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Common bean (*Phaseolus vulgaris* L.) landraces in Catalonia, a Mesoamerican germplasm hotspot to be preserved

By E. SÁNCHEZ[1], A. SIFRES[2], F. CASAÑAS[1]* and F. NUEZ[2]
[1]Escola Superior d'Agricultura de Barcelona, Campus Baix Llobregat, Av. Canal Olímpic s/n, 08860 Castelldefels, Spain
[2]Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universidad Politécnica de Valencia, Camino de Vera, 14, 46022 Valencia, Spain
(e-mail: francesc.casanas@upc.edu) (*Accepted 2 March 2007*)

SUMMARY
Several landraces of common bean with high organoleptic value have resisted the rapid expansion of improved cultivars in Catalonia, in north-eastern Spain. To establish strategies for their preservation and use, we employed RAPD and AFLP markers to investigate the genetic variability within 15 landraces and to identify their original gene pools. A higher percentage of Mesoamerican landraces was found in Catalonia (40%) than in the rest of the Iberian Peninsula, or in Europe (approx. 20%). This is probably due to the exclusion of Catalonia from early trade with the American colonies and stronger commercial links with the Caribbean during the nineteenth century. Our results confirm that Catalan consumers prefer white-seeded common bean varieties of Mesoamerican origin. The landrace 'Castellfollit del Boix' showed 69.6% polymorphic RAPD primers, with 53.2% polymorphic bands, while at the other extreme 'Tavella Brisa' showed 47.8% polymorphic primers with 25.3% polymorphic bands. An AFLP approach yielded similar results. The high genetic variability found in 'Castellfollit del Boix', one of the landraces most threatened, suggests a considerable amount of introgression from improved inbreds.

Most common bean (*Phaseolus vulgaris* L.) landraces cultivated in the Iberian Peninsula are of Andean origin (Álvarez *et al*., 1998; Rodiño *et al*., 2001; 2003). This situation is also generally true in Europe, as the first seeds to arrive were from South of the Andes, and later germplasm was brought from the Andes by merchants and sailors (Zeven, 1997).

Common bean landraces cultivated in Catalonia have scarcely been studied, and their origin and genetic variability are unknown. As elsewhere, their cultivation is seriously threatened by competition from improved varieties that dominate the markets. For instance, only four accessions of the landrace 'Tavella Brisa' and seven accessions of the landrace 'Castellfollit del Boix' remain under cultivation. In recent years, however, consumer interest has ensured a high market price for some of these landraces, providing producers with an incentive to maintain these crops. Thus, landraces with higher organoleptic value have resisted the rapid expansion of new cultivars, favouring their preservation through use, as recommended by the FAO (1996). Knowledge of the variability within landraces, and identification of their original gene pools and close relatives will help sustain this situation.

Random amplified polymorphic DNA (RAPD) markers have been widely used to assess genetic relationships and diversity in beans (Haley *et al*., 1994; Duarte *et al*., 1999; Maciel *et al*., 2001), while amplified fragment length polymorphism (AFLP) markers have also been applied widely and successfully in evolutionary research (Tohme *et al*., 1996; Caicedo *et al*., 1999; Maciel *et al*., 2003). Another technique that has been applied to evaluate genetic diversity in beans is the study of seed storage proteins, such as phaseolins. The predominance of the T phaseolin pattern in the Andean gene pool, and of the S phaseolin pattern in the Mesoamerican gene pool, helps to identify material and thus to ensure greater success in crosses, as some incompatibility between gene pools has been described (Singh and Gutiérrez, 1984; Gepts and Bliss, 1985). Molecular markers associated with each protein type are currently used (Kami *et al*., 1995).

The present study aims to assess: i) the origin of 15 Catalan landraces of common bean, using PCR-derived markers; and ii) the genetic variability within some of them, especially the landraces 'Tavella Brisa' and 'Castellfollit del Boix', using all available accessions still cultivated.

*Author for correspondence.

MATERIALS AND METHODS

Plant material

The most popular landraces of Catalan common beans were studied: 'Tavella Brisa' (four accessions), 'Castellfollit del Boix' (seven accessions), 'Ganxet' (six accessions), 'Genoll de Crist', 'Piula', 'Del Carme', 'Bermà', 'Del Carai', 'Pinta', 'Floreta', 'Sant Joana', 'Bitxo', 'Neu', 'Del Confit' and 'Sastre' (with one accession each). Three inbred control white beans in common use in Catalonia were also studied: 'Navy' and 'Great Northern' (Mesoamerican origin) and 'White Kidney' (Andean origin).

