# Genetic Diversity of Meat Quality Related Genes in Argentinean Pigs

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**Research**

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## Abstract

**Background:** The genetic influence on pork quality exists between breeds and within a breed. The variation is caused by a large set of genes and pork quality traits have a multi factorial background. Research into the genetics of meat quality found causative mutations associated with marked effects on pig meat value. The main objective of this study was to investigate the diversity of single nucleotide polymorphisms (SNPs) of meat quality-related genes in pigs from commercial and family farms in the North-West of Argentina. A screen for SNPs in RYR1, RN, CAST, and SOX6 candidate genes and the differentiation of their genotypes by PCR–RFLP was conducted.

**Results:** All genes were characterized by a high level of polymorphism and heterozygosity. Additionally, both populations, commercial and Creole pigs, showed genetic closeness for the analyzed SNPs and a moderate selection for RYR1 and RN loci consistent with their allele´s adverse effects.

**Conclusions:** The results obtained here highlighted the role of pig genotypes as a source of basic variability potentially affecting processed meat products as well as fresh meat.


## Background

Given the increasing global demand for meat, fast-growing species with a high food conversion rate, such as pigs, can contribute greatly to the development of the livestock subsector. According to The Food and Agriculture Organization (FAO), pork is a major source of protein for humans and accounts for a large percentage of world meat production. In this way, commercial pig production has increased significantly in recent decades. And more pigs, of the same small number of breeds, are raised on fewer and fewer farms, with an increase in the yield of products of animal origin. Large-scale production systems have reached a high level of uniformity as they are based on the same genetic material and therefore provide the same type of feed and infrastructure to animals. However, in developing countries, a large percentage of current pig herds continue to be kept under traditional small-scale production systems. These traditional production methods are a sample of the viability of alternative production systems, usually mixed agricultural systems linked to local markets. Nevertheless, in both types of production, there has not been significant focus on meat quality until the last few years. Meat quality depends on consumers´ subjective perceptions, who are demanding not only a carcass´s high lean content but also optimal tenderness, marble, aroma and acidity along with an attractive color and water holding capacity (WHC). Furthermore, the quality concept is related to sensory, nutritional, hygienic, technological and genetic components, as well as factors of cellular metabolism that influence meat attributes. Specifically, consumers' pork quality assessment is defined by the characteristics of sensory experience; such as tenderness, juiciness, flavor, texture, color, shear force, intramuscular fat (IMF), moisture content and protein content (Saini et al., 2018). Technological quality refers to WHC, firmness, intensity and homogeneity of color, pH and processing yield (Saini et al., 2018). Therefore in view of the market´s increasing requirements, the pork industry must focus on controlling every quality parameter along the whole production chain.

As already mentioned, genetic improvement strategies have focused on the production of animals with a rapid transformation of the feed consumed into lean meat and accelerated animal growth, which led to significant reductions in the intramuscular fat content in the carcass and changes in the composition of fatty acids that negatively affect the organoleptic characteristics of the meat (Wood et al., 2008). The genetic influence on pork quality exists between breeds and within a breed. The variation is caused by a large set of genes and pork quality traits have a multi factorial background (Gispert et al., 2000; Andersson 2001). Research into the genetics of meat quality found causative mutations associated with marked effects on pig meat value. Specifically, mutations in two major genes referred to Halotano and Rendement Napole. The one commonly known as the Halotano gen (HAL) is a major gene encoding the calcium release channel in the skeletal muscle sarcoplasmic reticulum called ryanodine receptor located on SSC6 (RYR1, Gene ID: 396718) (Fujii et al., 1991). Porcine stress syndrome (PSS) or malignant hyperthermia is an autosomal recessive disease originated by a mutation that causes a substitution C > T at 1843 nucleotide position in the RYR1 gene. Homozygous recessive animals (tt) with facing even minimum stress can show clinical signs such as tremors and muscle stiffness, increased body temperature and it may even lead to death, causing significant losses in primary production (Harrison, 1979). Further, tt animals turn in a pale, soft and exudative meat (PSE), which results in high losses in the industry. RYR1 not only affects the meat quality of tt swine but also it affects the quality of heterozygote (Ct) swine carcasses (Sather et al., 1991). Furthermore, Rendement Napole (RN) gene is also known to have a negative effect on meat quality; it is associated with the pork acidity (Naveau et al., 1986). RN or PRKAG3 gene maps to SSC15 and encodes for a specific isoform of the regulatory γ subunit of adenosine monophosphate-

activated protein kinase (AMPK γ3, Gene ID: 397149) (Milan et al., 1995; Milan et al., 2000). AMPK γ3 has a role in the metabolic plasticity of fast-glycolytic muscle and is primarily expressed in white (fast-twitch, type IIb) skeletal muscle fibers (Mahlapuu et al., 2004). Non-synonymous single nucleotide polymorphisms (SNPs) in this gene such as I199V and R200Q are associated with important pork quality traits (WHC and pH) (Ciobanu et al., 2001; Josell et al., 2003; Lindahl et al., 2004; Granlund et al., 2011). Both mutations are located in a highly conserved region of the cystathionine β- synthase domain which is believed to act as a sensor of cellular energy status (Ryan et al., 2012). Calpastatin (CAST, Gene ID: 397135) is also important in terms of the quality traits of pork. CAST is a specific inhibitor of μ- and m-calpain proteases, which are responsible for early postmortem muscle proteolysis (Huff-Lonergan et al., 1996; Goll et al., 2003). The calpain-calpastatin system plays an important role in the normal growth of skeletal muscles during the postnatal period (Melody et al., 2004). The activity of calpastatin is strongly associated with the meat tenderness and muscle growth rate, as well as the rate of postmortem proteolytic changes that make the meat tender (Barnoy et al, 1997). The function and localization of the calpastatin gene on porcine SSC2 (Ernst et al., 1998), where several quantitative trait loci (QTL) for meat and carcass quality have been mapped (Malek et al., 2001a and b; Geldermann et al., 2003; Stearns et al., 2005(a); Meyers et al., 2007), strongly suggest that this gene may be a good candidate for meat and carcass quality traits. Several CAST polymorphisms have been described including CAST 638 Ser>Arg and CAST 76872 G>A which have been associated with pork tenderness (Ciobanu et al., 2004, Gandolfi et al., 2011). *Calpastatin solubility and cellular location are influenced by phosphorylation by adenosine cyclic 3', 5'-monophosphate- dependent protein kinase (PKA) (Averna et al., 2001). Phosphorylation increased the proportion of CAST bound to membranes (Adachi et al., 1991) and decreased CAST inhibitory efficiency (Salamino et al., 1997). Therefore, CAST phosphorylation could influence proteolysis and may have an effect on tenderness and other related meat quality traits. Some of the sequence variation identified may be associated with differences in phosphorylation of CAST by PKA and may in turn explain the meat quality phenotypic differences (Cionabu et al., 2004). In addition,* muscle pH is an important factor influencing pork quality, which influences the extent of protein denaturation, thereby meat color and WHC that have significant impact on the purchasing decisions of consumers and processing yield of meat products. According to Heidt and col. (2013), SOX6 codes for a transcription factor that has a high heritability index. The versatility of this gene plays an important role in the specification of slow fiber during skeletal muscle differentiation by inhibiting the transcription of several sarcomeric genes (Hagiwara, 2011, Quiat et al., 2011); in addition it is associated with muscle growth and quality characteristics. Polymorphisms' at porcine SOX6 sequence (Gene ID: 397173) have been related to meat quality traits in commercial breed population (Pi and DuPi) (Zhang et al., 2015). In particular, a G>A substitution at 42812066 and a G>C at 43023574 nucleotide position were described at SSC2 intronic sequence (R. Zhang, personal communication, January 6, 2021) here named SOX6A and SOX6B, respectively. Muscle fiber is a determining factor for meat quality (Klont et al., 1998) and its biochemical and physiological properties derive in its divergent responses with respect to pre-slaughter stress, post-mortem pH decrease and quality of meat (Karlsson et al., 1999).

