Allelic frequencies of genes associated with productive traits in western Mexican boars

Frecuencias alélicas de genes asociados a rasgos productivos en cerdos sementales del occidente de México

Frequências alélicas de genes associados a características produtivas em cachaços do oeste Mexicano

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Abstract

The *ESR1, PRLR*, and *RYR1* genes have previously been associated with traits of productive interest. The objective of this study was to determine the allelic frequencies of genes associated with productive traits in boars from pig farms in western Mexico. A total of 140 boars of six breeds, Duroc, Hampshire, Landrace, Piétrain, and Yorkshire, and Yorkshire/Landrace crosses were sampled. The pigs were genotyped via polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. The two alleles of the *ESR1* gene were identified in the six breeds, but only BB homozygotes were recognized in Yorkshire pigs (0.2) and their crosses (0.05). The A and B alleles of the *PRLR* gene were distinguished in all the breeds studied, recognizing a considerable variability in the allele frequencies. Due to the allelic diversity and its effects evidenced in previous publications, it is suggested to evaluate the association of each genotype with the reproductive parameters to be improved in order to determine which genotype is more relevant in each population. In the *RYR1* gene, the mutant allele causing PSS was found in all the breeds studied, which can generate pigs with PSE meat. It is recommended that the selection of boars of any breed to be used as breeders includes a genotyping test. Knowing the genotypes in boars can be used as a way to select better breeders.

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Resumen

Los genes *ESR1, PRLR y RYR1* han sido previamente asociados con rasgos de interés productivo. El objetivo de este estudio fue determinar las frecuencias alélicas de genes asociados a rasgos productivos en sementales de granjas porcícolas del Occidente de México. Se muestrearon 140 sementales de seis razas: Duroc, Hampshire, Landrace, Piétrain, Yorkshire y cruces (Yorkshire/Landrace). Se genotipificó mediante las técnicas de reacción de cadena de polimerasa (PCR) y polimorfismos de longitud de fragmentos de restricción (RFLP). Se identificaron los dos alelos del gen *ESR1* en las seis razas, pero sólo se genotipificaron homocigotos BB en cerdos Yorkshire (0,2) y sus cruces (0,05). Los alelos A y B del gen *PRLR* se identificaron en todas las razas estudiadas reconociendo una considerable variabilidad en las frecuencias alélicas. En razón a la diversidad alélica y en sus efectos evidenciados en publicaciones previas se sugiere evaluar la asociación de cada genotipo con los parámetros reproductivos que se desean mejorar para determinar que genotipo es más relevante en cada población. En el gen *RYR1* se encontró el alelo mutante causante del PSS en todas las razas estudiadas, lo que puede generar cerdos con carne PSE. Se recomienda que la selección de sementales de cualquier raza que se utilizarán como reproductores incluya un test de genotipificación. El conocer los genotipos en cerdos sementales puede ser utilizado como una vía para la selección de mejores reproductores.

Palabras clave: genotipificación, *Sus scrofa domestica*, genes mayores

Resumo

Os genes *ESR1*, *PRLR* e *RYR1* foram previamente associados a características de interesse produtivo. O objetivo deste estudo foi determinar as frequências alélicas de genes associados a características produtivas em cachaços de granjas de suínos no oeste do México. Um total de 140 cachaços de seis raças, Duroc, Hampshire, Landrace, Piétrain e Yorkshire, e cruzamentos Yorkshire/Landrace foram amostrados. Os porcos foram genotipados por meio de técnicas de reação em cadeia da polimerase (PCR) e polimorfismo de comprimento de fragmento de restrição (RFLP). Os dois alelos do gene *ESR1* foram identificados nas seis raças, mas apenas homozigotos BB foram reconhecidos em porcos Yorkshire (0,2) e seus cruzamentos (0,05). Os alelos A e B do gene *PRLR* foram distinguidos em todas as raças estudadas, reconhecendo uma variabilidade considerável nas frequências alélicas. Devido à diversidade alélica e seus efeitos evidenciados em publicações anteriores, sugere-se avaliar a associação de cada genótipo com os parâmetros reprodutivos a serem melhorados, a fim de determinar qual genótipo é mais relevante em cada população. No gene *RYR1*, o alelo mutante causador da PSS foi encontrado em todas as raças estudadas, o que pode gerar suínos com carne PSE. Recomenda-se que a seleção de cachaços de qualquer raça para serem utilizados como reprodutores inclua um teste de genotipagem. Conhecer os genótipos em cachaços pode ser usado como forma de selecionar melhores reprodutores.

