Sources of variation for sustainable field pea breeding

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Summary

The pea crop (*Pisum sativum* L.) is a convenient source of plant protein for animal feeding. The objective of this research was to evaluate field pea breeding lines for agronomic performance and seed quality focussed to their use in a sustainable production. Thirty five field pea breeding lines and six elite cultivars were evaluated for their agronomic value in field in Spain upon 20 traits related to flower, cycle, plant architecture, productivity and seed quality. The lines showed significant differences in all the quantitative traits evaluated and three of them, namely MB-0307, MB-0308, and MB-0319 were chosen to be evaluated, together with the advanced cultivar ZP-1233, in field and in growing chamber for agronomic performance, seed quality and ability for sustainable production. The four accessions displayed high seed protein content that had significant effect of cropping density with averages of 253.6 g kg^{-1} under low cropping density of 60 plants m^{-2} and 259.1 g kg^{-1} under high cropping density of 90 plants m^{-2}, therefore, the low cropping density should be regarded as the most convenient. Average yield of the lines MB-0307, MB-0308 and MB-0319 and the cultivar ZP-1233 was fair (197.5 g m^{-2}), probably due to the absence of fertilizers and irrigation, aiming for the sustainability of the crop. Intercropping with rye and herbicide application resulted in no differences on the seed yield; therefore, the ability of the breeding lines to grow without herbicide seems to be demonstrated, while the germination of the seeds at low temperature was very good. These results indicate that field pea could be a new protein crop in the North of Spain to satisfy the demand of plant protein for animal feed, based upon adapted breeding lines that combine the ability for growing under sustainable conditions with other desirable agronomic traits maintaining an adequate productivity.

Introduction

Pea (*Pisum sativum* L.), a native legume from the Southwest of Asia, was one of the crops early cultivated by man, and wild pea can still be found in Afghanistan, Iran and Ethiopia. Pea is an annual, cool-season, pulse (legume) crop. Cultivation of pea has lead to a gradual separation of types: for vegetable use, for seed and fodder (field pea) (Duke 1981, Santalla et al. 2001), and the edible podded types which have evolved recently. Dry pea seeds have high levels of the essential amino acids lysine and tryptophan, which appeared in low amounts in cereal grains. Consequently, dry pea can supplement the low amount of protein present in food and feed processed from cereal grains. Pea flour is valued not only as a plant protein source but also because is unique functional properties. The use of plant proteins as functional ingredients in the food and feed industry is increasing and special attention has been paid to the use of peas because they are already accepted as a part of the human and animal diet throughout the world. Pea also contains proteases, tannins, lectins, etc. which may reduce livestock feed gain when their concentration is high in the diet. However, it has been shown that partial or complete replacement of soybean meal with pea screenings (in a barley diet for hogs) did not reduce growth rate or efficiency of feed conversion.

There is a chronic lack of home-produced plant protein sources for the animal feed industry in Europe reaching 76% deficit in 2003/2004 compared to 73% in 1999/2000 and 62% in 1990/1991 (AEP 2007). In Spain $43 \cdot 10^{3}$ ha of field pea crop averaged 1.34 Mg ha^{-1}. Therefore, acreage and yield are very low compared to other European countries such as France ($436 \cdot 10^{3}$ ha, 4.45 Mg ha^{-1}), United Kingdom ($85 \cdot 10^{3}$ ha, 3.50 Mg ha^{-1}) or Germany ($141 \cdot 10^{3}$ ha, 2.86 Mg ha^{-1}) (AEP 2004).

The pea plant requires cool, moist growing conditions and can withstand heavy frost; however, it succumbs quickly to heat, especially if combined with high humidity.

The use of commercial improved pea varieties is not a common practice in many areas of Spain which explains the low yield in this country. The main reason is the lack of varieties adapted to the humid conditions of some temperate areas as in the North and Northwest of Spain (Caminero 2002). Additionally, other limiting factors are diseases caused by bacteria (*Pseudomonas syringae* pv *pisi*) and fungi (*Erysiphe polygoni*, *Mycosphaerella pinodes, Fusarium* spp*., Aphanomyces euteiches*) as well as infection by crenate broomrape (*Orobanche crenata*) (Rubiales et al. 2005, Tivoli et al. 2006).

Most pea varieties grown in these Spanish regions are mixtures of pure lines and can be considered as unimproved adapted landraces (Santalla et al. 2001). Many of them have medium to long vines, medium size white flowers and cream or pale green coloured seeds with a smooth seed coat. The farmers save some seeds from the harvest for planting the next year. These traditional landraces or heirloom cultivars are an important genetic resource for plant breeders because of their considerable genotypic variation and their adaptation to environmental conditions after many years of cultivation (Amurrio et al. 1993).

The increasing interest to search for alternative sources of plant protein for animal feeding gives an opportunity for new uses of an ancient crop such as the pea. The Spanish pea collections were evaluated under different conditions in order to display the different uses of the accessions and to make possible the use of this germplasm for production and breeding (Amurrio et al. 1995, Amurrio et al. 2000, Santalla et al. 2001, Caminero 2002). Additionally, the cropping system should be assessed aiming for the crop sustainability by enhancing the use of local resources and having less need for external inputs (Crozat and Justes 2004).

The objective of this research was to evaluate field pea breeding lines for agronomic performance and seed quality focussed to their use in a sustainable production.