Considering the link between genetic background and quality attributes as an important step towards management of pork quality, the aim of this study was to revisit SNPs detection of meat quality traits in the context of the genetic variation in commercial and local Creole pig breeds providing information about the segregation of markers involved in determine the meat quality and giving some first insights into the variability genetic of these populations.

# Methods

### Animals and sample collection

A total of 242 unrelated animals including commercial breeds and Creole breeds from commercial and family farms at the North-West of Argentina (Northeast: 54° 54 50.64 S and 57°49 54.02 W Southeast: 32° 28 23.74 S and 58° 15 12.55 W Southwest: 32° 28 05.36 S and 59° 07 39.97 W and Northwest: 30° 52 42.18 S and 59° 03 43.23 W) were included in the present study. A total of 153 were commercial hybrid pigs from 12 different producers (the main of the tested animals are hybrids derived from crossing hybrids females Landrace x Yorkshire and a percentage of Chinese breeds with terminal hybrids males composed by different proportions of Duroc, Pietrain, Hampshire, Yorkshire and Landrance) and 89 were Creole from 10 different pig farms. The term Creole ("Criollo" in Spanish) is used to refer to descendants from the Iberian Peninsula (Elliott, 2007). The Creole pigs population in North-West Argentina, which is supposed to originate in the animals introduced by the Spaniards during the colonization having received since then numerous contributions from other exotic breeds (Revidatti et al., 2014). Hair bulbs samples were collected from the back of pigs, pulling strongly with the thumb, index and middle fingers. The hair bulbs of approximately 50 hairs were removed from each pig. Samples were labeled, transported and stored in plastic bags at room temperature until processed in the laboratory.

### DNA extraction

Genomic DNA was extracted using the cetyl-trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980, Sambrook et al., 2001). Briefly, about 15 bulbs were incubated in TE buffer, 10% SDS and proteinase K (1mg / ml) for 15 minutes at 37 ° C. Then, 5M NaCl and CTAB (0.7M NaCl, 10% CTAB, Genbiotech) were added and incubated at 65 °C for 10 minutes. Subsequently, chloroform: isoamyl alcohol was added in a 24: 1 ratio, and after centrifugation, DNA was precipitated from the aqueous phase with cold isopropanol. Then, washes were carried out with 70% ethanol, pellet allowed to dry at room temperature and resuspended in 15 µl of TE buffer. DNA concentration and purity (A260/A280 ratio) for each sample was assessed using a spectrophotometer. The measured DNA samples were stored at -80 °C until further analysis.

### PCR–RFLP analysis

In the present study seven SNPs of porcine meat quality-related genes were analyzed. A C1843T point mutation in the RYR1 gene (GenBank accession M91451.1) generating a substitution of arginine to a cysteine at position 615 in the aminoacidic sequence (p.Arg615Cys) (Fujii et al., 1991; Bonelli & Schifferli, 2001); two functional mutation at the PRKAG3 gene (GeneBank accession NM_214077.1), where the SNP (c.596G>T) at codon 199 cause an I>V amino acid substitution and the SNP at codon 200 (c.599G>A) a R>Q substitution (Milan et al., 1996; Josell et al, 2003; Lindahl et al., 2004); two SNP in CAST gene (GeneBank accession EU137105), one at p. Ser638Arg, A>C substitution at the nucleotide level (Ciobanu et al., 2004) and CAST G76872A SNP located at the nucleotide position 76872 at the intron 6 of CAST gene (G>A,) (Gandolfi et al., 2011), and two SNP at the transcription factor SOX6, the SOX6A 42812066 G>A (rs81358375) and SOX6B 43023574 G>C (rs321666676) (R. Zhang, personal communication, January 7, 2021). Genotyping of SNPs was done by PCR–RFLP procedure. PCR mix comprised: 1mM dNTPs, forward and reverse primers (10 pmol), nuclease free water, 10X green buffer and GreenTaq polymerase and DNA template in a final volume of 25 µl. The sets of forward and reverse gene-specific oligonucleotide primers were taken from literature and the optimization of appropriate annealing temperature with respect to each primer was determined by gradient PCR. Detailed information about SNPs along with six sets of forward and reverse gene-specific oligonucleotide primers, annealing temperature, restriction enzymes and amplicon size of each primer is given in Table 1. PCR amplification was performed in a conventional ESCO AERIS PCR thermocycler with the following cycling program: initial denaturation at 94 ° C for 5 min; 38 cycles of 94ºC for 30 sec, specific annealing temperature for each pair of primers, for 30 sec. and 72ºC for 30 sec, and a final extension at 72ºC for 7 min. The restriction enzyme (RE) digestion of PCR product was carried out in 0.2 ml tubes with a total reaction mixture of 20 µl by incubation at a temperature and time specified by enzyme manufacturer. The amplified and digested DNA fragments of SNPs were separated on 3% agarose gel with 0.1 µg / ml ethidium bromide visualized with UV transilluminator and photographed. The genotype of the individuals was determined for each polymorphism by analyzing the size of the fragments in RFLP.

### Genetic diversity and population genetic structure analyses

In order to quantify the genetic diversity of each breed allelic and genotype frequencies were calculated. After that, over n>5 animals per producer, allelic richness ($A_R$), minor allele frequency (MAF), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$) and Hardy-Weinberg equilibrium test (HWE) were estimated using GenAlEx software (Peakall and Smouse, 2012). To describe the distribution of genetic variability among different animal breeds and populations a Principal Component Analysis (PCA) was performed using Genalex software (Peakall and Smouse, 2012). To determine the population genetic structure Bayesian simulation procedure was implemented by Structure software (Pritchard et al., 2000). An analysis of molecular variance (AMOVA) was performed attending to different sources of variation: Model I) Between races and within races: Model II) Between races, between populations within races and within populations. Both models included a level within individuals. The level of genetic differentiation was estimated by means of Wright fixation index ($F_{ST}$). Also, inbreeding coefficient ($F_{IS}$) was estimated. AMOVA analysis, $F_{ST}$ and $F_{IS}$ indexes together to its statistical significance *p*-values were estimated by Arlequin software (Excoffier and Lischer, 2010). The statistical significance for the difference between populations of $H_E$, $H_O$, $A_R$ and $F_{IS}$ was evaluated by a pairwise *t*-test using the FSTAT software (Goudet, 1995). Linkage Disequilibrium (LD) was calculated with Arlequin and based on Lewontin and Kojima (1960), Slatkin (1993), and Excoffier and Slatkin (1995); using the EM algorithm with 2 initial condition and 10000 permutations. The differences were considered significant when $P < 0.05$.

## Results

### Polymorphism profiles

The 242 pigs were genotyped for the mentioned SNPs by PCR–RFLP procedure. PCR products of the expected size were obtained for each marker. **Figure 1** shows electrophoresis gel images of PCR–RFLP profile for RYR1, PRKAG3 or RN CAST and, SOX6 A and SOX6B. RYR1 had two genotypes i.e. dominant homozygote (CC) with a fragment size of 92 and 42 bp and heterozygote (Ct) with a fragment size of 134, 92 and 42 bp. Two mutations described at the PRKAG3 locus by Milan et al. (2000) and Ciobanu et al. (2001), namely I199V and R200Q, were analyzed by PCR-RFLP. A fragment size of 114, 81 and 33 bp; and 81 and 33 bp, respectively was observed for heterozygote QR and wild homozygote RR genotype Three genotypes were observed at PRKAG3 c.199I>V SNP locus: a restriction fragments size of 114, 82, and 32 bp was observed for VI, 114 bp for homozygote II. Due to the absence of recombination between these two neighboring codons (Milan et al., 1996), these two mutations yield three alleles for the RN gene: $RN^-$ (199V/200Q), rn+ (199V/200R) and rn* (199I/200R) (Josell et al., 2003; Lindahl et al 2004). Two SNPs at the CAST gene were analyzed, CASTSer638Arg (A>C) had three genotypes i.e. homozygote CC with a fragment size of 142 and 41 bp; heterozygote AC with fragment size of 183, 142 and 41 bp and homozygote AA with a fragment size of 183 bp. At CAST 76872G>A SNP site, two alleles i.e. A and G and all three genotypes i.e. GG, GA and AA were observed in the population. G76872A SNPs obtained restriction fragments were: 129 bp+247 bp for CAST g.76872 G allele; 376 bp (corresponding to the undigested amplicon) for CAST g.76872A allele. Finally, two SNPs for the SOX6 gene were studied SOX6A (42812066G>A) and SOX6B (43023574G>C). Homozygote AA, for SOX6A, show a fragment size of 305 and 97 bp, the heterozygote AG genotype differentiate three fragments 402, 305 and 97 bp whereas the homozygote GG 402 bp. SOX6B had three genotypes i.e. CC, CG and GG in present study showed the fragment size of 217 and 41 bp; 258, 217 and 41 bp; and 258 bp, respectively.

