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# Garden pea: agronomic, color and quality characterization using morphological and molecular data

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Espósito, M.A.[1,2,3]; Bermejo, C.[2,3]; Guindón, M.F.[3]; Palacios, T.[3]; Gatti, I.[2,3]


## ABSTRACT

During 2019 and 2020, 24 varieties of garden pea were sown under drip irrigation and dry-land conditions to evaluate the agronomic, grain color and quality traits. A molecular characterization was performed using SSR and SRAPS molecular markers. A high diversity at morphological and molecular levels was found among them. The variance components— genotypic ($CV_G$), phenotypic ($CV_P$) and environmental ($CV_E$) coefficients of variation and heritability in the broad sense ($H^2$)—were calculated. A $CV_G/CV_P$ ratio close to or greater than one indicates that selection based on phenotype can result in gain (traits C, PLH, PL, DFH, L, a, b; HUE, CRO, CI, Ca and Cb), while a low or intermediate ratio indicates that phenotypic selection will not be effective.

A Cluster analysis combining morphological and molecular data allowed the formation of five highly differentiated groups regarding expressed and underlying variability. Hybridization of members of the most distant Clusters may originate a segregating population with high variability to initiate a breeding program.


**Keywords:** garden pea, morphological and molecular characterization, grain quality.


## RESUMEN

*Durante 2019 y 2020 se sembraron 24 variedades de arveja tipo rugosa para consumo en fresco bajo riego por goteo y secano para evaluar las características agronómicas, de color y calidad del grano. Se realizó una caracterización molecular utilizando marcadores moleculares SSR y SRAPS. Entre ellas se encontró alta diversidad a nivel morfológico y molecular. Se calcularon los componentes de la varianza, los coeficientes de variación genotípico (CVG), fenotípico (CVP) y ambiental (CVE) y la heredabilidad en sentido amplio ($H^2$). Una relación CVG/CVP cercana o mayor que uno indica que la selección basada en el fenotipo puede resultar en una ganancia (rasgos C, PLH, PL, DFH, L, a, b; HUE, CRO, CI, Ca y Cb), mientras que una relación baja o intermedia indica que la selección fenotípica no será efectiva.*

*Un análisis de conglomerados en el que se combinaron datos morfológicos y moleculares permitió la formación de cinco grupos altamente diferenciados en cuanto a la variabilidad expresada y subyacente. La hibridación de miembros de los clústeres más distantes puede originar una población segregante con alta variabilidad para iniciar un programa de mejora genética.*


***Palabras clave:*** *arveja tipo rugosa, caracterización morfológica y molecular, calidad de grano.*

[1]Instituto Nacional de Tecnología Agrícola (INTA), Estación Experimental Agropecuaria (EEA) Oliveros, Ruta Nacional 11 km 353 (2206) Oliveros, Santa Fe, Argentina. Correo electrónico: esposito.maria@inta.gob.ar
[2]Universidad Nacional de Rosario (UNR), Facultad de Ciencias Agrarias, Cátedra de Mejoramiento Vegetal y Producción de Semillas, Maipú 1065 (2000), Rosario, Santa Fe, Argentina.
[3]Instituto de Investigaciones en Ciencias Agrarias de Rosario (IICAR)-CONICET, Campo Experimental Villarino, C.C. 14 (2125), Zavalla, Santa Fe, Argentina.
[4]CIUNR (Consejo de Investigaciones de la Universidad Nacional de Rosario) (CIUNR), Maipú 1065 (2000), 2.º piso oficina 209, Rosario, Santa Fe, Argentina.

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## INTRODUCTION

Pea (*Pisum sativum* L.) is one of the oldest domesticated crops in the world. The genus originated approximately 10,000 years ago in Ethiopia, where most subspecies were found (Zohary and Hopf, 2000). This specie has a wide variability and a huge number of cultivated varieties due to its popularity as a vegetable since ancient times. Round peas, or smooth peas, have a large amount of carbohydrates with a starch content between 44 to 49%; and, for this reason, they are used especially for dry grain. Wrinkled peas —also known as garden peas, table peas or English peas —have cotyledons with a lower starch content (between 28% and 34%), but a higher glucose and dextrin content. In this type of peas, the rate of conversion of sugars into starch during seed maturation occurs more slowly, facilitating harvest when embryos are immature and liquid endosperm is still present. The integuments are fully adhered to the cotyledons, which gives them a greater aptitude for freezing. The quality for fresh consumption depends largely on the maturity level at the time of harvest and decreases quickly once the filling process has started. The most important parameters for consumer acceptance are grain size, color, and sweet taste.

