubus*, *Alnus*). Wild bean population sizes are small (< 20 plants) thus constraining sample size. Seeds were multiplied in CIAT (Centro Internacional de Agricultura Tropical, Cali, Colombia).

Phaseolin analysis

Phaseolin was extracted from one seed of each plant as described in Gepts et al. (1986). Each sample was analyzed by one-dimensional sodium dodecyl sulphate 10% polyacrylamide-gel electrophoresis (SDS-PAGE).

DNA extraction

Two grams of leaves of 2-week-old seedlings were ground in a mortar with liquid nitrogen. Then 10 ml of extraction buffer (Tris-HCl 100 mM, pH 8; EDTA 50 mM, pH 8; NaCl 500 mM; β-mercaptoethanol 10 mM) and SDS at a final concentration of 1.5% were added. The mixture was heat-treated at 65°C for 20 min and potassium acetate was added to a final concentration of 1.2 M. Each sample was shaken, placed on ice for 30 min and then centrifuged at

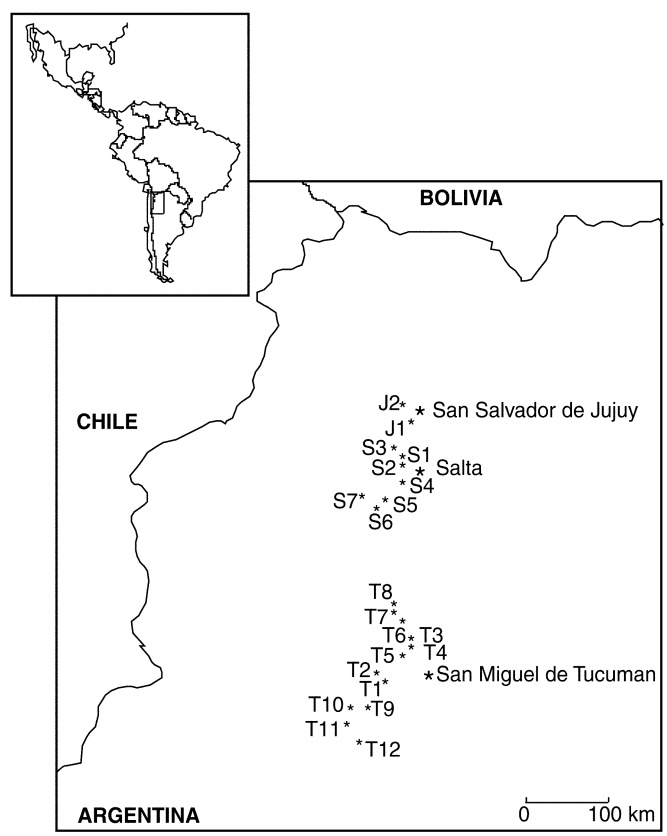

Fig. 1 The sampling sites of wild common bean in Argentina. *T1–T12*: populations of Tucuman; *S1–S7*: populations of Salta; *J1–J2*: populations of Jujuy

7000 rpm for 30 min. The supernatant was cleaned by filtration on Blutex films into a tube containing 1/2 vol of isopropanol. Each sample was placed at $-20°C$ for 30 min and then centrifuged at 9000 rpm for 20 min. Pellets were re-suspended in 1.4 ml of TE (Tris-HCl 100 mM, pH 8; EDTA 10 mM) and 100 µl of 3 M sodium acetate. Samples were treated with RNAse at 37°C for 1 h and then with phenol-chloroform-isoamyl alcohol (25:24:1). Phases were separated by centrifugation for 15 min. DNA was precipited with 2 vol of 100% ethanol for 5 min at room temperature. Samples were then centrifuged for 15 min. The pellets were washed with 70% ethanol and re-suspended in 200 µl of TE. Quantification of DNA was carried out in 0.8% agarose gels with the DNA concentration adjusted to 10 ng/µl.