### Allelic and genotypic frequencies

The allelic and genotypic frequencies of the studied markers for hybrids and Creole animals are summarized in Table 2. In both populations, the RYR1 SNP homozygote genotype tt was absent. Remarkably enough, it turns out that the commercial breed population showed a high percentage of Ct individuals (29.87%). Even so, the lower frequency of negative allele 1843T (t) indicates that possibility of PSE meat in both populations (22.41% and 14.945% for Creole and commercial breeds pigs, respectively). For the RN or PRKAG3 gene, three allelic variants were identified: $RN^-$ (199V-200Q), rn+ (199V-200R) and rn* (199I-200R) and the pigs studied showed the following five genotypes: RN-/RN-, RN-/rn+, RN-/rn*, rn+/rn+, rn+/rn* or rn*/rn*. The deleterious allele $RN^-$ was observed at PRKAG3 with 36.77% in local populations and with 24.35% in commercial breeds. Again, such an incidence was not expected in the last one. The $RN^-$ allele show considerable economic significance in the pig breeding industry, and most breeding companies would like to eliminate that mutation because of its negative effects on processing yield. Concerning the two CAST polymorphisms, the heterozygote's GA CAST g.G76872A and SA p.CASTSer638Arg genotypes were the most numerous in both breeds (with 51.72%, 52.87%, respectively for local pigs and 59.09% and 66.88%, respectively for commercial breeds) while less numerous were pigs with AA and SS genotype (about 8% and 6%, respectively). For SOX6 gene, both Creole and hybrids animals showed only the AA and AG genotypes. AG for SOX6A locus with the wild allele A being the most frequent (77.59% and 74.03%). All three genotypes were observed at $SOXB_{43023574G>C}$. SNP locus with 13.78% (GG), 71.26% (GC) and 14.94% (CC) frequency for Creole pigs and 24.03% (GG), 58.44% (GC) and 17.53% (CC) for hybrids animals where the mutant (C) allele was observed mainly in heterozygosis. The $SOX6A_{42812066G>A}$ SNP was associated with pH post mortem and CRA (being AA genotype favorable for fresh meat) and the SOX6B SNP was associated with meat color and pH (Zhang et al., 2015; Rodriguez et al., 2020).

### Genetic diversity parameters and population genetic structure

*Within-breed genetic diversity.* In order to determine the genetic diversity of commercial and Creole populations, standard indices of genetic diversity including average number of alleles per locus (Na), observed and expected heterozygosity ($H_O$ and $H_E$) minor Allele Frequency (MAF) and Hardy-Weinberg equilibrium (HWE) were estimated (Table 3). All SNPs were polymorphic; the number of allele per locus was 2, with the particular exception of RN, which showed 3 alleles ($RN^-$, rn+ and rn*). The MAF was higher than 0.2 for all the SNPs analyzed in Creole animals however was lesser than 0.2 only in RYR1 locus in hybrids animals. The average He was 0.455 for commercial breeds animals and ranged from a maximum value of 0.498 for the $SOX6B_{43023574G>C}$ locus to a minimum value of 0.255 for the RYR1 locus, whereas at the Creole animals the average He was 0.452, ranged from a maximum value of 0.659 for the RN locus to a minimum of 0.342 for RYR1 locus. The total heterozygosity values (Ho) exceeded the average heterozygosity level of 0.5 indicating high genetic diversity in both populations. Almost all loci deviated from HWE ($P < 0.05$) in both populations, ranged

between 0-0.037 in commercial and 0-0.104 in Creole animals, whereas $CAST_{G76872A}$ locus at Creole breeds resulted in equilibrium with $P$>0.05. Significant deviations in HWE in the studied loci correspond to an observed heterozygosity higher than expected heterozygosity. Therefore, both populations showed heterozygous excess and an $F_{IS}$ value of -0.244 for commercial and -0.310 Creole breeds (Table 4). Within-breed genetic diversity was reflected at the allelic richness (AR) observed 2.167 for both commercial and Creole animals. The same richness was observed when the subpopulation of each farm pigs were analyzed (data not showed).

*Between breed genetic differentiation.* The values of $F_{ST}$ and $F_{IS}$ indexes for each locus and for the set of loci for all populations are shown in Table 5. Genetic differentiation between and within breeds were evaluated (Model I). According to the values obtained by Weir & Cockerham estimators, the populations were not separated from a random mating model since the average $F_{IS}$ for all loci was -0.252. Negative $F_{IS}$ values observed in all loci are indicators of an excess of heterozygous although in no case were significant indicating no evidence of *inbreeding trends* between and within breeds. The global $F_{ST}$ value was 0.007 ($P$ = 0.002) suggesting a low genetic differentiation between subpopulations

Model II shows differentiation between, within breeds and between subpopulation of the breeds. $F_{ST}$ highest values were observed for RYR1 and RN locus (0.156 and 0.109 respectively) (Table 5), and the global $F_{ST}$ for this hierarchical model was 0.078 showing again the low genetic differentiation.

**Population genetic structure analysis**

Principal component analysis (PCA) was employed to explore the clustering of individuals of different populations. The first three principal components explained 22.60%, 19.93% and 17.22% of the total variation and the accumulated contributions of these three principal components explained 59.76% of genetic variation. Both populations are completely overlapped, which may suggest their genetic closeness for the analyzed SNPs. PCA was used in cluster analysis based on the genotype of each individual (Figure 3). The contributions of the first two principal components (pc) were 22.6% and 19.93% of the total variation, respectively, and their accumulated contributions, 42.53%. PCA showed no separate groups of genotypes. In addition, a Bayesian analysis using the multilocus genotype data was implemented in STRUCTURE software. The cluster results are shown in Figure 2. The grouping situation when K=2 was analyzed, meaning that it presupposed that all individuals originated from 2 ancestors or breeds. In well accordance with results obtained in the PCA and the neighbor-joining tree, the analyzed subpopulations were heterogeneous for the SNPs studied (Figure 2). Pairwise linkage disequilibrium between SNPs was used to measure the linkage disequilibrium (LD). This study provides an overview of LD patterns between SNPs related to meat quality in different subpopulations of Creole (11) and commercial pigs (13). It is observed that not all pairs of SNPs present statistically significant associations. In Creole pigs, 6 statistically significant associations out of 15 comparisons between pairs of SNPs were observed and in commercial breeds, only 2 out of 15 associations were statistically significant. Between the statistically significant associations, the SNPs $CAST_{G72872A}$ and $CAST_{S638A}$ present greater association in both populations ($P$=0.034). In Creole pigs, SNPs $RYR1_{1843C>T}$ x $CAST_{G72872A}$ has the lowest association ($P$=0.014); while in commercial breeds the lowest association was $CAST_{G76872A}$ x $SOX6A_{42812066G>A}$ (Table 6).