The general objective in any breeding program is to maximize the probability of creating and identifying superior genotypes that will become new successful cultivars containing all the desirable characteristics for specific production systems or specific markets. To achieve this, it is necessary to have a segregating population with high variability in the first place, that can be generated from crosses between parents with desirable traits.

With the advancement in technologies, and especially with the reduction of costs, the first step of any breeding program is the evaluation of the variation at both morphological and molecular level. Although morphological characters are sometimes helpful, they can be affected by environmental conditions. Molecular markers are useful to complement both the morphological and phenological characters because they are abundant, independent of environmental effects and they allow the identification of genotypes in the early stages of development (Tar'an *et al*., 2005; Bouhadida *et al*., 2013).

| Common name | Country of origin | Source |
|---|---|---|
| Accord | USA | USDA 635187+ |
| American Wonder | England | JIC 318* |
| Avon | France | JIC 1414 |
| Bolero | USA | USDA 635202 |
| Cuarentona | Argentina | USDA 162692 |
| Dante | USA | USDA 595591 |
| Duke of Albany | England | JIC 313 |
| Early Perfection 1322 | USA | JIC 520 |
| Early Sweet | USA | USDA 635190 |
| Eaton | England | JIC 1767 |
| Filigreen afila | Germany | JIC 1772 |
| Granada | USA | USDA 595579 |
| Green Sugar | USA | USDA 642175 |
| Gypsy | USA | USDA 595575 |
| Leo | USA | USDA 595573 |
| Multiviral resistent | USA | JIC 2619 |
| Panga | Zambia | JIC 2310 |
| Rapid | Hungary | JIC 622 |
| Rois des Conserves | France | JIC 350 |
| Superscout | USA | USDA 601010 |
| Suttons Early Giant | England | JIC 300 |
| Telephone Gold Straw | England | JIC 1720 |
| Trianon | France | JIC 1214 |
| Withan Wonder | Sweden | JIC 31 |

*JI: John Innes Centre, Norwich, UK. +USDA: U.S. National Plant Germplasm System (NPGS), USA.

**Table 1.** List of 24 varieties of garden pea used.

The genomic regions that contain simple repeat sequences (SSR) amplified by PCR (Polymerase Chain Reaction) constitute highly polymorphic DNA markers, known as microsatellites, created by Litt and Luty in 1989. They provide abundant information to calculate the distances between populations and also between individuals since they allow to distinguish the states of the alleles of each marker locus (data of alleles by locus) (Bruno and Balzarini, 2009).

On the other hand, the SRAP (Sequence-related Amplified Polymorphism) technique (Li and Quiros, 2001) has been recognized as a highly useful molecular marker system to characterize germplasm. It is a simple technique and has been widely used for studies of genetic diversity in peas (Wu *et al*., 2017; Trněný *et al*., 2018; Ferradini *et al*., 2019; Stavridou *et al*., 2020).

Currently, for autogamous crops, data is collected from various sources, such as observations from field experiments, molecular markers, and laboratory quality tests. The aim of the present work was to evaluate the variability in a set of 24 garden pea lines by analyzing the morphological, grain quality and color traits at a molecular level to select those appropriate to be used as parents to originate starting populations in breeding programs.

## MATERIALS AND METHODS

### Vegetal material

During 2019 and 2020, 24 varieties of garden pea (table 1) were sown in the Experimental Field of the Faculty of Agrarian Sciences of the UNR, located in Zavalla (33° S and 60° 53’ O) in a field experiment with two repetitions under drip irrigation and two in dry land. The plot was a 2 m long row with 40 plants and 0.7 m between rows.

### Morphological characterization

The agronomic traits analyzed on a plot basis were yield in g (Y), number of grains (GN), total weight of pods in g (PW), number of pods (PN), number of grains per pod (GPP), grain size in cm (C), pod length in cm (PL), pod width in cm (PW) and plant height in cm (PLH). The shelling percentage (SH) was calculated as the ratio Y/PW. Also, days from sowing to first harvest (DFH), days between first and second harvest (DFSH) and percentage of Y in first harvest (%YFH) were recorded.