Material and Methods

The collection of pea maintained at he Misión Biológica de Galicia - National Spanish Research Council (MBG-CSIC, Pontevedra, Spain) contains 237 accessions (Santalla et al. 2001). Some field pea breeding lines were derived by single plant selection within those accessions with an adequate profile for protein content. These lines were the basis for the field pea breeding program described in this research. This research involved three experiments: 1) screening of breeding lines, 2) evaluation of their agronomic performance and seed quality for feed, and 3) assessment for sustainable production.

Experiment 1: screening of breeding lines

Thirty-five field pea breeding lines and six commercial cultivars as control were evaluated in two winter field trials in 2000-2001 in Pontevedra (Spain, 42º 26' N, 8º 38' W, 20 masl, average temperature 18.6ºC, annual rainfall 1600 mm) and Lalín (Spain, 43º 00‘ N, 7º 33‘ W, 450 masl, average temperature 11.6ºC, annual rainfall 800 mm). The experimental design was a randomized complete block with two replications and 30 plants/plot spaced 0.25 m x 0.80 m (experimental cropping density of 5 plants m^{-2}). Fertilization and irrigation were not provided. Thirteen quantitative traits were measured or calculated on an average plot basis: first flower (days from sowing to the first flower open), flowering (days from sowing to 50 % of plants have at least one open flower), end of flowering (days from sowing to 50 % of plants end flowering), first dry pod (days from sowing to the first dry pod), seed maturity (days from sowing to 50 % of plants have pods with dry seeds), plant height (measured in centimetres), number of leaflets and number of tendrils, pod length (measured in millimetres), pods $plant^{-1}$, seeds pod^{-1}, seed diameter (measured in millimetres), and seed weight (g 100 $seeds^{-1}$). Six qualitative traits were also recorded: flower colour, shape of pod (I=small, straight and obtuse at end, IV=very long, wide and curved at end, V=long, straight with beak at the end) and seed (O=oval, S=spherical, G=without geometrical form), testa and cotyledon colour and cotyledon shape (W=wrinkled, S=straight, SD=straight with dimple) (Amurrio et al. 1993, 1995, Mateo-Box 1961).

Experiment 2: evaluation of the agronomic performance and seed quality for feed

After the screening, 24 breeding lines were grown in different sets in farmer fields in 10 locations (six under organic farming and four under conventional practices) in the Northwest of Spain in 2001-2002. This research permitted to point out three lines, namely MB-0307, MB-0308 and MB-0319. They were evaluated, together with the advanced cultivar ZP-1233 as control, in winter field trials in 2003, 2004 and 2005 in the season Pontevedra and Lalín according to an experimental design arranged as randomized complete blocks with two replications and two cropping densities: 60 plants m^{-2} and 90 plants m^{-2}. Each experimental plot had 25 m^2. Fertilization and irrigation were not provided. Agronomic value was assessed by five traits as described above: first flower, end of flowering, first dry pod, seed maturity, and seed yield (g m^{-2}). The seed quality for feed was evaluated as the content (expressed as g kg^{-1}) of protein, fibre and starch (AOAC, 2000).

Experiment 3: assessment for sustainable production

This experiment evaluated the ability of the breeding lines MB-0307, MB-0308, and MB-0319, with the cultivar ZP-1233 as a control, for growing under sustainable conditions such as low temperature, competition with weeds and intercropping.

The ability for seed germination under cold conditions was evaluated in a growing chamber. The seeds were sown in a sterile medium in plastic seed pots according to an experimental design arranged as randomized complete blocks with three replications and 10 seeds for each accession. Growing conditions resembled extreme winter weather conditions in Northern Spain: 12 h days at 5 ºC and 12 h nights at -1 ºC. Irrigation was provided when needed. The proportion of germination and the heat units accumulation until germination (Baskerville and Emin 1969) being 0ºC the threshold temperature were recorded on an average plot basis for each accession.

The performance of the four accessions under competition with weeds and intercropping with rye (*Secale cereale* L.) was evaluated in 2005 in a winter field trial in Castro de Rei - Lugo (Spain, 43º 09' N, 7º 29' W, 400 masl, average temperature 11.3ºC, annual rainfall 890 mm). The accessions were arranged according to a randomized complete block design with two replications and four treatments: a) control (check), b) intercropping with rye, c) pre-emergence (10 days after sowing) application of 4 L ha^{-1} diluted in 500 L water ha^{-1} pendimethalin herbicide, and d) post-emergence (40 days after sowing) application of 4 L ha^{-1} diluted in 500 L water ha^{-1} pendimethalin herbicide. Each experimental plot had 3 m^{2} with a crop density of 60 plants m^{-2} in sole crop and 40 plants m^{-2} plus 0.01 kg m^{-2} rye seeds. Fertilization and irrigation were not provided. Seed yield and three yield components were evaluated as described above: pods plant^{-1}, seeds pod^{-1}, and seed weight.

Statistical analysis

Analyses of variance were performed using the SAS statistical package (SAS Institute 2000) and considering environments as a random factor and lines as a fixed factor. Mean comparisons were performed by Student *t* test according to Steel et al. (1997).

Results

Screening of breeding lines

The table 1 shows the description of the studied accessions according to six flower, pod and dry seed qualitative traits. Mostly of the accessions (66 %) had white flower and pod type IV (51 %) or I (46 %) having only MB-0034 a pod type V. The six commercial cultivars and 20 lines had no geometrical seeds and only five commercial cultivars had wrinkled cotyledons. The most frequent colours were cream (46 %) for the testa and yellow (85 %) for the cotyledon, showing 18 lines and the cultivar Gloton the association of cream testa and yellow cotyledon.