# Discussion

Genetic characterization of pig breeds is essential to preserve their genetic variability, to advance conservation policies and to contribute to their promotion and sustainability. Genetic diversity studies generally focus on candidate genes related with reproduction, lipid, carbohydrate and protein metabolism, growth and development, cellular homeostasis, locomotor behavior and response to nutrient and a list of microsatellite markers recommended by the International Society for Animal Genetics / Food and Agriculture Organization of the United Nations working group (FAO, 2011). However, the aim of the present study was to analyze the genetic diversity of several loci related to porcine meat-quality traits. Seven polymorphisms were considered at four different genes including the so called “Halothane” or RYR1 gene (1843C>T substitution), “Rendement Napole” or RN gene (199I>V and 200R>Q substitutions in PRKAG3), Calpastatin (638S>A and 76872G>A) and SOX6 gene (SOX6A42812066G>A and SOX6B43023574G>C) (Table 2). To carry out this study, two pigs’ populations were considered based on the genetic background of the animals, the Creole or local adapted animals and those including highly selected commercial breeds. Based on PCR-RFLP fragment patterns results, alleles and genotypes frequencies were established. For RYR1 (1843C>T) SNP, genotypic frequencies observed were 55.17% and 70.13% homozygous normal CC, 44.83% and 28.87% of individuals heterozygous (Ct) for Creole and commercial breeds respectively, whereas no homozygous tt were detected in any of the analyzed populations. In four different provinces of Argentina (Córdoba,

Santa Fe, Chaco and Tucumán), Marini et al. reported similar frequency for “t” allele (19.6%) in a hybrid animal’s population derived from crossing hybrid females Landrace x Yorkshire with terminal hybrid males composed by different proportions of Duroc, Pietrain, Hampshire and Yorkshire (Marini et al., 2012). Genotypic frequencies observed were 65.0% homozygous normal (CC), 30.8% heterozygous (Ct) and 4.2% homozygous susceptible (tt). The frequency of allele t found for Creole (22.41%) and commercial breed pigs (14.945%) was noticeably high, even if the possibility to generate PSE meat is considered. A report describing the meat quality of commercial hybrid pigs in Argentina showed some little evidence of PSE on synthetic boar line (Lloveras et al., 2008). The harmful allele (t) of the RYR1 was linked with pork texture, influences the rate of pH fall by favouring calcium release in muscle cells which stimulate ATPase activity (Monin et al., 1999). Similar results for RYR1 SNP frequency were reported for commercial breed pig populations in Brazil, which is considered the biggest Latin America producer country (Bastos et al., 2001, Band et al.; 2005; Silveira et al., 2011). Among European local pig breeds as in most commercial European breeds (Fujii et al., 1991) the c.1843T mutant allele is scarce since many initiatives have been carried out to eliminate this allele (Muñoz et al., 2018). On the other hand, the protein encoded by the PRKAG3 gene is a regulatory subunit of the AMP activated protein kinase (AMPK), which plays a key role in the regulation of glucose and energy metabolism in skeletal muscle. Glycogen content is determinant of multiple meat quality traits, such as pH, meat color, drip loss or tenderness (Ciobanu et al., 2001). The amplified PCR product for PRKAG3 or RN (199I>V and 200R>Q) SNP was 114 bp in length, which was similar to the findings of Josell et al. (2003) and Lindahl et al. (2004). The three functionally alleles have been identified at the RN locus: 199V–200R (wildtype, rn+), 199V–200Q (RN-) and 199I–200R (rn*). The RN– allele was present in 36.77% of the Creole pigs with the RN-/rn* genotype as the most frequent (50.57%). For hybrids, 24.35% had RN– allele and also the RN-/rn* genotype was the most representative (36.36%). RN-/RN- genotype was the least frequent in both populations (2.29 Creole and 0.62% hybrids). Glycolytic potential is largely influenced by the PRKAG3 Q200 allele (RN–; Milan et al., 1996), which increases the glycogen content by about 70% in glycolytic muscles and causes the defect known as acid meat. High frequency of the mutated dominant RN– allele (RN-/RN-, RN-/rn+) in both populations indicated the possibility of acid meat and reflex that the SNP has not been eliminated from breeding populations yet. The PRKAG3 R200Q SNP appears in Hampshire breed or derived synthetic lines and the mutant allele was absent in several European local porcine breeds (Muñoz et al., 2018). On the other hand, the I allele (rn*) is widely reported to have a positive effect on pH, color and water holding capacity in diverse pig breed populations (Ciobanu et al., 2001; Lindahl et al., 2004, Otto et al., 2007). In both populations, the allele rn* is highly represented (37.35% and 42.21%). High frequencies of the rn* allele, were also reported in the bibliography for Iberian pigs (Muñoz et al., 2019) The V199I mutation, also involved in several meat quality traits, had been previously shown to segregate in different breeds, with allele I being less abundant in most breeds selected for muscularity such as Duroc, Landrace and Pietrain (Galve et al., 2013). The allele coding for isoleucine leads to lower glycogen content, being favorable for meat quality (Milan et al., 2000). Mexican pig populations showed similar RN gene frequency in Creole as well as certain commercial breed such as Yorkshire and Hampshire suggesting that no changes have arisen by artificial or natural selection (Carr et al., 2006; González Sarabia et al., 2011). Thus, selection against the two major genes RYR1 (t) and RN (RN-) alleles by genomic selection can potentially reduce the frequencies of the defective genes with high accuracy to enhance pork quality. Also, two calpastatin single nucleotide polymorphisms were genotyped by PCR-RFLP. For Ser638Arg SNPs (A>C), three genotypes were present in both populations, being the heterozygote AC the most frequent (52.87 Creole and 66.88 % Commercial pigs) and the CC under-represented (1.15 and 5.85%). Allele A of CAST p.Ser638Arg was the most frequent, as reported in French (Santé-Lhoutellier et al., 2012), Spanish (Gou et al., 2012) and Italian commercial pigs (Davoli et al., 2017). Based on the published literature, 638Arg/638Arg genotypes were associated with lower firmness desirable for fresh meat (Ciobanu et al., 2004). In studies conducted in Latin America, Mexican Creole pigs showed a lower frequency of the favorable allele A for CAST638S>A than in cuinos and Yorkshire pigs (González Sarabia et al., 2011). Allele frequencies in Creole pigs were 66.09 and 33.91% for CAST g.76872 G and A allele, respectively, and 62.67 and 37.33% for commercial breed populations. In both cases the homozygous genotype AA was the least abundant (8.05 and 7.79%). CAST g.76872 G>A genotype had a suggestive effect on drip loss, with a lower drip loss in pigs carrying AA genotype compared with the GG genotype in Northern Italy commercial pigs (Gandolfi et al., 2010). Based on the published literature, Ser638Arg could affect a PKA recognition consensus sequence. Ser638Arg substitution on phosphorylation of CAST by PKA as a result of changes in the PKA recognition sequence may potentiates the inhibitory activity of CAST (Ma et al., 1994). Therefore, 638Arg/638Arg genotypes were associated with lower firmness desirable for fresh meat (Ciobanu et al., 2004) and CAST g.76872 G>A genotype had suggestive effect on drip loss, with a lower drip loss in pigs carrying AA genotype compared with the GG genotype (Gandolfi et al., 2010). It was also observed that pig SOX6, located at chromosome 2, is embedded in or close to a number of reported QTLs (Lee et al., 2003; Stearns et al., 2005(a); Stearns et al., 2005(b); Harmegnies et al., 2006; Ai et al., 2012; Thomsen et al., 2004; Ruckert and Bennewitz, 2010). Even the available studies about porcine SOX6 are very limited; two SNPs located at intronic sequence have been reported to

be related to growth, carcass, and meat quality traits (Zhang et al., 2015). Allele frequencies for SOX6A42812066G>A, A and G, were 77.59 and 22.41% in Creole population, and 74.93 and 25.97% in commercial breed population, respectively. Genotype frequencies for SOX6A42812066G>A, AA and AG, were 55.17, 44.83% in Creole population, and 74.03 and 25.97 % in commercial breed population, respectively. In both populations, the GG genotype was missing. For the second SNP, SOX6B43023574G>C, allele frequencies G and C, were 49.91 and 50.59 in Creole population. For commercial pig populations they were 53.25 and 46.75%, respectively. Genotype frequencies for SOX6B43023574G>C, GG, GC, and CC, were 13.78, 71.26, and 14.94% in Creole population and 24.03, 58.44 and 17.53% for commercial breed populations, respectively. As for SOX6B43023574G>C, the CC genotype is missed from Pi and DuPi population, authors suggested it could be owing to its lethal mutation or molecular selection by farmers. On the other hand, SOX6A42812066G>A was associated were related to pH, CRA and color in Pietrain and DuPi (Duroc x Pietrain) populations and the Pi pigs carrying genotype AA of SOX6A42812066G>A have high pH and thick backfat. We have previously reported that SOX6A may influence pH and CRA, whereas allele C of SOX6B43023574G>C may be linked just to pH in Halothane free animals. So, the selection of A allele for SOX6A42812066G>A and C for SOX6B43023574G>C could improve the production of good quality fresh meat (Rodriguez et al., 2020)