DNA extraction

Plant DNA was isolated from fresh, young trifoliate leaves using the standard extraction method described by Doyle and Doyle (1990). DNA from 12 plants of each accession was used for RAPD analysis. For AFLP analysis, DNA was extracted from three seedlings of each accession. In the inbred controls, a single DNA sample was extracted from each variety. DNA bulks were created using equal fractions of material from each plant.

PCR reactions for RAPD and 15 bp phaseolin markers

Phaseolins, present either as a predominantly S (Sanilac, Mesoamerican) or T (Tendergreen, Andean) pattern, enabled the origin of each landrace to be classified into either of these two large groups of beans. A specific study of the multigenic family *Phs* was conducted using the 15-bp PCR assay, according to Kami *et al*. (1995). Specific PCR assays (upstream primer 5'-AGCATATTCTAGAGGCCTCC-3', downstream primer 5'-GCTCAGTTCCTCAATCTGTTC-3') amplified a region surrounding a 15-bp tandem repeat in members of the multigene family coding for phaseolins. This region includes part of the fourth exon, the entire third intron, and a small part of the third exon. S phaseolin genomic DNA yielded two PCR products, while T phaseolin genomic DNA gave three amplification products, depending on the number of 15-bp and/or 21-bp tandem repeats present. The PCR conditions were those proposed by Kami *et al*. (1995).

RAPD PCR reactions were performed with the following profile: 5 min DNA denaturation at 94ºC, followed by 40 cycles of 1 min denaturing at 94ºC, 1 min annealing at 42ºC, and a 2 min extension step at 72ºC, with a final extension cycle at 72º C for 5 min.

Each PCR reaction (final volume 30 µl), contained: *Taq*-LINUS 1x buffer (Cultek, Madrid, Spain), 2 mM $MgCl_2$, 100 µM dNTPs, 0.3 µM primers (26.6 ng each), 1 unit *Taq* DNA polymerase-LINUS (Cultek) and 20 ng template DNA. All samples, and a negative control without DNA, were run in parallel in the same Mastercycler® Personal Thermocycler (Eppendorf, Hamburg, Germany). Twenty-seven different primers (Operon Technologies, Alameda, OH, USA), previously described for their ability to detect genetic variability in common bean, were used in the RAPD reactions.

Amplification products were electrophoresed in 1.8% (w/v) agarose gels and detected by staining with 0.5 µg ml^{-1} ethidium bromide in 1x TBE buffer. RAPD bands and phaseolin fragment sizes were estimated using the Band Analysis programme of Gel Doc 2000 (Bio-Rad Laboratories, 2002).

AFLP reactions

AFLP products were generated using the Invitrogen (Carlsbad, CA, USA) AFLP Core Reagent according to the manufacturer's instructions. Six *Eco*R I and *Mse* I primer combinations, each containing three selective nucleotides, were used for selective amplification: E-AAC/M-CTC, E-AGC/M-CTT, E-ACT/M-CTT, E-ACG/M-CAG, E-AGC/M-CAA and E-ACC/M-CTC. PCR for pre-amplification was performed with the following profile: 2 min denaturation at 94ºC, then 20 cycles of 20 s denaturation at 94ºC, 30 s annealing at 56ºC, and 25 s extension at 72ºC, with a final extension step at 60ºC for 30 min. Reactions with selective nucleotides were performed with the following cycle profile: 2 min denaturation at 94ºC, 10 cycles of 20 s denaturation at 94ºC, 30 s annealing at 66ºC, and 25 s extension at 72ºC. The annealing temperature was reduced by 1ºC at each cycle, and was continued at 56ºC for a further 20 cycles. Finally, a 30 min extension step at 60ºC was performed.

PCR products were resolved using an ABI PRISM 310 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). Raw data were analysed using GeneScan 3.1.2 analysis software (Applied Biosystems) and the resulting GeneScan trace files were imported into Genographer (Benham, 2001).

Data analysis

RAPD and AFLP electrophoretic profiles were scored according to the presence (1) or absence (0) of a particular band, thereby generating a binary matrix. The similarity of all pair-wise combinations of the numerical profiles was determined using Dice's coefficient (Dice, 1945) and clustered by unweighted pair-group analysis using arithmetical averages (UPGMA; Sokal and Michener, 1958) using the NTSYSpc2.0 software package (Rohlf, 1996).