Samples of 300 g of grains were used to determine the variables related to quality. pH values were measured using a pH-meter

| Name | Forward primer sequence | Reverse primer sequence |
|---|---|---|
| | SSR | |
| AD148 | GAAACATCATTGTGTCTTCTTG | TTCCATCACTTGATTGATAAAC |
| AA200 | ACCGAAGAGCATTTTTCCTAAG | TCCATCAGTTCCCTAATTCACT |
| AA18 | CTGTAGACCAAGCCAAAAGAT | TGAGACACTTTTGACAAGGAGG |
| AA278 | CCAAGAAAGGCTTATCAACAGG | TGCTTGTGTCAAGTGATCAGTG |
| AA335 | ACGCACACGCTTAGATAGAAAT | ATCCACCATAAGTTTTGGCATA |
| AB23 | TCAGCCTTTATCCTCCGAACTA | GAACCCTTGTGCAGAAGCATTA |
| AC58 | TCCGCAATTTGGTAACACTG | CGTCCATTTCTTTTATGCTGAG |
| AD56 | GAAACATTGGTTGAAGAGCGAG | GTTGTCGCGTGAACACAAGTAA |
| AA5 | TGCCAATCCTGAGGTATTAACACC | CATTTTTGCAGTTGCAATTTCGT |
| AA23 | TTAGCTTGAAGCTCACACAAG | ACACTAGCTACTACAAATGAAGGC |
| D21 | TATTCTCCTCCAAAATTTCCTT | GTCAAAATTAGCCAAATTCCTC |
| AD61 | CTCATTCAATGATGATAATCCTA | ATGAGGTACTTGTGTGAGATAAA |
| | SRAP | |
| me2 | 5’-TGAGTCCAAACCGGAGC-3’ | |
| me3 | 5’-TGAGTCCAAACCGGAAT-3’ | |
| me4 | 5’-TGAGTCCAAACCGGACC-3’ | |
| me5 | 5’-TGAGTCCAAACCGGAAG-3’ | |
| em1 | | 5’-GACTGCGTACGAATTAAT-3’ |
| em2 | | 5’-GACTGCGTACGAATTTGC-3’ |
| em4 | | 5’-GACTGCGTACGAATTTGA-3’ |
| em5 | | 5’-GACTGCGTACGAATTGCA-3’ |

**Table 2.** Sequences of the SRAP and SSR primers used.

(*Hanna Instrument, Model Checker*). Titratable acidity in % (AcT) was measured using the A.O.A.C. (2000) method 942.15 as percentage of malic acid. The maturity index (MI) was calculated as the ratio of total soluble sugar determined on a juice sample of squeezed grains using a refractometer (Green Tech) expressed as percentage (Brix°) and the AcT. Vitamin C content in $mg*100g^{-1}$ (VitC) was calculated by iodometry, according to the methodology proposed by Ciancaglini *et al*. (2001), as ascorbic acid content, using a 24.1 mM iodine solution as an oxidizing agent and a freshly prepared 1% (w / v) starch solution as an indicator. The content of chlorophylls a and b in $mgAl^{-1}$ (Ca and Cb respectively) were determined using the spectrophotometric method proposed by Hansmann (1973).

To analyze the color of the grains, the attributes of hue (HUE) and chroma (CRO) of the Munsell color system and parameters L, a, and b of the CIELAB color system were measured on 600 dpi digital images taken with a Samsung CLX 3300 scanner in samples of 50 pea grains for each experimental plot, using the Tomato Analyzer (TA) software (Rodríguez *et al*., 2010). The L parameter provided a value for the Luminance or brightness of the sample. Parameter a indicated the zone of variation between red and green of the spectrum. Parameter b referred to the zone of variation between yellow and blue of the spectrum. The colorimetric index (CI) was calculated as CI = (1.000 x a) / (L x b).

### Molecular characterization

Extraction of DNA: young leaves of each of the lines studied were collected and stored in a freezer at -80°C. The total genomic DNA was extracted starting from 0.10 g of plant tissue using the CTAB (cetyltrimethylammonium bromide) method described by Doyle and Doyle (1990). After a treatment with RNase, the quantity and quality of the DNA was evaluated by electrophoresis in 1% agarose gels. The intensity of the bands was compared with a DNA standard (100 ng/µl) by analyzing the digital images of the gels with the GelAnalyzer v2010 program.

SSR markers: 12 microsatellite primer combinations developed for pea by Burstin and Loridon (Burstin *et al*., 2001; Loridon *et al*., 2005) were used (tale 2). The PCR reaction (20 µl final volume) consisted of 15ng of genomic DNA, 0.2 mM of dNTPs, 0.5 µM of each primer, 1.5 mM of MgCl2, 1X of Taq DNA polymerase buffer, and 1 unit (U) of Taq DNA polymerase (PB-L, Bio-Logical Products®). The amplifications were carried out in a MyCyclerTM thermocycler (BIO-RAD), initiating with a 5 min denaturation at 94ºC, 35 cycles of 3 steps: 30 sec at 94ºC, 30 sec at the corresponding hybridization temperature, 30 sec at 72ºC and ending with a final elongation step of 5 min at 72ºC.