Pig genetic diversity within populations is variable and consequently had quite variable heterozygosities on different chromosomal regions that may reflect the relatively long time of breeding and selection for the pig (Zhang and Plastow, 2011). When all analyzed loci were considered, the average expected heterozygosity (HE) values were 0.452 and 0.455 for Creole and commercial breeds and the average of observed heterozygosity (HO) were 0.588 and 0.56, respectively, indicating that high levels of genetic diversity exist in the breeds analyzed. Genetic variability at the different loci in each population may suggest a low level of artificial selection. In several reported studies for local Latin American breeds, the genetic diversity analysis is mostly based on microsatellite markers recommended by FAO/ISAG. Although the heterozygosity obtained here mediated by RFLPs analysis is not equivalent, the values resembled those found for other local populations/ breeds such as North East Argentina Creole pigs (MA Revidatti, 2009, Ph.D. thesis, University of Córdoba, Spain), Mexican Hairless pigs (Canul et al., 2005), Cuban Creole pigs (Martínez et al., 2005) Uruguayan pig breed Pampa Rocha (Montenegro et al., 2015) and Brazilian breeds such as Monteiro, Moura, and Piau (Sollero et al.,2009). In a recent study performed by Muñoz et al. (2019), European autochthonous breeds values for HO and HE were 0.297 (±0.053) and 0.303 (±0.054), respectively, values considerably lower than those reported previously for European cosmopolitan and Chinese pig breeds (Laval et al., 2000; Luetkemeier et al., 2010) with an average of about 0.5, but similar to those reported for some European local breeds (Herrero-Medrano et al., 2014). Also, the average expected heterozygosity was above 0.63 for Portugal native breeds and Landrace, ranging between 0.56 and 0.59 for other native breeds, Large With and Pietrain pigs, and below 0.5 in Duroc populations (Vicente et al., 2008). Chinese population had much higher diversity, ranging from 0.700 to 0.876 from 18 Chinese pig breeds (Yang et al., 2003). In contrast to many reports where the HE was much higher than the HO, in the present study the He was lower in both populations. Considering European populations, Muñoz et al. (2018) described the diversity of several polymorphisms on meat production candidate genes in European local pig breeds. HO and He values per locus ranged from 0.024 to 0.414 and from 0.025 to 0.415, respectively, with overall values of 0.240 for both parameters (including RYR11843C>T with a HO: 0.048 HE: 0.053; PRKAG3 199I>V HO: 0.399 HE: 0.388 and CAST76872G>A HO: 0.295 HE: 0.389). In addition, Mexican hairless pigs showed similar average heterozygosities for the RYR11843C>T and CASTG76872A loci but lower for the RN199I>V/200R>Q (González Sarabia et al., 2011), and Mexican commercial Yorkshire breeds showed a He value of 0.49 for RYR11843C>T, 0.49 for RN199I>V/200R>Q and 0.23 for CAST76872 G>A (González Sarabia et al., 2011). These values are higher than those obtained here for RYR11843C>T and lower for RN and CAST76872 G>A loci.

It is generally accepted that a locus is polymorphic when the most common allele has a frequency lower than 0.95. MAF is actually the second most frequent allele value. The average within-breed MAF ranged from 0.15 for RYR1 to 0.46 for SOX6B in commercial breeds and 0.2 for RYR1 to 0.49 for SOX6B in Creole breeds, suggesting that the total of SNPs analyzed is polymorphic. In accordance with the well-known negative effect of the t allele, lowest MAF values corresponded to RYR1 in both populations. Selection against porcine stress syndrome are currently performed in commercial hybrid pig breeds (RYR1 free lines) and farmers as well. Considering the SNPs with two (RYR1, CAST and SOX6) and three loci (RN), within-breed genetic diversity was reflected at the allelic richness (AR) observed as 2.167 for both commercial and Creole animals.

In both populations, SNPs genotypic frequencies do not agree with Hardy-Weinberg expectations for $p<0.01$, except for local pigs-CAST76872G>A loci ($p= 0.104$), which showed reduced heterozygosity. FST is directly related to the variance in allele frequency among populations and, conversely, to the degree of resemblance among individuals within populations (Holsinger and Weir, 2009).

If FST is small, it means that the allele frequencies within each population are similar; even if the variance among populations is the same as the variance within populations, there is no population substructure. Although a low genetic differentiation was found (global FST = 0.078), the deviations for HWE together with the FST values for RYR1 and RN loci differ from zero significantly (0.155 and 0.109) suggesting some genetic differentiation which may derivate from artificial selection effect over these two major genes (Table 5, Model II). The average FST of all loci was 0.077, which means that most of the genetic variation was kept within breeds and only a little of the genetic variation exists between populations. Negative FIS coefficients, which measures the departure of genotype frequencies within populations from Hardy–Weinberg proportions ranging from -0.370 to -0.150, were estimated for commercial and local breeds, suggesting an excess of heterozygous in well accordance with the HE obtained. Therefore, here both populations had excess heterozygous and a negative inbreeding coefficient which may indicate absence of inbreeding for the loci analyzed. This could indicate a tendency to exogamy, crossing between individuals of different breeds, which has an effect on the fitness of the offspring. Negative values indicate random mating among the individuals of the subpopulations but do not necessarily imply lower values of the total inbreeding coefficient which takes into account the accumulated inbreeding along the generations. Domestic animal diversity is the basic material for genetics and breeding studies and it is an important form of insurance which enables responses to as-yet-unknown future challenges. To analyze what has happened to the pig's population history (e.g., breeding history, selection, genetic drift, mutation), linkage disequilibrium (LD) - the nonrandom association of alleles at different loci - for each marker was estimated. This study provides an overview of LD patterns between SNPs related to meat quality in different studied populations. Commercial breed pigs present less LD as they were derived from different and distant genetic lines, in contrast to Creole pigs that are commonly obtained by crossing closer lines that have a small effective population size. LD is mostly observed in certain SNPs on the same chromosome (CASTG76872A x CASTS638A and CASTG76872A x SOX6A42812066G>A)), probably as a product of recombination events. Differences in LD among populations result from the differences in population history and demography. Population genetic factors can produce LD through a variety of processes such as natural selection, strong genetic drift, admixture and new mutations and inbreeding creates LD for the same reason as population subdivision. Because of recent common ancestry inbreeding augments the covariance between alleles at different loci (Montgomery Slatkin, 2008). In the present study, the negative FIS value, the possible absence of inbreeding, the size of the populations, and the contribution of different genetic lines to commercial pigs are in accordance with the observed LD in such population as well as with the small population size and evolutionary history that would reinforce Creole pig´s lower LD values.

Based on the SNPs analyzed, a neighbor-joining tree was constructed to resolve the pigs' phylogenetic relationships. The tree showed that the local and commercial breeds under study were not distinguished from each other. Both breeds plot very close to each other, which may indicate that they share common ancestry. Principal component analysis (PCA) was also carried out to illustrate the population relationships. PCA did not show any visualization of groups formed by individuals belonging to the same breeds, which is in agreement with the neighbor-joining tree results. In addition, when two ancestral populations (K=2) are assumed, it is observed that the analyzed subpopulations are heterogeneous for the SNPs studied. Burgos-Paz and col. data suggest that Creole pigs have undergone a dramatic introgression with international-breed pigs. Modern village pigs in the Americas are the result of many independent colonization and introgression events, maybe including a direct Chinese introgression (Burgos-Paz et al., 2012). It could explain the few differences observed here between both studied populations.