Palavras-chave: genotipagem, *Sus scrofa domesticus*, genes principais.

Introduction

The swine industry is very important at the global level since pork represents the second-most consumed source of animal protein in the world (Lebret and Čandek-Potokar, 2022). The development of molecular genotyping techniques in pigs has accelerated genetic progress and increased the precision of selection for productive traits that are difficult to improve, such as fertility, weight gain, meat quality, and disease resistance (Yin *et al*., 2024). Identifying the polymorphisms associated with these desirable traits in different breeds of pigs is essential for improving productive performance in production systems (Tan *et al*., 2017). Some candidate genes have been reported for their association with characteristics of productive interest. Estrogen receptor 1 gene (*ESR1)* is involved in the activation of the estrogen response in different tissues, including the ovaries, uterus, pituitary gland, and mammary gland, which modulate processes such as ovulation, pregnancy establishment, and sexual receptivity (Muñoz *et al*., 2007). The *ESR1* gene is located on chromosome 1 of the pig genome, for which the *PvuII* polymorphism with alleles A and B has been described (Rothschild *et al*., 1991). Alleles A and B of this polymorphism have been associated with an increase in the number of piglets born alive, revealing variability in the different breeds of pigs studied (Drogemuller *et al*., 2001; Goliášová and Wolf, 2004). Another gene of interest encodes the prolactin receptor (*PRLR)*, which is activated by the binding of prolactin and has important functions in the formation of the corpus luteum, progesterone synthesis, estrous cycle regulation, and milk production (Sabev, 2019), which implies the importance of this gene in the reproductive performance of sows. The *PRLR* gene is located on chromosome 16 in region 16q2.2-2.3, and alleles A and B have been described (Vincent *et al*., 1997). A positive effect of the AA genotype has been shown, with an increase in the number of live-born piglets (Rothschild *et al*. 1996; Kmieć *et al*., 2001). The gene encoding ryanodine receptor 1 (*RYR1*), also known as the halothane gene (hal) or porcine stress gene, plays a major role in the transport of Ca^{2+} in muscle cells (Luerce *et al*., 2009). A transversion mutation (C/T) at position 1,843 on chromosome 6 causes a change from an arginine to a cysteine (Fujii *et al*., 1991). The heterozygous (Nn) and homozygous mutant (nn) genotypes are susceptible to porcine stress syndrome (PSS) or malignant hyperthermia, which leads to the postmortem manifestation of pale, soft, and exudative meat (PSE) (Houde *et al*., 1993). Genetic studies of boars in Mexico are scarce because of multiple factors, such as restrictions due to the biosecurity of pig farms, the rapid rotation of animals, and the low importance given to analyzing gene tests. Genotyping by means of PCR-RFLP offers the opportunity to identify genetic aptitudes in boars to choose those that possess improvement-related polymorphisms for breeding (Hernández-López *et al*., 2006). Therefore, the objective of this study was to determine the allelic frequencies of genes *ESR1*, *PRLR* y *RYR1* in boars from pig farms in western Mexico.

Materials and methods

Ethical statement

The study was carried out in accordance with the internal regulations of bioethics of the University Center for Biological and Agricultural Sciences, University of Guadalajara, Mexico No. CC/CN 11-12/001/2012.