The amplification products were screened using electrophoresis on 2.5% agarose gels in 1X Tris-acetate buffer solution and visualized by SYBR® Safe staining (Thermo Fisher Scientific®, Waltham, MA, USA), following the methodology proposed by Kumari *et al*. (2013).

SRAP markers: 6 combinations (me2-em1, me3-em5, me4-em2, me5-em2, me5-em4, and me5-em5) were generated from 4 forward and 4 reverse primers (table 2). The PCR reaction (20 µl final volume) consisted of 15 ng of genomic DNA, 0.2 mM of dNTPs, 0.5 µM of each primer, 1.5 mM of MgCl2, 1X of Taq DNA polymerase buffer, and 1 unit (U) of Taq DNA polymerase (Invitrogen ™, 5U/µl). Amplifications were carried out in a MyCyclerTM thermal cycler (BIO-RAD). The cycling protocol was: 5 min at 94°C; 5 cycles of three steps: 1 min at 94°C, 1 min at 35°C and 1 min at 72°C; in the next 35 cycles, the annealing temperature was raised to 50°C; ending with an elongation step of 10 min at 72°C. After denaturation at 94°C for 5 minutes, the amplification products were screened using electrophoresis in polyacrylamide gels at 6% (m/v), using 15 µl of buffer (98% (v/v) formamide, 10 mM EDTA, 0.01% (m/v) bromophenol blue and 0.01% (m/v) of xylene cyanol. The visualization of the PCR products was performed by staining with silver nitrate at 1% (m/v), following the protocol of Bassam *et al*. (1991).

### Statistical analysis

An Analysis of Variance was carried out using a split-split plot design, with two repetitions, assigning the year effect to the main plot, the irrigation effect to the subplot and the genotype effect to the sub-subplot. The software InfoStat (Di Rienzo *et al*. 2015) was used to perform the analysis.

The variance components, as well as the genotypic ($CV_G$), phenotypic ($CV_P$) and environmental ($CV_E$) coefficients of variation, were calculated according to Burton (1952) and expressed as percentages of the mean. In addition, heritability in the broad sense ($H^2$) was estimated.

The calculation of genetic merit was performed using the best linear unbiased predictor (BLUP), according to the model proposed by Robinson (1991):

$$y=X\beta +Zu +e$$

where y is a vector of n measured variables, β is a vector of p unknown fixed effects parameters, X and Z are known matrices and u and e are vectors of q and n respectively unobserved variables of random effects

Thus, the combination of the average values obtained with the values predicted by BLUP were the genotypic values (GV).

$$GV = m + bi,$$

where m is the general mean of the study and bi is the predicted value of genotype i.

SSR and SRAPS were scored for the presence (1) and absence (0) of the corresponding band among the genotypes in the form of a binary matrix, following the methodology proposed by Bruno and Balzarini (2009). The comparison of individual profiles was performed using the Roger's modified distance and the Percentage of polymorphic loci was calculated as:

$$P = \frac{np_j}{n_{total}}$$

Where npj is the polymorphic loci number and ntotal is the total number of loci

The statistical program BIO-R (Biodiversity Analysis with R for Windows.) Version 1.0 (Pacheco *et al*., 2016) was used for all the molecular data analyses.

The cluster analysis was performed using Ward's method and Euclidean distance for GV of morphological data; modified Roger's distance for molecular data and Gower distance for morphological and molecular data combined. The software InfoStat (Di Rienzo *et al*., 2015) was used for all the analyses.

## RESULTS

The Analysis of Variance showed significant differences ($p < 0.01$) for variables GPP and %YFH and highly significant differences ($p < 0.001$) for the rest of the variables.