Differences among accessions were significant for all the quantitative traits evaluated (Table 2). Significant differences among locations were found for all traits while accessions by location interaction was significant for first flower, first dry pod, plant height, pod length, pods plant^{-1}, seeds pod^{-1}, seed diameter and seed weight. The coefficients of variation were low except for pods plant^{-1}.

The table 3 displays the mean comparison for the quantitative traits evaluated. Earliness is indicated by days to first flower, days to flowering, days to end of flowering, days to first dry pod and days to seed maturity. The lines MB-0034, MB-0050, MB-0052, MB-0109, MB-0315, and MB-0319, and the commercial cultivars Atlas, Lotus, Rondo and Utrillo, had the best global scores for earliness. The architecture of plant was revealed by plant height, number of leaflets and number of tendrils. In the North of Spain, farmers demand medium-tall plants, having as many tendrils as leaflets, to get the best canopy on field. Therefore, the lines MB-0007, MB-0010, MB-0013, MB-0024, MB-0100, MB-0307, MB-0312 and MB-0319, and the commercial cultivars, Alderman and Gloton, displayed adequate plant architecture for field pea production. The productivity was denoted by the yield components such as pod length, pods plant^{-1}, seeds pod^{-1}, and seed diameter and weight. The most productive lines were MB-0013, MB-0014, MB-0015, MB-0307, MB-0308, MB-0309, MB-0310, MB-0312 and MB-0314.

Evaluation of agronomic performance and seed quality for feed

The table 4 shows the analysis of variance for three breeding lines, MB-0307, MB-0308, MB-0319, and the advanced cultivar ZP-1233. The effect of years and locations and their interaction were significant for all the traits except for fibre content that presented a significant effect of location, while cropping density had a significant effect for protein and starch. Significant differences among accessions were found for first flower, first dry pod, protein, fibre and starch content. Accession by year interaction was significant for first flower, fibre and starch content while accession by location was significant for fibre as well as accession by year by cropping density and accession by location by cropping density. Average values for the traits evaluated are displayed in table 5. The earliness of the four accessions was very similar, and the protein content of the three breeding lines was higher than the advanced cultivar ZP-1233, while the seed yield showed acceptable values for all the accessions evaluated.

Assessment for sustainable production

No significant differences among accessions were found for germination under cold conditions. The 83.3 % of the seeds of the breeding lines MB-0307, MB-0308, MB-0319 and the advanced cultivar ZP-1233 germinated well after an average of 54.2 accumulated heat units

Under the field conditions of the winter experiment, the advanced cultivar ZP-1233 showed a severe lack of adaptation that resulted in no useful data. Therefore, ZP-1233 was disregarded for the data analysis. The table 6 shows significant differences both among treatments for seed weight and among accessions for seeds pod^{-1} and seed weight and significant interaction accessions by treatments for seed weight. The mean comparison of the accessions under competition with weeds and intercropping with rye is shown in table 7. The seed yield was acceptable for the three breeding lines and under the different treatments evaluated.

Discussion

The plant phenotype could be only a modest predictor of its genetic potential for breeding, therefore, the valuable genes within germplasm collections should be searched directly, according to Tanksley and McCouch (1997). In the case of complex and adaptive traits, characterization and evaluation data should be useful for the management of germplasm collections (Tohme et al. 1995; Amurrio et al. 2000). Selection of peas in small farms and gardens over a long period of time has contributed to the development of landraces adapted to (specific) particular environments. Pea landraces are sometimes grown as unimproved populations or mixtures in many small farms in the North of Spain and Portugal and they may have multiple uses (Santalla et al., 2001).

Pea landraces from different areas of Spain and Portugal have intra-landrace variation both in phenotype and isozyme profile (Amurrio et al. 1993, Varela et al. 1997) that provides an opportunity for breeding for different food and feed purposes. The field pea breeding lines evaluated were selected within landraces that exhibited a good performance in previous studies (Amurrio et al., 1993). Mostly of the lines combined white flower and cream or green testa and yellow or green cotyledon, that are the most appropriated for their use in animal feeding (Mateo-Box 1961).

Significant location effect and accessions by locations in the experiment 1 could be due to wide differences between the environmental conditions. Temperature during the cool season was lower in Lalín than in Pontevedra and rainfall was higher in Pontevedra than in Lalín. Similar results were reported by Amurrio et al. (1993) in an evaluation of pea germplasm in quite different environments: greenhouse-winter planting open field-winter planting and open field-spring planting. These results agree with those of Snoad and Arthur (1974), Biarnès (2000), and Magallanes (2000). None of the commercial cultivars evaluated showed a good productivity, based on yield components probably, due to a poor adaptation to the area of production. This indicates the need for using adapted germplasm for field production in this area.