## Conclusions

Pig populations analyzed in this work show a high genetic variability at the level of the meat quality markers $RYR1_{1843C>T}$, $RN_{199I>V/200R>Q}$, $CAST_{76872G>A}$, $CAST_{638S>A}$, $SOX6A_{42812066G>A}$ and $SOX6B_{43023574G>C}$, and slightly pronounced genetic differentiation. Meat quality is important both to consumers and to the meat processing industry. As quality is currently a very important aspect for meat consumers and there is not much information available to producers, these pork quality predictive molecular markers could be useful for genetic selection programs to determine the appropriate market for specific pork products. Consequently, these results highlighted the role of pig genotypes as a source of basic variability potentially affecting processed meat products as well as fresh meat. Therefore, the farm´s meat final destination will define how selection should be made. Phenotypic traits like pH, the shear force of cooked meat, fat thickness, estimated carcass lean meat and, weight losses over dry-cured ham processing are recognized as key factors to determine pork quality cuts intended for fresh consumption and dry-curing. Most genetic selection in commercial swine is conducted over productive characters markers, only few for quality traits, nevertheless the use of marker-assisted selection is especially interesting for meat attributes because the improvement of meat quality using conventional selection methods turns difficult since most traits of interest can only be measured after slaughter and, therefore, only

information on relatives can be used for selection. On the other hand, knowledge of the structure of livestock populations in terms of variability between and within breeds is essential to establish conservation priorities and strategies (Caballero and Toro, 2002), with the long-term objective of maintaining genetic diversity for future generations (Notter, 1999). Therefore, the valuation of their genetic diversity and possible relationships with other breeds represents a great step towards the development of conservation and improvement programs.

## List Of Abbreviations

SNP: single nucleotide polymorphisms

FAO: Food and Agriculture Organization

ISAG: International Society for Animal Genetics

WHC: water holding capacity

IMF: intramuscular fat

HAL: Halotane gene

PSS: Porcine stress syndrome

PSE: pale, soft and exudative meat

RN: Rendement Napole gene

PRKAG3: γ subunit of adenosine monophosphate-activated protein kinase

CAST: Calpastatin gene

QLT: quantitative trait loci

*PKA: adenosine cyclic 3', 5'-monophosphate- dependent protein kinase*

CTAB: cetyl-trimethyl ammonium bromide

TE: Tris-EDTA

PCR*: Polymerase Chain Reaction*

RFLP:  Restriction Fragment Length Polymorphism

$A_R$: allelic richness

MAF: minor allele frequency

$H_O$: observed heterozygosity,

$H_E$: expected heterozygosity

HWE: Hardy-Weinberg equilibrium

PCA: Principal Component Analysis

AMOVA: analysis of molecular variance

$F_{ST}$: Wright fixation index

$F_{IS}$: inbreeding coefficient

AT: Annealing temperature

AS: amplicon size

RE: restriction enzyme

LD: Linkage Disequilibrium

N: sample size

Na: allelic number per locus

# Declarations


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### Author contributions

Conceptualization: M.L., V.R.R, M.E.B and M.V.G.; Data curation: V.R.R. and M.L.; Formal analysis: V.R.R., M.L., R.F., M.E.B and M.V.G.; Funding acquisition: M.L; Investigation: V.R.R., J.I.M., L.A.Z., R.F. and M.L.; Methodology: V.R.R, J.I.M., L.A.Z. and M.L.; Project administration: M.L.; Resources: M.L. and V.R.R; Supervision: M.L.; Writing, review & editing: M.L., V.R.R., M.E.B and M.V.G. All authors read and approved the final manuscript.


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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author or reasonable request.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Conflict of Interest

No conflict of interest declared.

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## Tables

**Table 1.** Details of SNPs

| Gene SNP | Primer sequence (5'–3') | AT (°C) | AS (bp) | RE | PCR–RFLP pattern (bp) | References |
|---|---|---|---|---|---|---|
| RYR1 1843C>T | F:GTGCTGGATGTCCTGTGTTCCCT R:CTGGTGACATAGTTGATGAGGTTTG | 52.0 | 134 | HhaI | 134//92+42 | Marini et al.; 2012 |
| $RN_{200R>Q}$ | F:GGAACGATTCACCCTCAACT R: AGCTCTGCTTCTTGCTGTCC | 52.0 | 114 | MbiI | 114//82+32 | Martínez-Quintana et al.; 2006 |
| $RN_{199I>V}$ | F:GGAACGATTCACCCTCAACT R: AGCTCTGCTTCTTGCTGTCC | 52 | 114 | Hsp91 | 114//81+33 | Martínez-Quintana et al..; 2006 |
| CAST 638S>A | F:CCTTTGTTGTGTTCTCTGAGG R:AAACCTATTTTCAGGGATATGGG | 52.5 | 183 | PvuII | 183//142+41 | Ciobanu et al.; 2004 |
| CAST 76872G>A | F:TTCCCATAGCCCACAAGAAG R:AATGAGCAGCCAACATCAGA | 50.0 | 376 | HinfI | 376//247+129 | Gandolfi et al.:2010 |
| SOX6A 42812066G>A | F:CCAGTCCATCCTTTCCTTGA R:GTTTCCAAAAGGGAATGCAG | 58.0 | 402 | BSMBI | 402//305+91 | Zhang et al.; 2015 |
| SOX6B 43023574G>C | F:CAATGCCATCGTTGAGTCTG R:GTTGTACTGCACATCTTCTCCCTGTTGGATCGTCT | 50.6 | 258 | BSMBI | 258//217+41 | Zhang et al.; 2015 |

Annealing temperature (AT), amplicon size (AS), restriction enzyme (RE), and PCR-RFLP pattern of each primer used.

**Table 2.** Allelic and genotypic frequencies at different SNPs sites in pigs from North-West of Argentina

| | | Commercial breeds (N= 153) | | Creole breeds (N=87) | |
|---|---|---|---|---|---|
| Gene SNP | Genotype | Genotype frequency (%) | Allelic frequency (%) | Genotype frequency (%) | Allelic frequency (%) |
| RYR1 | CC | 70.13 | C= 85.06 | 55.17 | C=77.59 |
| 1843C>T | Ct | 29.87 | t= 14.94 | 44.83 | t=22.41 |
| RN | $RN^-/rn^*$ | 36.36 | $RN^-$=24.35 | 50.57 | $RN^-$=36.77 |
| 199I>V/200R>Q | $rn^+/rn^*$ | 23.38 | rn*=42.21 | 14.94 | rn*=37.35 |
| | $rn^+/rn^+$ | 16.23 | $rn^+$ = 33.44 | 9.19 | $rn^+$=25.86 |
| | $RN^-/rn^+$ | 11.04 | | 18.39 | |
| | rn*/rn* | 12.34 | | 4.59 | |
| | $RN^-/RN^-$ | 0.65 | | 2.29 | |
| CAST | GG | 33.12 | G=62.67 | 40.23 | G=66.09 |
| 76872G>A | GA | 59.09 | A=37.33 | 51.72 | A=33.91 |
| | AA | 7.79 | | 8.05 | |
| CAST | CC | 5.85 | S=39.29 | 1.15 | S=27.59 |
| 638S>A | CA | 66.88 | A=60.71 | 52.87 | A=72.41 |
| | AA | 27.27 | | 45.98 | |
| SOX6A | AA | 48.05 | A=74.03 | 55.17 | A=77.59 |
| 42812066G>A | AG | 51.95 | G=25.97 | 44.83 | G=22.41 |
| SOX6B | GG | 24.03 | G=53.25 | 13.78 | G=49.41 |
| 43023574G>C | GC | 58.44 | C=46.75 | 71.26 | C=50.59 |
| | CC | 17.53 | | 14.94 | |