| | Mean | $\sigma^2_F$ | $CV_P$ | $\sigma^2_G$ | $CV_G$ | $\sigma^2_E$ | $CV_E$ | $H^2$ | $CV_G/CV_E$ |
|---|---|---|---|---|---|---|---|---|---|
| Y | 685.0 | 116218.5 | 49.8 | 31021.1 | 25.7 | 109768.8 | 48.4 | 0.27 | 0.53 |
| GN | 1530.7 | 925784.2 | 62.9 | 234440.7 | 31.6 | 591044.1 | 50.2 | 0.25 | 0.63 |
| PW | 1314.3 | 377449.1 | 46.7 | 88694.6 | 22.7 | 344893.3 | 44.7 | 0.23 | 0.51 |
| PN | 283.4 | 16836.7 | 45.8 | 2700.1 | 18.3 | 14136.6 | 42.0 | 0.16 | 0.44 |
| GPP | 5.3 | 1.2 | 20.8 | 0.03 | 3.3 | 1.1 | 20.0 | 0.02 | 0.16 |
| C | 10.1 | 0.8 | 8.7 | 0.6 | 7.7 | 0.1 | 3.3 | 0.78 | 2.35 |
| PL | 6.9 | 1.7 | 19.1 | 1.0 | 14.6 | 0.7 | 12.3 | 0.58 | 1.18 |
| PW | 1.2 | 0.1 | 20.1 | 0.02 | 11.6 | 0.04 | 16.4 | 0.33 | 0.70 |
| SH | 51.7 | 47.2 | 13.3 | 16.1 | 7.8 | 26.0 | 9.9 | 0.34 | 0.79 |
| PLH | 90.7 | 909.4 | 33.2 | 714.0 | 29.5 | 138.8 | 13.0 | 0.79 | 2.27 |
| DFH | 112.7 | 50.5 | 6.3 | 31.1 | 5.0 | 19.7 | 3.9 | 0.62 | 1.26 |
| DFSH | 9.0 | 9.1 | 33.5 | 0.7 | 9.1 | 7.8 | 31.1 | 0.07 | 0.29 |
| %YFH | 44.4 | 551.6 | 52.9 | 1.8 | 3.0 | 294.1 | 38.7 | 0.01 | 0.08 |
| AcT | 1.7 | 0.0 | 10.0 | 0.01 | 5.5 | 0.02 | 8.3 | 0.30 | 0.66 |
| pH | 7.2 | 0.1 | 3.7 | 0.02 | 1.8 | 0.04 | 2.8 | 0.23 | 0.64 |
| MI | 393.9 | 12688.6 | 28.6 | 2687.9 | 13.2 | 11762.4 | 27.5 | 0.21 | 0.48 |
| Vit C | 62.5 | 288.9 | 27.2 | 19.9 | 7.1 | 269.0 | 26.3 | 0.07 | 0.27 |
| L | 57.4 | 0.7 | 1.5 | 0.3 | 0.9 | 0.3 | 0.9 | 0.37 | 0.96 |
| a | 1.6 | 1.1 | 64.6 | 0.6 | 48.7 | 0.3 | 34.7 | 0.57 | 1.40 |
| b | 7.8 | 0.9 | 12.0 | 0.6 | 10.3 | 0.2 | 6.0 | 0.70 | 1.71 |
| HUE | 102.6 | 67.4 | 8.0 | 38.2 | 6.0 | 19.2 | 4.3 | 0.57 | 1.41 |
| CRO | 8.1 | 0.6 | 9.8 | 0.4 | 8.2 | 0.2 | 5.3 | 0.71 | 1.57 |
| CI | 3.9 | 7.0 | 67.7 | 3.8 | 49.9 | 2.0 | 35.6 | 0.54 | 1.40 |
| Ca | 10.2 | 6.3 | 24.6 | 3.1 | 17.3 | 3.0 | 17.0 | 0.49 | 1.02 |
| Cb | 3.8 | 1.2 | 29.0 | 0.6 | 19.9 | 0.6 | 20.3 | 0.47 | 0.98 |

Y: yield (g); GN: number of grains; PW: total weight of pods (g); PN: number of pods; GPP: number of grains per pod; C: grain size (cm); PL: pod length (cm); PW: pod width (cm); SH: shelling percentage (%); PLH: plant height (cm); DFH: days to first harvest; DFSH: days between first and second harvest, %YFH % Y in first harvest, AcT: titratable acidity (%); MI: maturity index; pH: potential of hydrogen; VitC: vitamin C content (mg / 100g); L (luminosity), a, and b; parameters of the CIELAB and HUE (hue) and CRO (chroma or saturation): parameters of Munsell systems of color; CI: colorimetric index; Ca and Cb: content of chlorophylls a and b respectively ($mgAl^{-1}$).

**Table 3.** General mean, phenotypic ($\sigma^2_F$), genotypic ($\sigma^2_G$) and environmental ($\sigma^2_E$) variance; coefficients of phenotypic ($CV_P$), genetic ($CV_G$) and environmental ($CV_E$) variability and heritability in the broad sense ($H^2$).