The significant differences among lines 24 permitted to choose a set of breeding lines with adequate scores to continue the breeding programme. Eleven breeding lines were disregarded based upon their poor performances, for instance accessions with low values for yield components such as pods plant^{-1} or with long pods but a low number of seeds per pod. An approach to participatory varietal selection (Witcombe et al. 1996) was made by farmer's assessment of the field pea breeding lines under real farm practices as wide as possible. Compared to conventional plant breeding, participatory varietal selection is more likely to produce farmer-acceptable products, particularly for marginal environments as many on the North of Spain are. In this case, the three breeding lines, namely PHA-0307, PHA-0308 and PHA-0319, gained the consensus of farmers and they were included in the next field and laboratory experiments, together with the advanced cultivar ZP-1233. This advanced cultivar has medium-tall plants and displays conventional leaves. According to Caminero (2002), who reported the evaluation of this cultivar in the central highlands of Spain, ZP-1233 represents a reliable control to compare to the well-adapted breeding lines with conventional leaves too.

As expected, years and locations had significant effects and interaction on the agronomic traits evaluated in the experiment 2, but no effect of cropping density was detected for these traits. The major pea seed constituents such as protein, fibre and starch content have the potential to be very interesting ingredients for a variety of food and non-food products, and the three seed quality traits showed significant differences among accessions. The protein content is a valuable character for the use of dry pea in animal feeding (Froidmont and Bartiaux-Thill 2004, Soto-Navarro et al. 2004, Arganosa et al. 2006). The three breeding lines, namely MB-0307, MB-0308 and MB-0319 had no differences for this trait but all of them displayed higher protein content than the cultivar ZP-1233. The protein value of these seeds should be regarded as a very good one compared to the average protein content of 239 g kg^{-1} reported by Gueguen and Barbot (1988). Protein content presented significant effect of cropping density with averages of 253.6 g kg^{-1} under low cropping density of 60 plants m^{-2} and 259.1 g kg^{-1} under high cropping density of 90 plants m^{-2}. Despite the significance of this difference, it seems that in practical terms the average protein content is similar in both densities maybe because N uptake by plants was not affected by the cropping density when N is not a limiting factor. It means that a cropping density of 60 plants m^{-2} result in an adequate canopy and it must be regarded as the most convenient with the additional value of seed saving at sown.

Average yield in this experiment was 197.5 g m^{-2} that is a fair value compared to 370.0 g m^{-2} reported by Santalla et al. (2001) and 360.5 g m^{-2} by Tar'an et al. (2005). Nevertheless, the agronomic practices should be regarded since in this experiment neither fertilizers nor irrigation were provided aiming for the sustainability of the crop.

The field pea crop is not currently established in the North of Spain in spite of the many unproductive lands that could be devoted to this crop, aiming for a sustainable and local plant protein for feed. The results of the experiment 3 were unexpected since only seed weight showed a significant effect of treatments and together with seeds pod^{-1} significant differences among accessions and accession by treatment interaction. Intercropping and herbicide application both in pre-emergence and post-emergence resulted in no differences among the seed yield of the accessions assayed with values quite similar to those obtained in absence of any treatment. Therefore, the ability of the breeding lines to grow in sustainable conditions with no-herbicide seems to be demonstrated. Crozat and Justes (2004) and Brisson et al. (2007) experimented with intercropping barley/pea and they found a reduction in pea yield together with relevant agro-ecological benefits as reductions both of weeds and level of diseases. However, it is hard to understand why the intercropping with rye did not result in yield reduction as is usual in associate cropping systems (Santalla et al. 1994). Other valuable result was the response of the accessions to germinate at sub-optimal temperature. The pea germplasm from the northwest of the Iberian Peninsula averaged 11.0 days to emergence in field in Pontevedra, as reported by Amurrio et al. (1993) and 19.2 days according to a later study by Amurrio et al. (1995). Therefore, the response of the accessions to low temperature during germination, that averaged 27.1 days when the 83.3% of the seeds were successfully germinated, should be considered very good although a further validation under field conditions should be done before to extend the pea crop to cold areas in the North of Spain.

The phenotypic selection for agronomic performance within pea landraces applied in this work has resulted in a group of very good field pea breeding lines. The results indicate that field pea could be established as a new protein crop in the North of the Iberian Peninsula to satisfy the demand of plant protein for animal feed. The promotion of this crop should be based more upon adapted breeding lines than commercial unadapted cultivars. Further breeding is needed to release new field pea varieties that combine the adaptation and ability for growing under sustainable conditions with other desirable agronomic traits. Regarding to the cropping system, medium cropping density and monoculture of pea without herbicide application should be a chance for field pea in the farms in Northern Spain maintaining an adequate productivity.

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References

AEP (2004) Statistics and economics. Supply and demand. EU deficit in protein sources. www.grainlegumes.com

AEP (2007) Statistics and economics. Supply and demand. EU deficit in protein sources. www.grainlegumes.com

Amurrio JM, De Ron AM, Escribano MR (1993): Evaluation of *Pisum sativum* landraces Northwest of the Iberian peninsula and their breeding value. Euphytica 66: 1-10

Amurrio JM, De Ron AM, Zeven AC (1995) Numerical taxonomy of Iberian pea landraces based on quantitative and qualitative characters. Euphytica 82: 195-205

Amurrio JM, De Ron AM, Hernández-Nistal J (2000) How to identify edible-pod pea varieties in a germplasm collection. *Pisum* Genetics 32: 56-57

AOAC (2000) Official methods of analysis. 17th ed. AOAC International. Gaithersburg, MD, USA

Arganosa GC, Warkentin TD, Racz VJ, Blade S, Phillips C, Hsu H (2006) Prediction of crude protein content in field peas using near infrared reflectance spectroscopy. Can. J. Plant Sci. 86: 157-159

Baskerville GL, Emin P (1969) Rapid estimation of heat accumulation from maximum and minimum temperatures. Ecology 50: 514-517

Biarnès V (2000) Genotype x environment interaction for pea yield. Grain Legumes 27: 8-9

Brisson N, Corre-Hellou G, Dibet A, Launay M, Crozat Y (2007) Evaluation of the STICS crop model within the EU INTERCROP project. Grain Legumes 45: 10-12

Caminero C (2002) Adaptación a la siembra invernal y tolerancia al frío en guisante (*Pisum sativum* L.). Doctoral Thesis. University of León, León, Spain

Crozat Y, Justes E. (2004) How to exploit the ecological benefits of grain legumes in cropping systems? Grain Legumes 40: 16-17

Duke JA (1981) Handbook of legumes of world economic importance. Plenum Press, New York. 345 pp.