RAPD PCR procedure

PCRs were conducted in 25 µl of a mix containing 30 ng of DNA, 200 µM of each dNTP (Pharmacia, Saclay, France), 400 µM of a single decamer primer from Operon Technology (Alameda, Calif., USA) or Bioprobe Systems (France), 2.5 µl of $10\times$ *Taq* DNA polymerase buffer and 1.5 units of *Taq* DNA polymerase (Appligène, Illkirch, France). Amplifications were carried out in a Perkin Elmer Cetus 9600 thermocycler programmed for 2 min at 92°C followed by 40 cycles of 30 s at 92°C, 1 min at 35°C and 1 min at 72°C. A ramp between annealing and elongation was added (3 s/°C) to prevent the de-annealing of the primer (Kresovich et al. 1992). A negative control, containing all reaction products except genomic DNA, was run with each amplification. Amplifications were conducted at least twice for each sample to check for repeatability. Amplification products were separated on 1.4% agarose gels, containing 0.4 µg/ml of BEt, for 2 h 30 min at 5 V/cm. Fragments were visualized under UV light.

Resistance tests

Plants were tested for their resistance or susceptibility to six strains of *C. lindemuthianum*. These strains had been isolated from cultivated beans of different origins and were characterized for their virulence against cultivars possessing known resistance genes (Table 1).

Five seeds of each plant were sown in vermiculite for 1 week and then spray-inoculated with a 5×10^6 spores/ml suspension. Spore suspensions were prepared by flooding 10-day-old fungus cultures with distilled water. Conidia were dislodged by scraping the culture surface with a spatula. The concentration was adjusted to 5×10^6 spores/ml with a hemacytometer. The inoculated plants were incubated in a growth chamber at 19°C, a 12-h day length and saturated humidity. Symptoms were scored 7 days after inoculation and reactions classified as resistant (R) or susceptible (S).

Scoring procedures

Common bean is considered to be an autogamous species (Brücher 1988). All individuals were thus assumed to be homozygous for all the markers studied (Bonnin et al. 1996). Phaseolin types are coded by a group of tightly linked genes which segregate as a Mendelian unit (Brown et al. 1981). The different types were thus considered as different alleles of a unique locus. Phaseolin types have been shown to be co-dominant and no heterozygotes were observed, which confirms a low outbreeding level (Brown et al. 1981). Mendelian inheritance of RAPD markers has been shown in many species (Echt et al. 1992; Kazan et al. 1993; Adam-Blondon et al. 1994). Each amplified fragment was considered as a locus with two alleles: presence (1) or absence (0). For resistance, a single-locus analysis was conducted assuming that resistance to each strain was an independent factor. A multi-locus analysis was also performed representing each plant by a six-component vector.

Table 1 The six strains of *C. lindemuthianum* analyzed

Geographical origin	Strain number	Putative genotype[c]
Europe	9	II III^a III^b IV V
Brazil	2	2 III^a 3^b IV V
Colombia	21	2 III^a 3^b IV 5
Rwanda	3616	II III^a 3^b 4 V
Costa Rica	100	2 III^a 3^b 4 5
Colombia	80	II III^a III^b IV V

[c] Genotype was determined after inoculation on a differential host-set of cultivars that possess the resistance genes Co_2, Co_3^a, Co_3^b, Co_4 and Co_5. Roman numbers indicate the presence of the corresponding avirulence gene and Arabic numbers the virulence gene

Data analysis

For resistance and RAPD data, polymorphism levels and associated standard deviations (SDs) were calculated. For each population and province, an unbiased estimate of genic diversity was obtained (Nei 1978) using BIOSYS software (Swofford and Selander 1989).

An analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was used to partition variability into its hierarchical components (variability between individuals within populations, among populations within provinces, and among provinces) and to test the significance of variance components. This procedure is based on an analysis of variance using distances between haplotypes. The distance chosen was a Euclidean metric equivalent to the number of differences between two individuals in their multilocus profile. Subdivision was quantified with *F*-statistic analogs called ϕ-statistics: ϕ_{sc} represents the differentiation between populations of the same province and ϕ_{ct} represents the differentiation between province. ϕ_{st}, which represents the differentiation between populations of the whole sample, is given by the relation $(1-\phi_{st})=(1-\phi_{sc})(1-\phi_{ct})$. The AMOVA software also provided estimates of ϕ_{st} between pairs of populations. This analysis was conducted on phaseolin types, RAPD multilocus profiles, and resistance phenotypes.