Collection of samples

The execution of the laboratory tests was carried out at the Institute of Animal Biotechnology of the University of Guadalajara, Camino Ramón Padilla Sánchez # 2100, Las Agujas, Zapopan, Jalisco, Mexico. For this study, 140 samples of boars were taken from different pig farms located in the western region of Mexico, which is composed of temperate, mountainous, and humid tropic agroecological zones. Among these pigs, 19 were of the Duroc breed, eight were Hampshire, 13 were Landrace, 21 were Piétrain, 15 were Yorkshire, and 64 were Yorkshire/Landrace crosses.

DNA extraction

Approximately 3 mL of peripheral blood was collected from each animal in a vacutainer® tube with ethylenediaminetetraacetic acid (EDTA) via puncture of the external jugular vein according to NOM-060-SAG/ZOO-2020 (Secretaría de Agricultura y Desarrollo Rural, 2020). DNA extraction was performed via the Quick-DNA™ Universal Kit (Zymo Research, USA).

PCR-RFLP

To perform DNA amplification, we used Thermo Scientific™ PCR kits (Takara Bio Inc., Japan) with a 20 µL reaction mixture containing ~100 ng of blood lysate, 0.5 µL of DreamTaq® DNA Polymerase, 2 µL of 1x PCR buffer with 20 mM $MgCl_2$, 1 µL of 10 mM dNTP mixture, and 5 pmol of both primers, with the remaining volume consisting of double distilled water (ddH_2O). Amplification of the DNA fragments was carried out in a Techne® TC-5000 Thermal Cycler (Techne Inc., USA) via the following PCR program: initial denaturation for 5 min at 95 °C, followed by 35 cycles of 94 °C for 30 s, annealing for 30 s at the temperature listed for each gene in Table 1, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min as described by Ayala-Valdovinos *et al*. (2017). For PCR-RFLP, the primers and enzymes shown in table 1 were used to amplify the regions of interest in *ESR1, PRLR*, and *RYR1.* The amplified products were analyzed via 4% agarose gel electrophoresis, stained with Gel Red (Biotium, Hayward, USA), and photographed under ultraviolet light.

Estimation of genotype and allele frequencies for each gene

To estimate genotype and allele frequencies, we applied the direct counting method (Baltian *et al*., 2011).

Results and discussion

In the present study, the 140 boars included in the research were genotyped using the PCR-RFLP technique for the three polymorphisms evaluated. The results indicated that the pig breeds in the western region of Mexico presented different genotype and allelic frequencies for each gene studied, as shown in table 2**.**

ESR1

The B allele is associated with a higher number of piglets per litter (Drogemuller *et al*., 2001). Boars with the homozygous genotype BB were found only in the Yorkshire breed and in Yorkshire/Landrace hybrid pigs. The allelic frequency of Yorkshire boars found in our study was A: 0.63 and B: 0.36, similar to that reported by Lemus-Flores *et al*. (2009), who reported frequencies of A: 0.62 and B: 0.38; despite the higher frequency of the A allele, the groups with a higher presence of the B allele were the maternal lines (Lemus-Flores *et al*., 2009). In the Yorkshire/Landrace hybrid pigs in our study, the A: 0.82 and B: 0.18 allele frequencies were similar to those reported by Rempel *et al*. (2010) and Duifhuis-Rivera *et al*. (2019) of A: 0.79 and 0.62 and B: 0.21 and 0.37 in Yorkshire/Landrace/Duroc and Yorkshire/Landrace crosses, respectively. The B allele has been reported more frequently in Chinese breeds (Wu *et al*., 2006), so it is speculated that this allele could have been introduced in some European breeds through crosses with pigs of Chinese breeds (Rothschild *et al*., 1996).