Froidmont E, Bartiaux-Thill N (2004) Suitability of lupin and pea seeds as a substitute for soybean meal in high-producing dairy cow feed. Anim.Res. 53: 475-487

Gueguen J, Barbot J (1988) Quantitative and qualitative variability of pea (*Pisum sativum* L.) protein composition. J. Sci. Food Agric. 42: 209-224

Magallanes J (2000) Mejora genética de poblaciones de guisante vaina (*Pisum sativum*) para la producción de líneas puras de valor hortícola. Doctoral Thesis University of Santiago de Compostela, Spain

Mateo-Box JM (1961) Leguminosas de grano. Salvat Editores. Barcelona, Spain

Rubiales D, Moreno MT, Sillero J (2005) Search for resistance to crenate broomrape (*Orobanche crenata* Forsk.) in pea germplasm. Genet. Resour. Crop Evol. 52: 853-861

Santalla M, De Ron AM, Escribano MR (1994) Effect of intercropping bush bean populations with maize on agronomic traits and their implication for selection. Field Crops Res. 36: 185-189

Santalla M, Amurrio JM, De Ron AM (2001) Food and feed potential breeding value of green, dry and vegetable pea germplasm. Can. J. Plant Sci. 81: 601-610

SAS Institute (2000) SAS/STAT User's Guide. Version 6. 4th ed. SAS Institute Inc. Cary, North Carolina, USA

Snoad B, Arthur AE (1974) Genotype-environment interactions in peas. Theor. Appl. Genet. 44: 222-231

Soto-Navarro SA, Williams GJ, Bauer ML, Lardy GP, Landblom DG, Caton JS (2004) Effect of field pea replacement level on intake and digestion in beef steers fed byproduct-based medium-concentrate diets. J. Anim. Sci. 82: 1855-1862

Steel RGD, Torrie JH, Dickey DA (1997) Principles and procedures of statistics. A biometrical approach (third ed.). McGraw-Hill, New York, USA.

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277: 1063-1066

Tar'an B, Zang C, Warkentin T, Tullu A, Vanderberg A (2005) Genetic diversity among varieties and wild species accessions of pea (*Pisum sativum* L.) based on molecular markers, and morphological and physiological characters. Genome 48: 257-273

Tivoli B, Baranger A, Ávila CM, Banniza S, Barbetti M, Chen WD, Davidson J, Lindeck K, Kharrat M, Rubiales D, Sadiki M, Sillero JC, Sweetingham M, Muehlbauer FJ (2006) Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. Euphytica 147: 223-253

Tohme J, Jones P, Beebe S, Iwanaga M (1995) The combined use of agroecological and characteristics data to establish the CIAT *Phaseolus vulgaris* core collection. In: Hodgkin T, Brown ADH, van Hintum TJL, Morales EAV (Eds.) Core collections of plant genetics resources. IPGRI-John Wiley & Sons. Chinchester, UK.

Varela M, Hernández J, Amurrio JM, De Ron AM (1997) Comparación entre clasificaciones basadas en la variación agronómica e isoenzimática en *Pisum sativum*. Acta Hortic. 17: 307-313.

Witcombe JR, Joshi A, Joshi KD, Sthapit BR (1996) Farmer participatory crop improvement. I. Varietal selection and breeding methods and their impact on biodiversity. Exp. Agric. 32: 445-460

Table 1. Description for 35 field pea breeding lines and six commercial cultivars.

Lines	Flower colour	Pod shape	Seed shape	Testa colour	Cotyledon colour	Cotyledon shape
MB-0006	white	IV	O	cream	yellow	S
MB-0007	white	IV	O	cream	yellow	S
MB-0009	white	IV	O	cream	yellow	S
MB-0010	white	IV	O	cream	yellow	S
MB-0012	white	IV	S	cream	yellow	S
MB-0013	white	I	G	cream	yellow	SD
MB-0014	white	IV	G	cream	yellow	SD
MB-0015	white	I	S	cream	yellow	S
MB-0024	white	I	O	cream	yellow	S
MB-0026	white	IV	O	cream	yellow	S
MB-0034	white	V	G	brown	yellow	SD
MB-0050	white	I	G	cream	yellow	SD
MB-0052	white	I	G	cream	yellow	SD
MB-0053	purple	I	G	brown	yellow	SD
MB-0098	purple	IV	G	mottled	yellow	SD
MB-0099	purple	IV	G	mottled	yellow	SD
MB-0100	purple	IV	G	mottled	yellow	SD
MB-0102	purple	IV	G	mottled	yellow	SD
MB-0103	purple	I	G	brown	yellow	SD
MB-0107	purple	IV	G	brown	yellow	SD
MB-0109	white	I	S	green	green	SD
MB-0306	purple	IV	G	brown	yellow	SD
MB-0307	white	IV	O	cream	yellow	S
MB-0308	white	IV	S	cream	yellow	S
MB-0309	white	I	S	cream	yellow	S
MB-0310	white	I	S	cream	yellow	S
MB-0311	white	IV	G	brown	yellow	SD
MB-0312	purple	I	O	cream	yellow	S
MB-0313	purple	I	G	brown	yellow	SD
MB-0314	purple	I	G	brown	yellow	SD
MB-0315	white	I	S	cream	yellow	S
MB-0316	purple	IV	G	brown	yellow	SD
MB-0317	purple	I	G	brown	yellow	SD
MB-0318	white	I	G	mottled	yellow	SD
MB-0319	purple	I	G	brown	yellow	SD
ALDERMAN	white	IV	G	green	green	W
ATLAS	white	IV	G	green	green	W
GLOTON	white	I	G	cream	yellow	SD
LOTUS	white	I	G	green	green	W
RONDO	white	IV	G	green	green	W
UTRILLO	white	IV	G	green	green	W