Associations between phaseolin type, RAPD alleles, and resistance factors were tested by using Fisher's exact test provided by the GENEPOP software (Raymond and Rousset 1995). Correlations between matrices of distances obtained for the different traits and geographical distances were calculated using Mantel's (1967) test.

Results

Phaseolin type

Three different phaseolin types were identified among the 128 plants: T and J2 types, which had been previously described (Gepts et al. 1986; Gepts 1990), and a novel four-banded type (J4 type). Type T was predominant in our sample (97 plants) whereas types J2 and J4 were present in 14 and 17 plants respectively (Table 2). The novel type J4 was relatively widespread, occuring in 7 out of 21 populations.

The province of Salta was monomorphic for phaseolin type T while intrapopulation variability was observed in Tucuman and Jujuy. Analysis showed that

Table 2 Geographical distribution, phaseolin types, RAPD and resistance phenotypes of 21 wild common bean populations in Argentina

Population	Number of plants	Province	Phaseolin type[a]	RAPD haplotypes[b]	Resistance phenotypes[c]
T1	8	Tucuman	T, J2, J4	1, 2, 3	M, N, T
T2	3	Tucuman	T	2, 4	A, E
T3	7	Tucuman	T, J2	5, 6, 7, 8, 9, 10, 11	F, I, M, N, T
T4	3	Tucuman	J2, J4	12, 13, 14	E, T
T5	5	Tucuman	J4	10, 15, 16	M, N
T6	11	Tucuman	T	3, 17	A, D, H, I, N, P, R
T7	3	Tucuman	T	3, 13, 18	A, B
T8	10	Tucuman	T, J2, J4	3, 12, 13, 17, 19	E, G, H, I
T9	3	Tucuman	T	16, 19	I, N, T
T10	9	Tucuman	T, J4	3, 10, 12, 13, 15	L, T
T11	2	Tucuman	T, J4	17	N
T12	2	Tucuman	T	13, 14	C, D
S1	5	Salta	T	17	N, S
S2	5	Salta	T	2, 17	E, M, N, R, S
S3	5	Salta	T	2, 17	N, T
S4	4	Salta	T	17	J, O
S5	4	Salta	T	17	H, L, Q, S
S6	4	Salta	T	2, 17, 20	I, N, P
S7	4	Salta	T	17	L, N, S, T
J1	24	Jujuy	T, J4	2, 3, 11, 13, 14, 17, 18, 21, 22, 23, 24, 25, 26, 27	H, I, L, N, R, S, T
J2	7	Jujuy	T, J4	3	K, M, N

[a] Three phaseolin types were observed: T, J2 and J4
[b] 27 RAPD phenotypes were identified: 1–27
[c] 20 resistance phenotypes were identified: A–T

Table 3 Analysis of molecular variance (AMOVA) for phaseolin types, RAPD and resistance phenotypes in 21 populations of wild common bean, located in three provinces of Argentina

Marker	Source of variation	% Of total variance	*P*-value[a]	ϕ-statistics
Phaseolin type	Among provinces	0	0.43	$\phi_{ct} = -0.020$
	Among populations within provinces	58.4%	< 0.001	$\phi_{sc} = 0.573$ $\phi_{st} = 0.564$
	Among individuals within populations	43.6%	< 0.001	
RAPD	Among provinces	11.4%	0.011	$\phi_{ct} = 0.114$
	Among populations within provinces	21.1%	< 0.001	$\phi_{sc} = 0.238$ $\phi_{st} = 0.325$
	Among individuals within populations	67.5%	< 0.001	
Resistance	Among provinces	0	0.38	$\phi_{ct} = -0.007$
	Among populations within provinces	43.8%	< 0.001	$\phi_{sc} = 0.435$ $\phi_{st} = 0.431$
	Among individuals within populations	56.9%	< 0.001	

[a] *P*-value: probability of obtaining a larger variance component by chance under the null hypothesis that the variance component is zero (estimated from 1000 permutations)

43.6% of the total variance was attributable to individual differences within populations (Table 3). The differentiation among populations was highly significant ($P < 0.001$) whereas no differentiation was found among provinces.