The reliability and robustness of the dendrograms were tested by bootstrap analysis, with 100 replications, to assess branch support using PHYLIP software (Felsenstein, 1994). A consensus dendrogram was constructed from the patterns using PHYLIP (v.3.5c). A limit of 50% was used to indicate statistical support for the topology of a node (Highton, 1993).

RESULTS AND DISCUSSION

Landrace origins by RAPD analysis

As a first approach to the origin of the Catalan landraces, a RAPD study was conducted on 'Ganxet', 'Castellfollit del Boix', 'Tavella Brisa' and 'Genoll de Crist', the four landraces most appreciated by consumers, and the three control inbreds. The 27 primers used generated 121 bands, each between 660 – 2,450 bp. Twenty-three primers generated 92 polymorphic bands. Thus, in spite of the pre-selection of primers, and the inclusion of germplasm from two different gene pools, only 85% of primers and 76% of bands were polymorphic.

In the dendrogram, 'Castellfollit del Boix' was grouped with 'Great Northern' and both were grouped with 'Tavella Brisa' (Figure 1). 'Navy' and 'Ganxet' were also related, although less strongly, with these three varieties (Figure 1). The control 'White Kidney' was most closely related to the landrace 'Genoll de Crist' (Figure 1). The Mesoamerican controls 'Navy' and 'Great Northern', together with the landraces 'Tavella Brisa', 'Castellfollit del Boix' and 'Ganxet', were clustered in the same area of the dendrogram, while the Andean control 'White Kidney' and the landrace 'Genoll de Crist' were also clustered (Figure 1).

Seed morphology suggested close relationships between 'Tavella Brisa' and 'Navy', between 'Castellfollit del Boix' and 'Great Northern', and between 'Ganxet' and 'White Kidney'. RAPD results

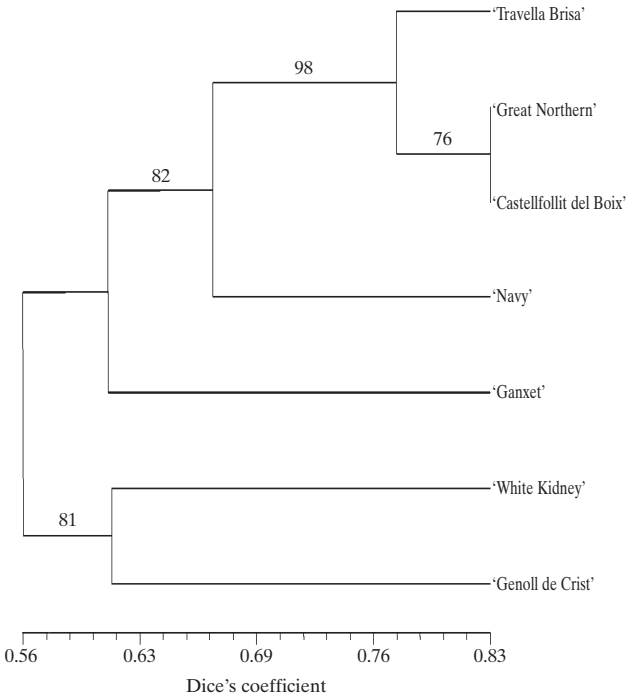

FIG. 1
UPGMA dendrogram of common bean varieties obtained using Dice's coefficient based on RAPD marker data, showing similarities between the common bean controls 'Great Northern', 'Navy' and 'White Kidney' and the Catalan landraces 'Tavella Brisa', 'Castellfollit del Boix', 'Ganxet' and 'Genoll de Crist'. Bootstrap values (percentages over 100 replications) are indicated at each node when they were higher than 50%.