The Landrace pigs included in the present study presented frequencies of A: 0.88 and B: 0.11. These results coincide with those reported by Kmieć *et al*. (2002), Wu *et al*. (2006) and Kapelański *et al*. (2013), who reported frequencies of 0.94, 0.87, and 0.93 for allele A and 0.06, 0.13, and 0.07 for allele B, respectively. The low presence of the B allele in Landrace in this study as in other previous studies, could be explained because the A allele has been associated to improve some reproductive performance traits of boars (Terman *et al*., 2006), which by the selection of these traits, could consequently decrease the frequency of the B allele. The allelic frequencies for Piétrain pigs found in our study (A: 0.81 and B: 0.19) are similar to those reported by Gunawan *et al*. (2011) and Hunyadi-Bagi *et al*. (2016); the frequencies were equal in both studies, with values of 0.90 and 0.10 for alleles A and B, respectively.

Table 1. The primers used for the genotyping of *ESR1*, *PRLR* and *RYR1*.

Gen	Primers (5´-3´)	Tm (°C)	PCR product (bp)	RE	Reference
ESR1	F- GACAGCTTCCCTGCAGATTC R- TTCATCATGCCCACTTCGTA	55 °C	BB: 55, 65 AB: 55, 65, 120 AA: 120	*PvuII*	Drogemuller *et al*. (2001)
PRLR	F-CGTGGCTCCGTTTGAAGAACC R-CTGAAAGGAGTGCATAAAGCC	57 °C	BB: 104, 59 AB: 104, 85, 59, 19 AA: 85, 59, 19	*AluI*	Drogemuller *et al*. (2001)
RYR1	F-CCACACCCTCCCCGCAAGTGC R-GCCAGGGAGCAAGTTCTCAGTAAT	58 °C	NN: 95, 49 Nn: 144, 95, 49 nn: 144	*HhaI*	Luerce *et al*. (2009)

F = forward, R = reverse, RE = restriction enzyme, Tm = annealing temperature

Table 2. Genotype and allelic frequencies of the ESR1, PRLR, and RYR1 genes.

Gene	Breed	No	Genotypic frequency (No)			Allelic F.	
			AA	AB	BB	A	B
ESR1	Duroc	19	0.95 (18)	0.05 (1)	0 (0)	0.97	0.03
	Hampshire	8	0.88 (7)	0.13 (1)	0 (0)	0.95	0.07
	Landrace	13	0.77 (10)	0.23 (3)	0 (0)	0.88	0.11
	Piétrain	21	0.62 (13)	0.38 (8)	0 (0)	0.81	0.19
	Yorkshire	15	0.47 (7)	0.33 (5)	0.2 (3)	0.63	0.36
	Crosses	64	0.69 (44)	0.27 (17)	0.05 (3)	0.82	0.18
Gene	**Breed**	**No**	**AA**	**AB**	**BB**	**A**	**B**
PRLR	Duroc	19	0.47 (9)	0.47 (9)	0.05 (1)	0.70	0.29
	Hampshire	8	0.38 (3)	0.38 (3)	0.25 (2)	0.57	0.44
	Landrace	13	0.23 (3)	0.62 (8)	0.15 (2)	0.54	0.46
	Piétrain	21	0.57 (12)	0.33 (7)	0.1 (2)	0.74	0.27
	Yorkshire	15	0 (0)	0.47 (7)	0.53 (8)	0.24	0.77
	Crosses	64	0.22 (14)	0.53 (34)	0.25 (16)	0.49	0.52
Gene	**Breed**	**No**	**NN**	**Nn**	**nn**	**N**	**n**
RYR1	Duroc	19	0.89 (17)	0.11 (2)	0 (0)	0.95	0.06
	Hampshire	8	0.62 (5)	0.38 (3)	0 (0)	0.81	0.19
	Landrace	13	0.92 (12)	0.08 (1)	0 (0)	0.96	0.04
	Piétrain	21	0.29 (6)	0.52 (11)	0.19 (4)	0.55	0.45
	Yorkshire	15	0.93 (14)	0.07 (1)	0 (0)	0.97	0.03
	Crosses	64	0.73 (47)	0.22 (14)	0.05 (3)	0.84	0.16