Table 2. Mean squares of the analysis of variance and coefficient of variation for the agronomic traits evaluated in 35 field pea breeding lines and six commercial cultivars.

Source [a]	d.f.[b]	First flower (days)		Flowering (days)		End of flowering (days)		d.f.[b]	First dry pod (days)		d.f.[b]	Seed maturity (days)	
L	1	277222.5	**	325171	**	546975.1	**	1	568290.9	**	1	577299.0	**
R (L)	2	22.5		50.8		22.4		2	1.8		2	8.5	
A	40	514.9	**	259.9	**	386.8	**	40	64.2	**	40	42.8	**
A x L	40	83.6	**	49.5		17.8		40	19.2	*	40	8.4	
Error	80	36.5		35.5		14.5		78	10.8		79	9.7	
C.V. (%)[c]		4.7		4.3		2.1			1.8			1.6	

Source [a]	d.f.[b]	Plant height (cm)		d.f.[b]	Leaflets (nr)		d.f.[b]	Tendrils (nr)		d.f.[b]	Pod length (mm)	
L	1	22894.9	**	1	0.75	*	1	2.19	*	1	1294.3	**
R (L)	2	5333.9	**	2	1.33	**	2	1.15	*	2	419.8	**
A	40	6627.9	**	40	4.21	**	40	10.85	**	40	295.2	**
A x L	40	1072.7	**	40	0.20		39	0.34		39	39.2	*
Error	73	374.3		80	0.17		79	0.33		77	19.4	
C.V. (%)[c]		13.1			8.23			9.14			5.9	

Source [a]	d.f.[b]	Pods plant^{-1}		d.f.[b]	Seeds pod^{-1}		d.f.[b]	Seed diameter (mm)		d.f.[b]	Seed weight (g 100 seeds^{-1})	
L	1	119276.6	**	1	14.8	**	1	8.44	**	1	147.0	**
R (L)	2	7200.9	*	2	1.1		2	0.98	**	2	2.7	
A	40	5504.2	**	40	1.1	**	40	0.47	**	40	36.3	**
A x L	37	2763.3	**	39	0.9	*	34	0.44	**	29	10.4	**
Error	74	1005.9		77	0.4		54	0.12		42	3.5	
C.V. (%)[c]		44.7			10.8			4.50			9.1	

*, ** significant at $p \leq 0.05$, $p \leq 0.01$ respectively
[a] L=Locations, R=Replications, A=Accessions
[b] d.f.=degrees of freedom
[c] C.V.= Coefficient of Variation

Table 3. Mean comparison and range of variation for the agronomic traits in 35 field pea breeding lines and six commercial cultivars.

Accessions	First flower (days)	Flowering (days)	End of flowering (days)	First dry pod (days)	Seed maturity (days)	Plant height (cm)	Leaflets (nr)	Tendrils (nr)	Pod length (mm)	Pods plant^{-1}	Seeds pod^{-1}	Seed diameter (mm)	Seed weight (g 100 seeds^{-1})
MB-0006	129	136	177	180	190	135.2	5	5	80.6	41.9	5.8	7.8	22.2
MB-0007	128	137	179	182	192	156.9	5	6	82.6	51.6	5.7	7.4	27.2
MB-0009	124	131	177	181	193	169.1	5	6	82.1	61.3	6.0	7.9	27.6
MB-0010	123	132	179	184	192	145.8	6	6	83.6	39.7	6.3	8.1	27.9
MB-0012	137	144	181	186	194	160.7	6	6	62.6	108.2	6.2	6.9	14.2
MB-0013	138	148	184	190	194	149.4	5	6	67.7	108.8	6.7	7.2	17.4
MB-0014	133	142	183	188	195	187.6	5	5	65.7	134.6	6.1	7.3	17.5
MB-0015	137	147	184	188	194	182.3	5	6	67.2	114.9	7.0	7.8	19.2
MB-0024	130	135	175	185	192	151.9	5	6	75.1	51.8	6.4	7.8	23.6
MB-0026	137	144	181	186	193	166.9	6	7	70.3	71.2	5.3	7.9	23.2
MB-0034	110	126	161	181	188	69.8	4	7	72.6	16.1	5.9	7.9	16.5
MB-0050	110	130	162	178	184	60.7	4	6	64.4	8.30	5.1	8.0	21.7
MB-0052	108	125	166	178	184	55.7	4	7	72.9	7.40	5.5	7.8	20.9
MB-0053	137	142	189	190	196	185.5	5	6	68.5	87.4	5.4	7.9	21.2
MB-0098	129	135	184	186	194	176.1	5	6	81.9	59.9	6.7	8.0	23.9
MB-0099	132	139	184	186	195	170.2	5	6	82.3	63.5	6.6	7.8	24.9
MB-0100	132	139	177	186	193	155.2	4	5	71.6	64.3	6.6	7.9	22.7
MB-0102	131	140	174	186	194	168.9	5	5	73.1	44.5	6.5	8.1	23.7
MB-0103	143	151	182	188	194	161.5	5	6	71.7	53.8	5.6	7.4	18.0
MB-0107	142	152	187	191	195	174.1	5	6	75.8	91.2	6.0	7.5	18.6
MB-0109	109	125	160	154	187	61.8	0	20	59.9	5.70	4.9	7.5	18.1
MB-0306	130	140	180	188	194	161.5	5	6	82.2	86.0	6.7	7.4	22.0
MB-0307	128	138	182	184	194	157.3	6	6	77.2	70.5	6.8	7.4	26.0
MB-0308	125	136	183	186	194	167.0	6	6	74.8	107.0	6.4	7.6	18.5
MB-0309	131	143	187	189	196	195.0	6	6	70.4	146.7	5.4	7.5	24.6
MB-0310	125	141	183	187	195	166.3	5	7	67.4	110.4	5.3	7.7	24.5