RAPD analysis

Sixty primers of kits C, D E and F (Operon Technology or Bioprobe Systems) were screened with ten plants. Fifty four primers gave amplification products and five gave reproducible polymorphisms. Seven polymorphic products were obtained among the 128 plants tested (Table 4) and 27 multilocus profiles were identified (Table 2). Two RAPD phenotypes were predominant with frequencies calculated on the whole sample of 0.29 and 0.19. The other profiles had lower frequencies that ranged between 0.008 and 0.06. The 27 profiles were distributed differently among the 21 populations. Gene-diversity calculations showed that Salta was less polymorphic (0.120; SD 0.062) than Tucuman and

Table 4 Attributes of the oligonucleotide primers used for generating random amplified polymorphic DNA markers from 128 wild common bean plants in Argentina

Primer	Nucleotide sequence (5′ → 3′)	Number of amplified products	Number of polymorphic markers
C10	TGTCTGGGTG	11	2
D8	GTGTGCCCCA	7	2
E12	TTATCGCCCC	8	1
E18	GGACTGCAGA	10	1
F10	GGAAGCTTGG	13	1
Total		49	7

Table 5 Frequencies of the 20 resistance phenotypes identified against six strains of *C. lindemuthianum* in the 128 wild common bean plants of Argentina

Resistance phenotype	Strains of *C. lindemuthianum*[a]						Frequency
	9	2	3616	21	100	80	
A	S	S	S	S	S	S	0.03
B	**R**	S	S	S	S	S	0.008
C	S	**R**	S	S	S	S	0.008
D	**R**	**R**	S	S	S	S	0.016
E	S	**R**	**R**	S	S	S	0.08
F	**R**	**R**	**R**	S	S	S	0.008
G	S	**R**	S	**R**	S	S	0.008
H	S	**R**	**R**	**R**	S	S	0.04
I	**R**	**R**	**R**	**R**	S	S	0.07
J	S	**R**	S	**R**	**R**	S	0.023
K	**R**	**R**	S	S	**R**	S	0.008
L	S	**R**	**R**	**R**	**R**	S	0.03
M	**R**	**R**	**R**	S	**R**	S	0.047
N	**R**	**R**	**R**	**R**	**R**	S	0.27
O	S	S	S	**R**	**R**	S	0.008
P	S	**R**	**R**	S	**R**	S	0.016
Q	S	**R**	**R**	S	**R**	**R**	0.008
R	**R**	**R**	**R**	**R**	S	**R**	0.023
S	S	**R**	**R**	**R**	**R**	**R**	0.08
T	**R**	**R**	**R**	**R**	**R**	**R**	0.22

[a]S = susceptible, **R** = resistant

Jujuy [0.304 (SD 0.075) and 0.346 (SD 0.075) respectively]. Intrapopulation genic diversity ranged from 0 to 0.345; six populations were monomorphic (T11, S1, S4, S5, S7, J2) while others presented up to 14 RAPD phenotypes (J1) (Table 2).

An analysis of molecular variance showed significant population subdivision at both the province and the population level, with substantial polymorphism at the intrapopulation level (67.5% of the total variance; Table 3). The distances between pairs of populations were significantly different from zero at the 5% level, except between populations from Salta. Associations between pairs of RAPD loci were tested using GENEPOP software: 5 associations out of the 70 calculated were significant at the 5% level.

Resistance to anthracnose

Six strains of *C. lindemuthianum* were inoculated on the 128 plants**.** Twenty resistance phenotypes were identified. These phenotypes showed between one and six resistance factors in the six strains tested (Table 5). Two phenotypes (N and T) were the most common with frequencies calculated on all populations of 0.27 and 0.22 respectively. The 18 other phenotypes had lower frequencies. All populations, except T11, were polymorphic for resistance and had between two and seven resistance phenotypes (Table 2).