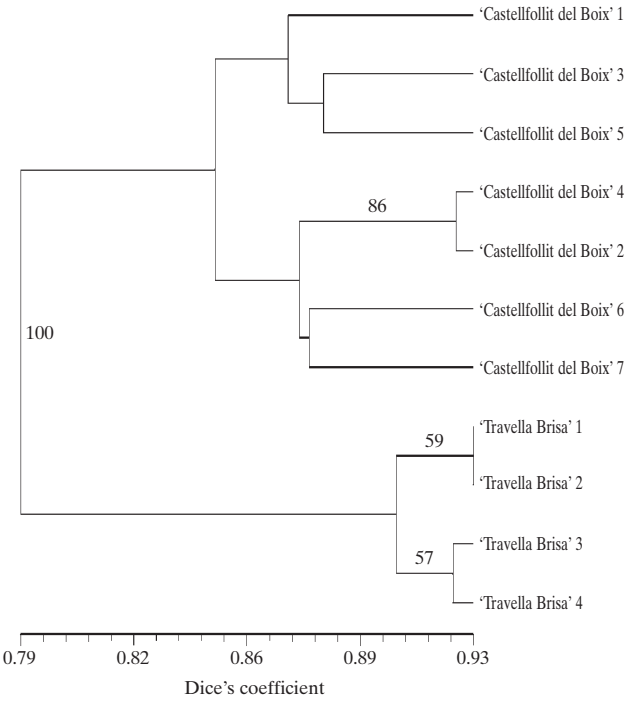

FIG. 2
UPGMA dendrogram of common bean varieties obtained using Dice's coefficient based on RAPD marker data, showing similarities between different accessions of the Catalan landraces of common bean 'Tavella Brisa' and 'Castellfollit del Boix'. DNA was extracted from a bulk of 12 plants for each accession. Bootstrap values (percentages over 100 replications) are indicated at each node when they were higher than 50%.

(Figure 1) confirmed the high genetic similarity (Dice's coefficient = 0.827) between 'Castellfollit del Boix' and 'Great Northern'. Thus, the origin of this landrace must be the Mesoamerican gene pool ('Durango') to which 'Great Northern' belongs (Singh *et al*., 1991; Voysest, 2000).

According to the RAPD analysis (Figure 1), 'Tavella Brisa' showed most similarity with 'Great Northern' (Dices's coefficient = 0.803), followed by 'Navy' (Dice's coefficient = 0.663). 'Ganxet' was at a similar distance from both the Mesoamerican controls, although it was most similar to 'Great Northern' (Dice's coefficient = 0.586; Figure 1). These preliminary results pointed to a Mesoamerican origin for 'Tavella Brisa', 'Castellfollit del Boix' and 'Ganxet', although 'Ganxet' could have some Andean components. Santalla *et al*. (2002) suggested that 'Ganxet' was an intermediate Mesoamerican-Andean form, with some isoenzymes in common with each gene pool.

RAPD approach: variability

Having established the origin of the four most popular Catalan landraces, we undertook a study of genetic variability within two of these landraces, those with the fewest number of accessions still cultivated by farmers (four of 'Tavella Brisa', and seven of 'Castellfollit del Boix').

Almost half (47.8%) of the RAPD primers detected polymorphism in the four accessions of 'Tavella Brisa', with 25.2% polymorphic bands. In 'Castellfollit del Boix', 69.6% of the primers detected polymorphism in the seven accessions, with 53.3% polymorphic bands. The minimum similarity in 'Castellfollit del Boix' was between accession 1 and accession 6 (Dice's coefficient = 0.810), lower than that found in 'Tavella Brisa' between accession 3 and accession 2 (Dice's coefficient = 0.882; Figure 2).

The percentage of polymorphic primers (69.6%) recorded with 'Castellfollit del Boix' was closer to the percentage found within the gene pool (Haley *et al*., 1994; Jacinto *et al*., 2003) than within the race. For instance, Miklas and Kelly (1992) found 34% and 40% polymorphic primers between landraces of the 'Durango' and Mesoamerican races, respectively. Although 'Castellfollit del Boix' has been referenced since the eighteenth century, the high polymorphism present today is probably due to gene flow that occurred during the second-half of the twentieth century. 'Great Northern'-type material, with a similar seed form, and cultivated extensively in Catalonia, could have been a source for crosses and seed mixtures in the recent past. It is extremely difficult to discriminate the traditional landrace from its hybrids with 'Great Northern'-type germplasm at the seed and/or plant levels, and this has probably favoured the high polymorphism in these accessions.

'Tavella Brisa' has been referenced since the nineteenth century in association with the culture of maize. It began to be cultivated in monoculture without physical support (canes or props) around the mid-twentieth century and this change in cropping system probably placed a strong selection pressure on the landrace. Later, during the 1990's, different 'Navy'-type commercial varieties from the USA began to be cultivated in the area and represent a possible source of introgression. Furthermore, the number of more-or-less independent populations that are still in culture is very low (four accessions).

With 47.8% of the primers producing polymorphism, and 25.2% polymorphic bands, the estimated genetic variability in 'Tavella Brisa' is within the range described for a market class (Haley *et al*., 1994). Two reasons may account for the different picture with 'Castellfollit del Boix'. First, the selection pressure that occurred when cropping with maize was discontinued must have decreased variability. Second, although 'Tavella Brisa' is cultivated without physical support, it has a type II-III growth habit (i.e., some climbing ability), while commercial 'Navy'-type varieties recently introduced into the area are clearly type I (i.e., no climbing ability).