Table 3 presents the mean values; phenotypic ($\sigma^2_F$), genotypic ($\sigma^2_G$) and environmental ($\sigma^2_E$) variances; genotypic ($CV_G$), phenotypic ($CV_P$) and environmental ($CV_E$) coefficients of variation; heritability in the broad sense ($H^2$) and the $CV_G/CV_P$ ratio for each variable.

The phenotypic variance was greater than the genetic variance for all the variables, being the difference between them very high for variables %YFH, GPP, Vit C and DFSH; and low for variables C, PLH, PL, DFH and all the traits related to grain color. When comparing the genetic variance with the environmental one, it was higher for the variables C, PLH, PL, DFH, a, b, HUE, CRO, CI and Ca, but it presented lower values than the environmental variance for the rest of the variables. Variables C and PLH showed the greatest differences. The $H^2$ values found were moderately high for DFH, b, CRO, C and PLH; medium for Cb, Ca, CI, a, HUE and PL and low for the rest of the variables.

In the molecular marker analysis, a total of 121 polymorphic bands were obtained. The percentage of polymorphic loci found was 90%.

For the Genotypic Values (GV) of morphological data, seven Clusters were identified in the Cluster Analysis using the Ward's method and Euclidean distance (fig. 1). The most distant Clusters were 7 and 5, with a distance of 9.74.

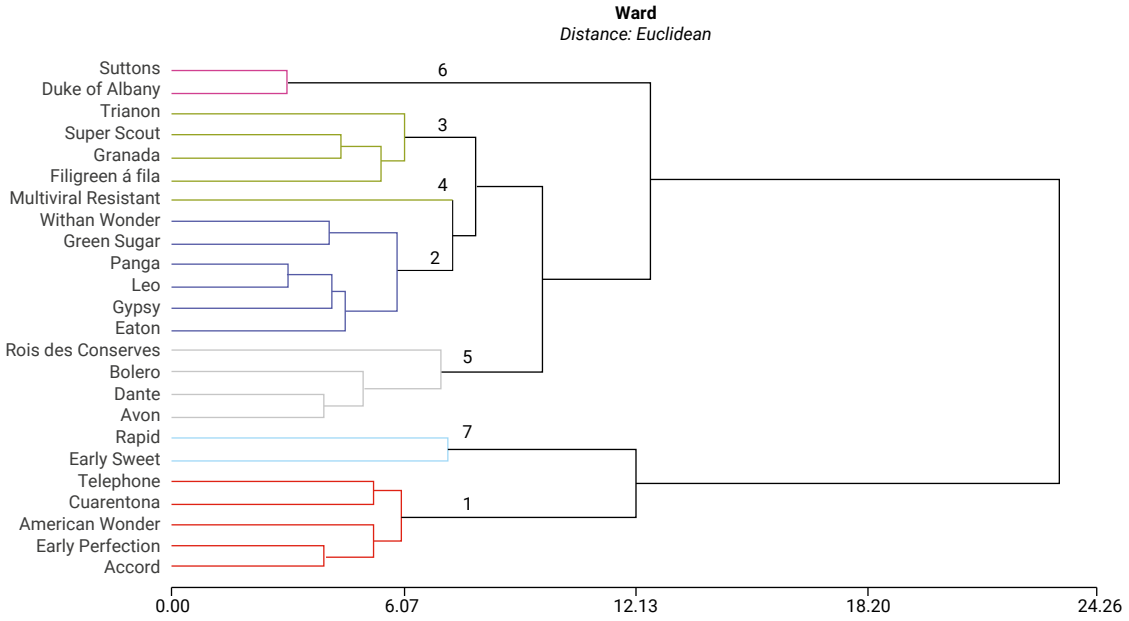


**Figure 1.** Cluster Analysis of 24 garden pea varieties using GV of morphological data (Ward's method and Euclidean distance).