Diversity indices calculated using BIOSYS software, were 0.347 (SD 0.056) for Tucuman, 0.286 (SD 0.068) for Salta, and 0.199 (SD 0.074) for Jujuy. At the intrapopulation level, diversity varied between 0 and 0.356. Associations between the six resistance factors were not significant at the 5% level. The AMOVA procedure showed that the variation among provinces was not significant (Table 3). The variance was almost equally divided between the among-populations/within-provinces and the among-individuals/within-populations components, which were both significant.

Correlations between phaseolin types, RAPD markers, resistance factors and geographic distances

Statistical associations were tested between phaseolin types, RAPD alleles, and resistance factors. Most of the combinations tested were in random association.

Correlations between the four distance matrices were tested using Mantel's (1967) tests. A significant positive correlation ($P < 0.005$) was found between RAPD data and linear geographical distance with a weak correlation coefficient (0.2), but no significant correlation was found between the other matrices.

Discussion

RAPD and phaseolin type

Three phaseolin types were identified in this study. Type T is common in the wild and cultivated Andean gene pools while type J2 has only been described from wild Argentinian common bean accessions (Bannerot and Debouck 1992). Although two other phaseolin types previously identified in wild common bean from Argentina (types C and H) were not observed in this study, a new phaseolin type (J4) was identified (Toro et al. 1990). Few wild accessions from Argentina are represented in the common bean germplasm in CIAT

so that studies of new accessions of the Andean gene pool have resulted in the identification of new phaseolin types (Koenig et al. 1990).

Low levels of polymorphism were detected using the RAPD technique. Sixty primers were tested and only five gave polymorphic bands. Yet, this technique has been shown to be more powerful in detecting polymorphism compared to RFLPs or isozymes (Dawson et al. 1993; Liu and Furnier 1993). These results were different from those obtained in natural populations of the selfing species *Hordeum spontaneum* and *Medicago truncatula* where higher levels of polymorphism were observed (Dawson et al. 1993; Bonnin et al. 1996).

For both markers, the polymorphism observed was low compared to that of Mexico where more than 20 different phaseolin types have been identified (Bannerot and Debouck 1992) and where substantial isozyme polymorphism has also been observed (Koenig and Gepts 1989). We detected no polymorphism in our sample using ten different isozyme systems (data not shown). The Andean gene pool appears to be less polymorphic than the Meso-american one. This finding is in agreement with the hypothesis that the species *P. vulgaris* originated in Central America and subsequently dispersed to the Andes (Koenig et al. 1990).

For both molecular traits, high levels of intrapopulation variability were observed: 67.5% of the total diversity for RAPD markers and 43.6% for phaseolin type. These values are compatible with the average value of within-population variability (56%) found in selfing species (Hamrick 1983).

Significant interpopulation differentiation was observed for both types of molecular markers. Such a subdivision is frequently observed in autogamous species for isozyme markers (Hamrick 1983; Loveless and Hamrick 1984). The ϕ_{st} values given by the AMOVA procedure are 0.564 for the phaseolin type and 0.325 for the RAPD data. The range of Fst generally observed in autogamous species is very large: $0.026 < \text{Fst} < 0.78$ with a mean of 0.24 (Heywood 1991).

Matrices of ϕ_{st} obtained with the AMOVA software show that populations from Tucuman and Jujuy are genetically similar even though they are geographically the most distant. This result can most easily be explained by the strong population subdivision and the pre-eminence of genetic drift due to small population size.