In short, the landrace is easily identified at the plant level. At the pod level, the landrace is also easily distinguishable. The commercial 'Navy'-type has a green pod that turns yellow during ripening, whereas 'Tavella Brisa' has a green pod that turns purple. The Catalan name 'Tavella Brisa' refers to the purple pod, similar to the skin of pressed red grapes. Furthermore, farmers consider landraces to be clearly different from the commercial varieties they cultivate, and take care to keep both types separate in their fields, selecting within 'Tavella Brisa' to preserve the colour of the pod. Although the type II-III growth habit presents several difficulties during culture, the landrace is considered to be organoleptically superior to the commercial varieties.

AFLP approach: origin and variability

An AFLP analysis was conducted to confirm the results on origin and variability established by RAPD markers (Table I). For this analysis, 'Ganxet' samples were not bulked, because they had been chosen to represent the variability among 'Ganxet' accessions in a previous study on morphological traits (Casañas *et al*., 1997).

AFLP clustering (Figure 3) showed a distribution similar to RAPD-based clustering (Figure 1), although 'Navy' exchanged positions with 'Ganxet'. The low level of dissimilarity between the two groups of entries makes this reasonable, when the markers are different. So, the Mesoamerican origin of 'Tavella Brisa', 'Castellfollit del Boix' and 'Ganxet' was confirmed by the AFLP approach. The close relationship between 'Castellfollit del Boix' and 'Great Northern' (Figure 1; Figure 3) is also clearly seen. The Andean origin of 'Genoll de Crist' was also confirmed (Figure 1; Figure 3).

As with RAPDs, the AFLP approach also showed higher genetic variability in 'Castellfollit del Boix' than in 'Tavella Brisa' (Figure 3). 'Ganxet' seems to lie between these two varieties, although we have registered > 300 accessions and 'Ganxet' undoubtedly has a wider distribution area than 'Castellfollit del Boix' and 'Tavella Brisa'. If the entries used in the present study are representative of the variability in 'Ganxet' (Casañas *et al*., 1997), we may have a situation similar to that found in 'Tavella Brisa'. 'Ganxet' has an easily-recognisable, hooked form of seed. Farmers and consumers therefore reject materials that differ greatly from this standard, and any spontaneous crosses with closely-related varieties such as 'Great Northern', which is also cultivated in Catalonia, tend to be eliminated. The grouping of 'Ganxet' accessions, based on AFLPs, is consistent with that made on agronomic and morphological data (Casañas *et al*., 1997).

TABLE I

Total number of scored bands and number of polymorphic bands from the differentAFLP primer combinations used to assess genetic diversity in 21 genotypes of common bean

AFLP primer pair combination	Total number of scored bands	Number of polymorphic bands
*Eco*R I-AAC / *Mse* I-CTC	34	13
*Eco*R I-AGC / *Mse* I-CTT	17	7
*Eco*R I-ACT / *Mse* I-CTT	37	24
*Eco*R I-ACG / *Mse* I-CAG	30	16
*Eco*R I-AGC / *Mse* I-CAA	27	13
*Eco*R I-ACC / *Mse* I-CTC	29	18
Total	174	91