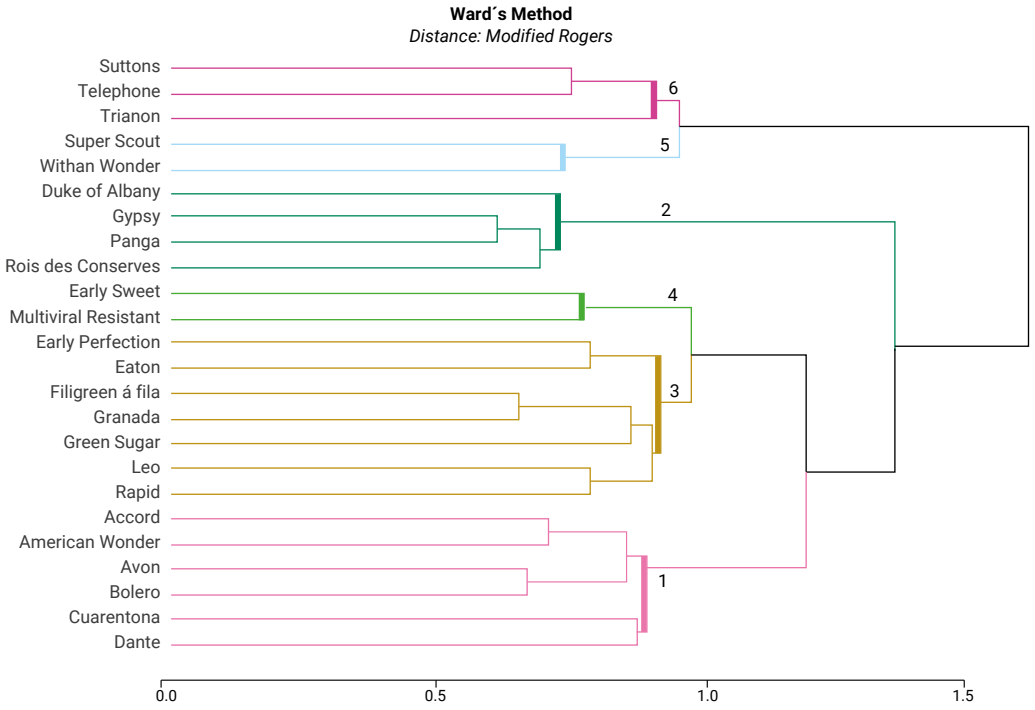


**Figure 2.** Cluster Analysis of 24 garden pea varieties using molecular data (Ward's method and Modified Roger's distance).

Using molecular data, six Clusters were obtained with Ward's method and Modified Roger's distance (fig. 2). The most distant Clusters were 2 and 5, with a distance of 0.73.

Combining GV of the morphological and molecular markers data, five Clusters were obtained using Ward's method and Gower distance (fig. 3). The most distant Clusters were 1 and 2, with a distance of 0.76.

## DISCUSSION

Genetic variability is a determining factor and a prerequisite for breeding programs (Tiwari and Lavanya, 2012) and its evaluation allows to identify the best parents to hybridize within the available germplasm. In turn, knowing the heritability values of the traits of interest and the estimation of the genetic parameters is of primary importance to ensure the success of the program (Esiyok *et al*., 2011).

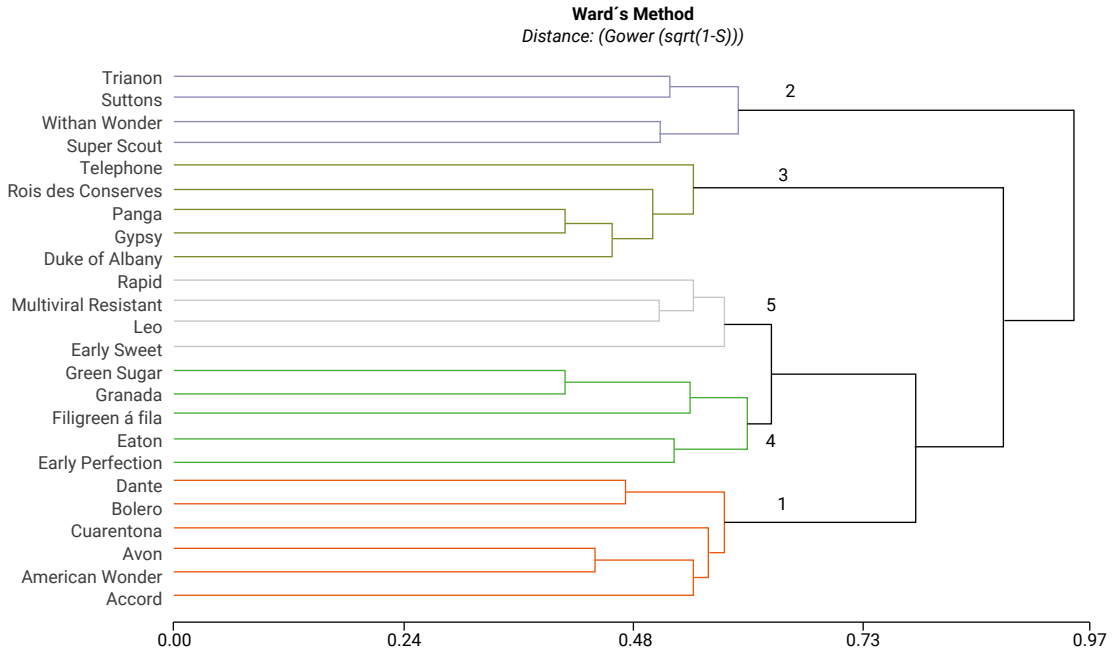


**Figure 3.** Cluster Analysis of 24 garden pea varieties using GV of morphological and molecular data (Ward's method and Gower distance).