**Table 3**: **Genetic diversity**.

| Gene SNP | Commercial breeds (N=153) | | | | | | Creole breeds (N=89) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Na | MAF | $H_O$ | $H_E$ | $A_R$ | HWE | Na | MAF | $H_O$ | $H_E$ | $A_R$ | HWE |
| RYR1 1843C>T | 2.000 | 0.150 | 0.301 | 0.255 | 2 | 0.029 | 2.000 | 0.219 | 0.438 | 0.342 | 2 | 0.008 |
| RN 199I>V /200R>Q | 3.000 | 0.242 | 0.706 | 0.650 | 3 | 0.000 | 3.000 | 0.264 | 0.843 | 0.659 | 2 | 0.000 |
| CAST 76872G>A | 2.000 | 0.373 | 0.588 | 0.468 | 2 | 0.001 | 2.000 | 0.343 | 0.528 | 0.451 | 2 | 0.104 |
| CAST 638S>A | 2.000 | 0.392 | 0.667 | 0.477 | 2 | 0.000 | 2.000 | 0.281 | 0.539 | 0.404 | 2 | 0.002 |
| SOX6A 42812066G>A | 2.000 | 0.258 | 0.516 | 0.383 | 2 | 0.000 | 2.000 | 0.230 | 0.461 | 0.355 | 2 | 0.005 |
| SOX6B 43023574G>C | 2.000 | 0.467 | 0.582 | 0.498 | 2 | 0.037 | 2.000 | 0.494 | 0.719 | 0.500 | 2 | 0.000 |
| Averange | 2.167 | 0.313 | 0.560 | 0.455 | 2.167 | | 2.167 | 0.305 | 0.588 | 0.452 | 2.167 | |

N=Sample size, Na= allelic number per locus, MAF= minor allele frequency, $H_O$= observed heterozygosity, $H_E$= expected heterozygosity, $A_R$= allelic richness, and HWE= Hardy-Weinberg equilibrium.

**Table 4:** Genetic variability between breeds.

| Parameter | Commercial breeds | Creole breeds | P* |
|---|---|---|---|
| $H_O$ | 0.559 | 0.584 | 1.000 |
| $H_E$ | 0.449 | 0.446 | 0.674 |
| $A_R$ | 2.167 | 2.167 | 0.160 |
| $F_{IS}$ | -0.244 | -0.310 | 0.663 |

$H_O$= Observed Heterozygosity, $H_E$= Expected Heterozygosity, $A_R$= Allelic Richness and $F_{IS}$= inbreeding coefficient.

**Table 5:** Genetic differentiation: $F_{IS}$ and $F_{ST}$ values.

| | Model I | | Model II | |
|---|---|---|---|---|
| **Locus** | $F_{ST}$ (*P*) | $F_{IS}$ (*P*) | $F_{ST}$ (*P*) | $F_{IS}$ (*P*) |
| $RYR1_{1843C>T}$ | 0.012(0.036) | -0.218 (1) | 0.155(0.000) | 0 |
| $RN_{199I>V\ /200R>Q}$ | 0.014 (0.006) | -0.153 (0.999) | 0.109(0.000) | 0 |
| $CAST_{76872G>A}$ | 0 | -0.223 (1) | 0.023(0.002) | 0 |
| $CAST_{638S>A}$ | 0.022 (0.002) | -0.374 (1) | 0.049(0.000) | 0 |
| $SOX6A_{42812066G>A}$ | 0 | -0.327 (1) | 0.029(0.000) | 0 |
| $SOX6B_{43023574G>C}$ | 0 | -0.264 (1) | 0.108(0.000) | 0 |
| **Global** | 0.007 (0.002) | -0.252 (1) | 0.078(0.000) | 0 |

**Table 6:** Pairwise LD of SNP loci.

| SNP Pair | Commercial breeds | | Creole breeds | |
|---|---|---|---|---|
| | LD (*P*) | $X^2$ | LD (*P*) | $X^2$ (*P*) |
| $RYR1_{1843C>T}$ x $RN_{199I>V/200R>Q}$ | 0.534 | 1.340 | 0.010 | 9.962 (**) |
| $RYR1_{1843C>T}$ x $CAST_{76872G>A}$ | 0.057 | 3.914 | 0.014 | 6.447 (*) |
| $RN_{199I>V/200R>Q}$ x $CAST_{76872G>A}$ | 0.650 | 0.915 | 0.074 | 5.339 |
| $RYR1_{1843C>T}$ x $CAST_{638S>A}$ | 0.055 | 4.065 | 0.009 | 5.339 (**) |
| $RN_{199I>V/200R>Q}$ x $CAST_{638S>A}$ | 0.394 | 1.952 | 0.191 | 3.525 |
| $CAST_{76872G>A}$x $CAST_{638S>A}$ | 0.000 | 23.254 (***) | 0.003 | 8.896 (**) |
| $RYR1_{1843C>T}$ x $SOX6A_{42812066G>A}$ | 0.619 | 0.248 | 0.153 | 2.279 |
| $RN_{199I>V/200R>Q}$ x $SOX6A_{42812066G>A}$ | 0.489 | 1.461 | 0.010 | 9.979 (**) |
| $CAST_{76872G>A}$ x $SOX6A_{42812066G>A}$ | 0.055 | 3.914 | 0.468 | 0.579 |
| $CAST_{638S>A}$ x $SOX6A_{42812066G>A}$ | 0.002 | 9.500 (**) | 0.365 | 0.875 |
| $RYR1_{1843C>T}$ x $SOX6B_{43023574G>C}$ | 0.119 | 2.583 | 0.005 | 0.010 (**) |
| $RN_{199I>V/200R>Q}$ x $SOX6B_{43023574G>C}$ | 0.243 | 2.923 | 0.130 | 4.380 |
| $CAST_{76872G>A}$ x $SOX6B_{43023574G>C}$ | 0.41931 | 0.67740 | 0.285 | 1.244 |
| $CAST_{638S>A}$ x $SOX6B_{43023574G>C}$ | 0.06683 | 3.49142 | 0.400 | 0.786 |
| $SOX6A_{42812066G>A}$ x $SOX6B_{43023574G>C}$ | 0.91356 | 0.00216 | 0.504 | 0.503 |

LD= linkage disequilibrium. LD significance level ($X^2$ and p value) between each pair of SNPs under study. Significance level (*) *P*<0.05; (**) *P*<0.01 and, (***) *P*<0.001.