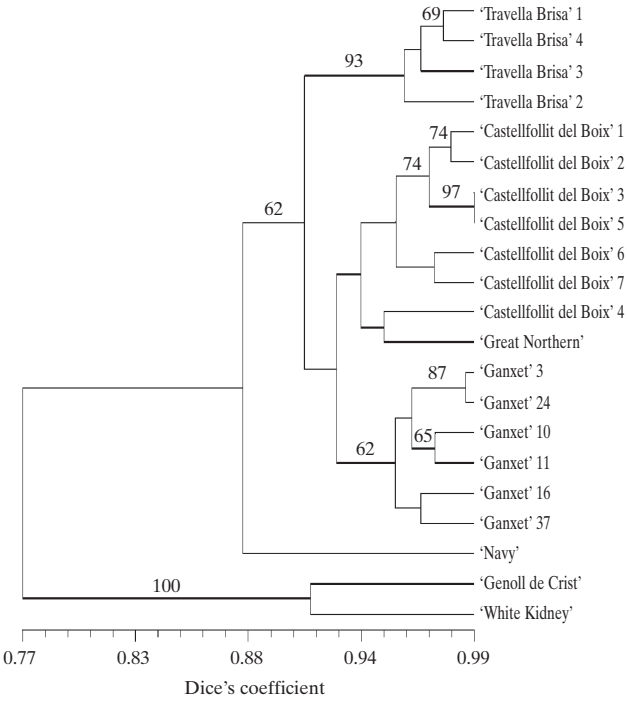

FIG. 3

UPGMA dendrogram of common bean varieties obtained using Dice's coefficient based on AFLP marker data, showing similarities between the common bean controls 'Great Northern', 'Navy' and 'White Kidney' and different accessions of the Catalan landraces 'Tavella Brisa', 'Castellfollit del Boix', 'Ganxet' and 'Genoll de Crist' . Bootstrap values (percentages over 100 replications) are indicated at each node when they were higher than 50%.

The origin of Catalan landraces and a portrait of the present situation

A study of the multigenic family *Phs*, using 15-bp PCR assays, was conducted to complete the investigation into the origin of the Catalan landraces still present in local markets.

Iberian consumers prefer beans of Andean origin (Rodiño *et al*., 2001), probably for historical reasons of ecological adaptation (Zeven, 1997). Nevertheless, compared to the 76% of landraces of Andean origin in Portugal (Rodiño *et al*., 2001) or the 80% in Spain reported by Rodrigo (2000), only 60% of the landraces grown in Catalonia were found to be of Andean origin (Table II). Furthermore, around 75% of landraces of common bean of Andean origin have been reported in Italy (Piergiovanni *et al*., 2000; 2006; Sicard *et al*., 2005) and in Switzerland (Eichenberger *et al*., 2000).

Tension with the Spanish monarchy led to the exclusion of Catalonia from the exploration and conquest of America. Moreover, the War of

TABLE II
Probable genetic origin of 15 different Catalan landraces of common bean according to PCR-derived RAPD markers for phaseolin types

Local name of landrace	Genetic origin
'Piula'	Mesoamerican
'Del Carme'	Andean
'Barmà o Buenos Aires'	Andean
'Del Carai'	Mesoamerican
'Pinta'	Andean
'Floreta'	Andean
'Santa Joana'	Mesoamerican
'Bitxo'	Andean
'Neu'	Andean
'Del Confit'	Andean
'Sastre'	Andean
'Genoll de Crist'	Andean
'Ganxet'	Mesoamerican
'Castellfollit del Boix'	Mesoamerican
'Tavella Brisa'	Mesoamerican

Independence in North America, and Charles IV's policies that led to wars with France and England, severely hampered commercial interchange. Therefore, it was not until 1778 that trade with America opened-up to Catalonia. Finally, in the nineteenth century, Catalonia began trading with America, mainly with Caribbean countries such as Mexico, Cuba and Puerto Rico.

Many Catalans who went to trade with these Caribbean countries settled there and established businesses. On their return to Catalonia, they brought new customs and dishes, such as chicken with rice, coffee and cigars (Espuga, 2003). Perhaps the predominance of this late-blooming commercial relationship with the Caribbean can account for the high proportion of Mesoamerican gene-pool varieties of bean in Catalonia.

At present, the most successful varieties are the local landraces 'Ganxet' and 'Castellfollit del Boix', and the foreign, improved commercial varieties 'Navy' and 'Great Northern', all of Mesoamerican origin. Catalan consumers prefer white-seeded varieties, whether in traditional landraces ('Sant Joana',' Del Carai', 'Del Confit', 'Castellfollit del Boix', 'Tavella Brisa' or 'Ganxet'), or in improved varieties ('Great Northern', 'Navy' and 'White Kidney' types).

The S phaseolin locus is linked to the *P* gene that accounts for white seeds (Basset, 1991). This would explain the coincidence of the white colour and S phaseolin in most Mesoamerican material. The above-mentioned commercial relationship between Catalonia and the Caribbean might provide clues for this preference, as repeated introductions of Mesoamerican material would have found a favourable habitat, especially near the coast.

It is not clear if the Andean varieties of bean still present in Catalonia came directly from America, or arrived from Spain by land. The coincidence of some Catalan types with Spanish or Portuguese types suggests the entry of Andean landraces elsewhere in the Iberian Peninsula. Spain, considered a secondary centre of diversification (Santalla *et al.*, 2002), has been a persistent source of (mainly Andean) varieties that partially overlapped with direct entry of Mesoamerican germplasm in Catalonia.

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