Hedau *et al*. (2018) analyzed 33 lines of garden peas for grain quality traits and agro-morphological properties and found that the magnitude of genetic variance for all variables was greater than the environmental one. These findings are similar to those in previous reports (Tiwari and Lavanya, 2012; Jaiswal *et al*., 2015). The results obtained here agree with these findings for the variables Ca; variables related to color, such as a, b, HUE, CRO and CI; and the agronomic variables DFH, PL, C and PLH.

When the relationship $CV_G/CV_E$ tends to or is greater than one, the genetic variance is the major contributor to the phenotypic variation of the trait rather than the environmental variance, and there is a chance to obtain gain by selection. Variables %YFH, GPP, VitC, DFSH, PN, MI, PW and Y presented low $CV_G/CV_E$, indicating that the variations observed for these variables were mainly due to the environment; therefore, selection of these variables based on the phenotypic value will not be effective. On the other hand, GN, pH, AcT, PW and SH presented an intermediate relationship, while the rest of the variables presented a relationship close to or greater than one, the highest being for C (2.25) and PLH (2.27), indicating that, for these variables, selection based on phenotype can result in gain per selection.

Another interesting population parameter to analyze is the heritability in broad sense ($H^2$), which is the portion of the phenotypic variation transmitted from parent to offspring. The greater the heritable variation, the greater the possibility of fixing a character through selection methods (Sharma and Bora, 2011).

According to Singh (2001), values of $H^2$ greater than 80% are very high, from 60% to 79% moderately high, from 40% to 59% medium and values lower than 40% are considered low. In the present work, the $H^2$ values found were moderately high for DFH, b, CRO, C and PLH; medium for Cb, Ca, CI, a, HUE and PL; and low for the rest of the variables. In this way, those variables where the genetic variance was the major contributor to the phenotypic variation and present additive gene actions are the ones that will produce a response to selection. Once these conditions are met, genotypes with favorable values in these specific traits can be used as parents for the breeding program.

Some varieties were always located together in the same cluster when analyzing the grouping in terms of the expressed variability (morphological characters), the underlying variability (molecular markers) and the two of them combined; for example, Cuarentona, American Wonder and Accord (Clusters 1, 1 and 1 respectively); Green Sugar and Eaton (Clusters 2, 3 and 4 respectively); Granada and Filigreen afila (Clusters 3, 3 and 4 respectively) and Bolero, Dante and Avon (Clusters 5, 1 and 1 respectively) this indicates that these varieties are very similar.

On the other hand, there are no coincidences in the varieties located in the most distant Clusters. The most distant varieties regarding expressed and underlying variability were those in Cluster 2 (conformed by Trianon, Suttons, Withan Wonder and Super Scout) and Cluster 1 (Dante, Bolero, Cuarentona, Avon, American Wonder and Accord). The hybridization of the members of these Clusters may originate a segregating population with high variability to initiate a breeding program.

## CONCLUSIONS

A high diversity among the set of 24 garden pea varieties studied was found at morphological and molecular levels. The variables %YFH, GPP, VitC, DFSH, PN, MI, PW and Y presented a low $CV_G/CV_P$ ratio, indicating that the variation observed in these variables was due to a high influence of the environment on their expression. In this case, the selection of these characters based on the phenotypic value will not be effective. On the other hand, GN, pH, AcT, PW and SH presented an intermediate relationship, while the rest of the variables presented a relationship close to or greater than one, being very high for C (2.25)

and PLH (2.27); thus, selection based on phenotype can result in gain per selection for these variables.

The cluster analysis combining morphological and molecular data allowed the formation of five highly differentiated groups regarding expressed and underlying variability. Hybridization using varieties from the most distant Clusters (Cluster 1 and Cluster 2) may originate a segregating population with high variability to initiate a breeding program.

## STATEMENTS AND DECLARATIONS

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### Competing interests

The authors have no relevant financial or non-financial interests to disclose.

### Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ileana Gatti and María Andrea Espósito. The first draft of the manuscript was written by Ileana Gatti and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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