# Figures

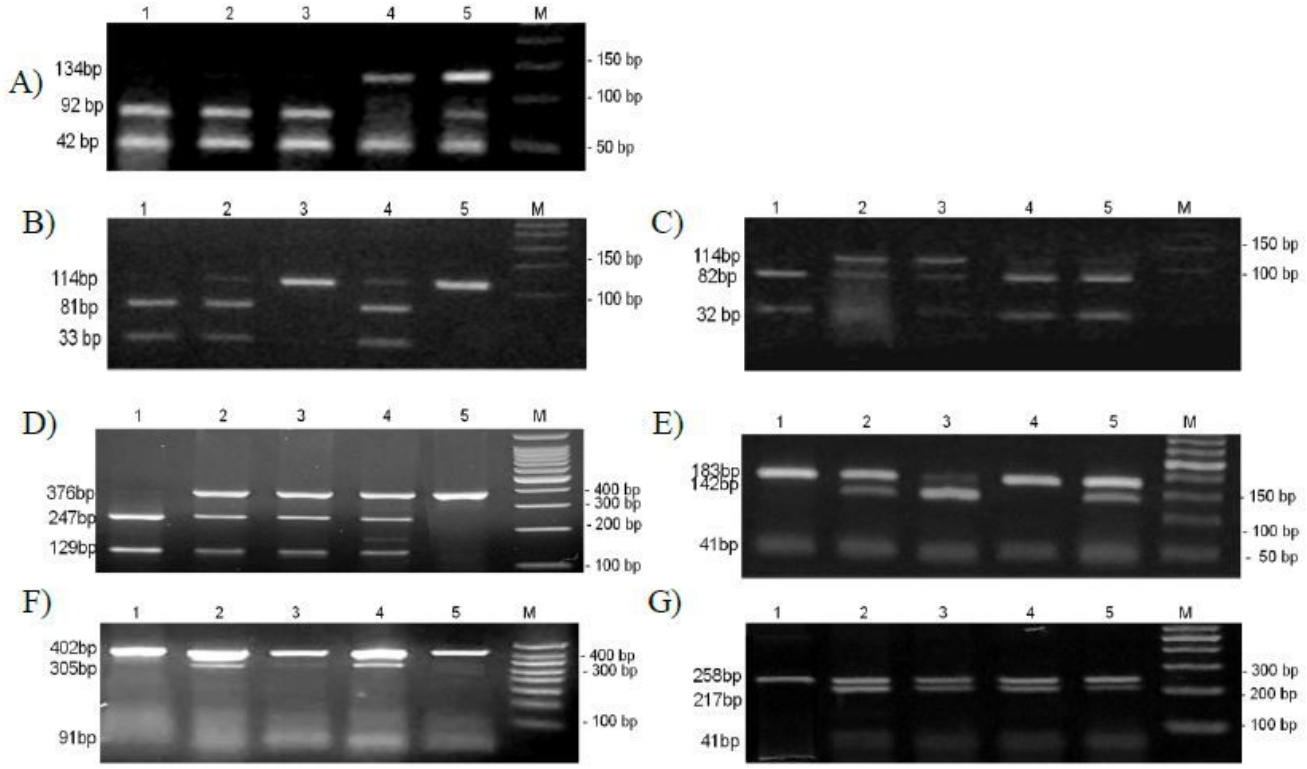
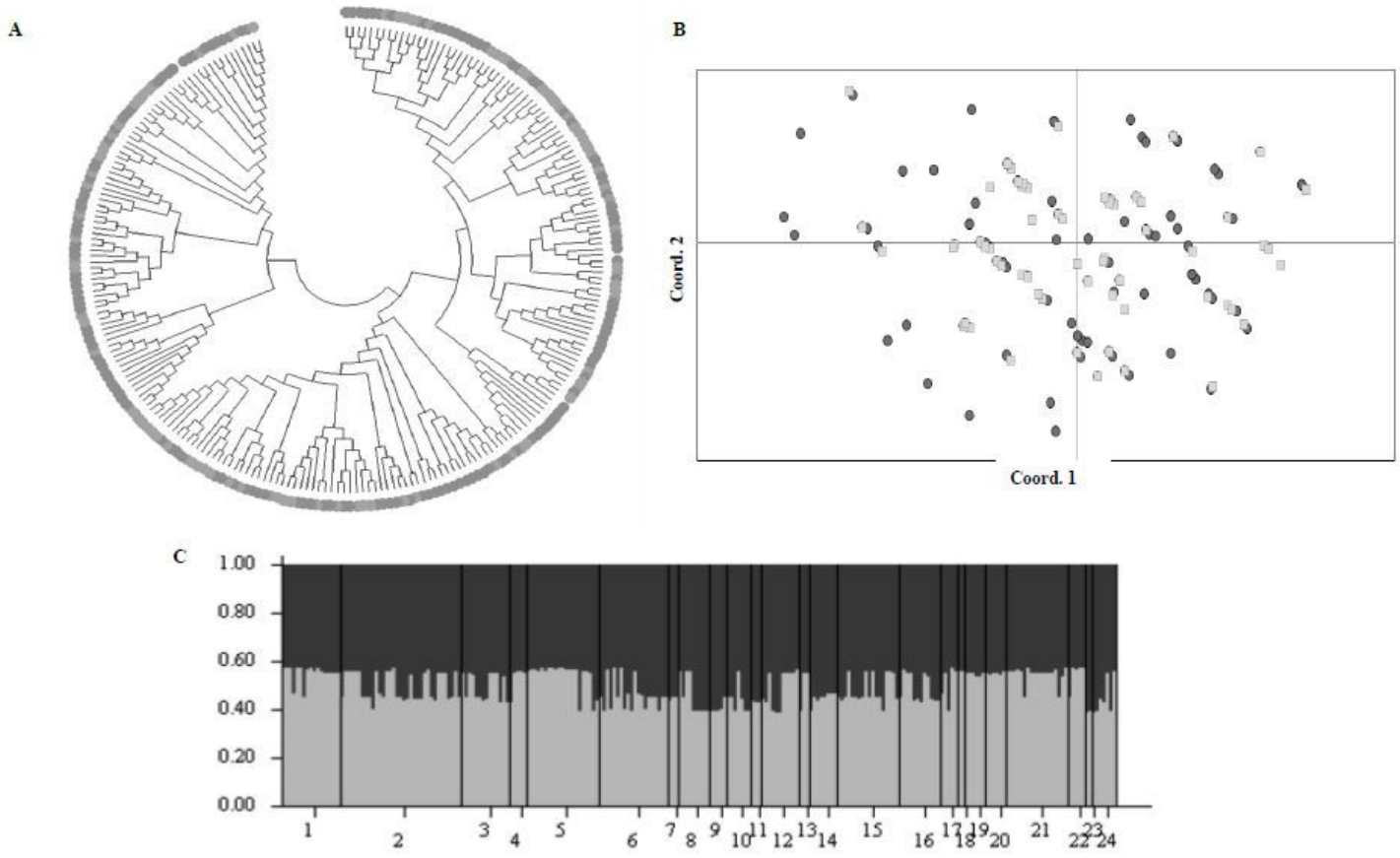



**Figure 1**

SNPs PCR-RFLP profile on 3% agarose gel. A) RYR11843C>T SNP by using HhaI. Lane M: 50bp DNA Ladder (Genbiotech, Cat# B041-50). Lanes 1-3: CC genotypes. Lanes 4, 5: Ct Genotype B) PCR-RFLP profile of PRK3G RN199I>V SNP by using Hsp91. Lane M: 50 bp DNA ladder (Genbiotech, Cat #L00607). Lane 1: VV genotype. Lanes 2, 4: VI genotype and, lanes 3, 5: II genotype. C) PCR-RFLP profile of PRK3G RN200R>Q SNP by using MbiI. Lane M: 100 bp DNA ladder (Genbiotech, Cat #L00607). Lanes 1, 4, 5: RR genotype. Lanes 2,3: RQ Genotype D) PCR-RFLP profile of CAST638S>A SNP by using PvuII. Lane M: 50bp DNA Step Ladder (Genbiotech, Cat# B041-50). Lane 3: SS genotype. Lanes 2, 5: SA Genotype. Lanes 1, 4: AA genotype. E) PCR-RFLP profile of CAST76872G>A SNP by using HinfI. Lane M: 100 bp DNA ladder (Promega). Lane 1: GG genotype. Lanes 2-4: GA genotype. Lane 4: AA genotype. F) PCR-RFLP profile of SOX6A42812066G>A SNP by using BSMBI. Lane M: 50 bp DNA ladder (Genbiotech, Cat #L00607). Lanes 2, 4: AG genotype. Lanes 1, 3, 5: AA genotype. G) PCR-RFLP profile of SOX6B43023574G>C SNP by using BSMBI. Lane M: 50 bp DNA ladder (Genbiotech, Cat #L00607). Lane 1: CC genotype. Lanes 2-5: GC Genotype




## Figure 2

Population structure analyses for all pig individuals. (A) Neighbor-joining tree for all individual pigs. (B) First and second principal components from a principal component analysis of all populations. The contributions of the first two principal components (Coord.) were 44.19% and 22.6%, (●) represents commercial breeds and (▯) Creole pigs (C) Population structure plots for each genotype is shown at K = 2. Each number corresponds to one pig farm and each bar represents an individual animal. Grey color represents Creole pigs and black color the Commercial breed populations