Table 3. Continuation

Accessions	First flower (days)	Flowering (days)	End of flowering (days)	First dry pod (days)	Seed maturity (days)	Plant height (cm)	Leaflets (nr)	Tendrils (nr)	Pod length (mm)	Pods plant^{-1}	Seeds pod^{-1}	Seed diameter (mm)	Seed weight (g 100 seeds^{-1})
MB-0311	127	138	184	187	195	182.7	5	7	77.4	92.5	5.8	7.9	21.1
MB-0312	118	137	187	189	196	147.3	5	6	76.1	75.1	6.0	8.0	28.1
MB-0313	123	141	188	188	195	184.4	6	6	80.0	74.9	6.2	7.6	18.4
MB-0314	134	141	185	188	195	189.4	5	6	67.2	105.6	5.8	7.2	18.5
MB-0315	109	124	175	178	189	162.0	5	6	73.3	111.7	5.5	7.6	18.2
MB-0316	141	159	188	194	196	180.2	5	7	77.9	55.7	6.8	7.8	19.2
MB-0317	139	145	188	188	196	163.6	6	6	61.5	138.9	6.1	7.1	12.0
MB-0318	134	142	187	188	195	160.8	6	6	61.0	121.4	6.6	7.1	13.0
MB-0319	109	113	139	148	159	149.1	6	6	80,1	51.4	6,7	6.4	20.0
ALDERMAN	120	134	156	183	192	112.3	4	6	94.4	3.8	6.3	8.6	26.7
ATLAS	131	139	164	181	188	70.5	6	7	81.7	15.7	6.7	7.9	21.5
GLOTON	114	127	182	184	195	152.5	4	5	72.8	71.7	6.3	7.0	21.8
LOTUS	106	122	161	161	169	54.7	4	5	63.9	13.0	6.4	7.7	15.6
RONDO	122	134	164	182	187	75.9	4	6	93.2	9.28	6.2	8.4	27.5
UTRILLO	115	127	156	181	189	67.4	4	5	95.2	6.02	6.2	8.0	19.3
Mean	126.3	137.1	176.7	183.1	191.3	145.0	4.9	6.3	74.5	67.1	6.1	7.6	21.1
Range of variation	106-143	113-159	139-189	148-194	159-196	57.7-195.0	0-6	5-20	59.9-95.2	3.8-146.7	4.9-7.0	6.9-8.6	12.0-28.1
LSD (0.05) [a]	8.5	8.4	5.4	4.7	4.4	28.2	0.6	0.8	6.3	47.1	0.9	0.5	3.5

[a] LSD=Least significant difference at $p<0.05$

Table 4. Mean squares of the analysis of variance and coefficient of variation for the agronomic and seed quality traits evaluated in three field pea breeding lines and one advanced cultivar.

Source [a]	d.f.[b]	First flower (days)		d.f.[b]	End of flowering (days)		First dry pod (days)		Seed maturity (days)		d.f.[b]	Seed yield (g m^{-2})	
Y	2	244332.00	**	1	62437.51	**	35297.01	**	36528.76	**	2	136072.6	**
L	1	5625.00	**	1	228.76	**	310.64	**	4641.01	**	1	254443.7	**
Y x L	1	517.56	**	1	1670.76	**	319.51	**	123.76	**	2	92995.8	**
R (Y x L)	5	26.04	**	4	2.73		3.67		486.26	**	6	22742.1	**
D	1	19.02		1	0.76		4.51		0.76		1	9338.6	
D x Y	2	5.13		1	2.64		0.14		0.01		2	21561.3	*
D x L	1	0.25		1	0.01		1.26		0.76		1	7014.1	
D x Y x L	1	0.56		1	3.51		0.76		0.01		2	15446.6	*
A	3	67.43	**	3	11.14		7.64	*	5.97		3	10519.3	
A x Y	6	62.21	**	3	20.60		2.81		5.56		6	2681.7	
A x L	3	14.29		3	2.60		0.76		5.97		3	5671.6	
A x Y x L	3	6.69		3	1.43		1.56		5.56		6	8200.0	
A x D	3	1.71		3	7.26		1.81		8.64		3	4422.5	
A x Y x D	6	6.12		3	7.47		4.68		5.39		6	3115.1	
A x L x D	3	2.79		3	1.43		3.81		8.64		3	9345.2	
A x Y x L x D	3	4.60		3	1.26		1.56		5.39		6	2516.3	
Error	30	5.29		24	3.25		2.45		4.87		35	4518.4	
C.V. (%)[c]		2.06			1.05		0.93		1.10			34.0	