Analysis of resistance

Six strains of *C. lindemuthianum* isolated from cultivated beans have been used to test resistance in wild common bean populations. The six isolates gave different patterns of virulence on the 128 plants tested. Twenty resistance phenotypes were identified and significant polymorphism was observed. The polymorphism for resistance to pathogens is quite widespread in natural plant populations (Burdon 1987) and could be due to the negative frequency dependent selection exerted by fungi. A high level of polymorphism was also observed at the population level (Table 3). Such an important intra population variability has already been noted in natural populations of *Triticum dicoccoides* for resistance to *Erysiphe graminis* f.sp. *tritici* (Moseman et al. 1984) and in populations of *Stylosanthes capitata* for resistance to *Colletotrichum gloeosporioides* (Lenné 1988). However, the strains of *C. lindemuthianum* used in the present study have been isolated from cultivated beans and may be quite different from those present in wild populations. Polymorphism for resistance is thought to be partly responsible for the maintenance of natural populations in the presence of the pathogen, though in our study we do not know if these particular strains exert a selective pressure on bean populations. The relatively high levels of polymorphism for these genes could also be explained by intense meta-population dynamics (Frank 1993; Gandon et al. 1996).

A high level of resistance was observed in these wild populations and represented 70% of the interactions (Table 5). This result is consistent with the exotic origin of the strains analysed. Indeed, several studies on various interactions, such as *H. spontaneum*/*Puccinia hordei*, *H. spontaneum*/*E. graminis hordei*, *Linum marginale*/*Melampsora lini* or *Amphicarpae bracteata*/*Synchitrium decipiens*, have shown that plants are more resistant to allopatric pathogens than to sympatric ones (Parker 1985; Nevo 1986; Lawrence and Burdon 1989). This result is in agreement with a theoretical model which predicts that local adaptation of pathogens would arise when host populations are more subdivided than pathogen populations (Gandon et al. 1996). Moreover, a high proportion of the plants were resistant to all the strains tested, suggesting that wild bean populations possess a wide resistance spectrum. These results demonstrate the potential importance of these wild common bean populations in the exploration for resistance to strains of pathogens threatening bean crops.

Significant subdivision for resistance was not observed at the province level**.** This reflects the fact that all six resistance factors identified in this study are present in all three provinces, leading to a weak ϕ_{ct}. On the other hand, within provinces the populations have different factors leading to significant ϕ_{sc} values. In other interactions, such as *L. marginale*/*M. lini* (Lawrence and Burdon 1989) or *A. bracteata*/*S. decipiens* (Parker 1985), a hierarchical geographical structure was observed for resistance. The origin of the isolates used in our study could be responsible for the result. The studies cited above were conducted with local pathogens which are likely to reveal different patterns. It would be interesting to compare our results with those on resistance to local strains of *C. lindemuthianum.*

Correlations between the different markers

No significant associations were found either between pairs of markers of the same type or between pairs of different markers. These results were not consistent with what is generally observed in autogamous species, where associations between traits are assumed to be important (Hedrick et al. 1978; Parker 1988). Partial outbreeding may occur in natural populations of common bean, though a more parsimonious explanation is that the very small size of the populations studied prevented us from detecting significant associations.

The correlation between RAPD distances and geographical distances was significant, though quite weak (0.2), whereas no correlation was found between geographical distances and other genetic distances. This difference in the correlation with geographical distance between RAPDs and resistance can most likely be attributed to differences in the selection regimes of these traits. Indeed, it is reasonable to assume that RAPDs are selectively neutral and therefore conform to isolation by distance. On the other hand, the distribution of resistance factors also depends on the distribution of *C. lindemuthianum* virulence genes, which does not follow a simple geographic pattern.

Differences were observed in the distribution of variability for the different markers but no subdivision was observed at the province level for resistance and phaseolin type. Moreover, the relationships between populations are different according to the trait studied. For example, populations of Salta, which are monomorphic for phaseolin and very similar for RAPDs, are quite variable for their reaction to *C. lindemuthianum* and furthermore present a good level of resistance. These results suggest the importance of the character chosen for the evaluation of plant population diversity. While an analysis of population structure for molecular markers may give indications about the history and biology of plant populations it does not necessarily reflect what may be observed for agronomic traits, even in the case of autogamous species where correlations between traits are generally strongest. Thus, a complete evaluation of diversity using different traits must be achieved before the establishment of a representative collection of a species.

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