Table 4. Continuation

Source [a]	d.f. [b]	Protein content (g kg^{-1})		Fibre content (g kg^{-1})		Starch content (g kg^{-1})	
Y	1	819.5	**	18.56		1478.5	**
L	1	633.9	**	369.94	**	2325.3	**
Y x L	1	7812.3	**	16.70		5312.7	**
R (Y x L)	4	256.1	**	19.89		241.5	
D	1	486.7	**	68.00		1137.4	**
D x Y	1	352.2	*	0.13		425.1	
D x L	1	50.9		21.33		115.6	
D x Y x L	1	227.0	*	115.96	*	433.4	
A	3	757.5	**	258.01	**	1339.8	**
A x Y	3	86.8		77.25	*	734.7	**
A x L	3	42.0		70.20	*	95.7	
A x Y x L	3	3.9		26.13		94.2	
A x D	3	39.1		32.28		78.6	
A x Y x D	3	41.6		72.88	*	99.8	
A x L x D	3	4.7		76.69	*	112.6	
A x Y x L x D	3	84.1		15.11		309.2	
Error	24	46.3		19.07		105.2	
C.V. (%) [x]		2.65		5.77		2.06	

*, ** significant at $p \leq 0.05$, $p \leq 0.01$ respectively

[a] Y=Years, L=Locations, R=Replications, D=Cropping density, A=Accessions

[b] d.f.=degrees of freedom

[c] C.V.= Coefficient of Variation.

Table 5. Mean and range of variation for the agronomic and seed quality traits evaluated in three field pea breeding lines and one advanced cultivar

Accession	First flower (days)	End of flowering (days)	First dry pod (days)	Seed maturity (days)	Seed yield (g m^{-2})	Protein content (g kg^{-1})	Fiber content (g kg^{-1})	Starch content (g kg^{-1})
PMB-0307	110	172	169	201	187	260	71	496
PMB-0308	112	173	169	199	221	258	73	498
PMB-0319	113	173	170	200	177	261	79	488
ZP-1233	110	171	169	200	205	246	79	511
Mean	112	172	169	200	197	256	76	498
Range of variation	58.0-156.0	129.0-208.0	139.0-196.0	160.0-236.0	177.1-221.2	225.0-297.0	60.1-89.3	442.0-533.0
LSD (0.05) [a]	1.48		1.14			4.9	3.2	7.5

[a] LSD= Least significant difference at $p<0.05$

Table 6. Mean squares of the analysis of variance and coefficient of variation for the agronomic performance traits evaluated under different field treatments in three field pea breeding lines and one advanced cultivar.

Source [a]	d.f.[b]	Pods $plant^{-1}$	Seeds pod^{-1}		Seed weight (g 100 $seeds^{-1}$)		Seed yield (g m^{-2})
T	3	0.87	0.10		7.28	**	5390.2
R	1	0.60	0.04		0.57		1730.9
A	2	1.46	0.70	*	7.53	**	5164.5
A x T	6	1.82	0.22		2.30	*	1328.8
Error	11	2.23	0.11		0.62		3755.1
C.V. (%)[c]		16.65	8.34		3.32		21.9

*, ** significant at $p \leq 0.05$, $p \leq 0.01$ respectively

[a] T=Treatments: check, intercropped with rye, with pre-emergence herbicide, with post-emergence herbicide, R=Replications, A=Accessions

[b] d.f.=degrees of freedom

[c] C.V.= Coefficient of Variation.

Table 7. Mean and range of variation for the agronomic performance traits evaluated under different field treatments in three field pea breeding lines and one advanced cultivar.

	Pods plant^{-1}	Seeds pod^{-1}	Seed weight (g 100 seeds^{-1})	Seed yield (g m^{-2})
Accession				
PMB-0307	9.1	4.1	24.1	297.1
PMB-0308	9.3	4.3	22.7	294.0
PMB-0319	8.5	3.7	24.6	251.6
Mean	9.0	4.0	23.8	280.9
Range of variation	8.5-9.3	3.7-4.3	22.7-24.6	251.6-297.1
LSD (0.05) [a]		0.4	0.9	
Treatment[b]				
A	9.5	4.0	23.2	301.1
B	8.7	4.2	25.4	237.9
C	9.1	4.0	23.4	301.7
D	8.7	4.0	23.1	283.0
Mean	9.0	4.0	23.8	280.9
Range of variation	8.7-9.5	4.0-4.2	23.1-25.4	237.9-301.7
LSD (0.05) [a]			0.9	

[a] LSD=Least significant difference at $p<0.05$

[b] A: control, B: intercropped with rye, C: with pre-emergence herbicide, D: with post-emergence herbicide