No = Number of animals. F = Frequency

The allelic frequencies of the Duroc pigs in our study (A: 0.97 and B: 0.03) differ from those reported by Short *et al*. (1997) and Hunyadi-Bagi *et al*. (2016), where no pigs carrying the B allele were found. In terminal line boars, the B allele was found to have low frequencies, this could be due to a selection directed at the reproductive traits of the boar, because the ESR1 gene is important in the initiation and maintenance of spermatogenesis. Terman *et al*., (2006) obtained similar frequencies in a population of Duroc x Pietrain boars A: 0.84 and B: 0.16, where the A allele is more common, since it is associated with ejaculates of greater volume, motility and concentration.

PRLR

The three genotypes for the *PRLR* gene were identified in the different breeds studied, with the exception of the homozygous AA genotype, which was not observed in Yorkshire pigs. In the case of the Duroc, Hampshire, Landrace and Piétrain breeds, the A allele presented the highest allele frequency, with values of 0.70, 0.57, 0.54, and 0.74, respectively. The highest frequency of allele A found is in line with the results reported by Vincent *et al*. (1998), in Duroc (A: 0.79) and Landrace pigs (A: 0.72); Drogemuller *et al*. (2001), in Duroc pigs (A: 0.82); Kmieć and Terman (2006), in Piétrain (A: 0.72), Duroc/Piétrain hybrid (A: 0.65), and Hampshire/Piétrain hybrid (A: 0.83) pigs. In contrast to the previous data, in the Yorkshire and hybrid pigs in our study, a higher frequency of the B allele was observed, with values of 0.77 and 0.52, respectively. These results agree with those reported by Vincent *et al*. (1998) in Yorkshire pigs (B: 0.63); Menčik *et al*. (2015) in Landrace/Large White hybrid pigs (B: 0.73). The AA genotype is associated with an increase in the number of piglets born and live-born piglets per litter (Vincent *et al*., 1998; Kmieć *et al*., 2001; Alonso *et al*., 2003; Epishko *et al*., 2009). In contrast, in different populations of pigs with the BB genotype, an increase in the number of live-born piglets has been reported (Mihailov *et al*., 2014; Menčik *et al*., 2015). The Yorkshire is a prolific breed, where it could be suggested that the frequency of the AA genotype would be higher, but there are studies where the BB genotype was associated with the best reproductive traits in pigs. The participation of the *PRLR* gene in a selection process as a marker should preferably be accompanied by the study of the relationship of the genotypes with the traits of the pigs that are desired to be improved, to determine which allele has an improving effect in each population (Mihailov et al., 2014). Inconsistencies have been described in other genes associated with reproductive traits between which variant is improving or not, and this does not detract from the effect of the different genotype in each population; the variations can be attributed to environmental, genetic, sample size and age differences (Rempel *et al*., 2010). In boars with the heterozygous genotype AB, an increase in the volume, concentration, and number of live spermatozoa per ejaculate was reported (Kmieć and Terman, 2006). Due to the variability in allele frequencies and the different effects described for the *PRLR* genotypes, it is suggested to identify which of the genotypes has the best effect on the reproductive traits of each population being investigated (Sabev, 2019).

RYR1

In many countries, genotyping for the *RYR1* gene mutation is mandatory. There is evidence that the *RYR1* sensitivity allele (n) has a great influence on the pH fall observed in PSE (pale, soft and exudative) meats. In pigs PSE meat is a major quality defect associated with abnormal post-mortem muscle acidification, usually

occurs in pigs that are genetically sensitive to stress when subjected to acute pre-slaughter stressors immediately prior to slaughter (Guàrdia *et al*., 2004). All boars with PSS must be eliminated from breeding schemes, as a result worldwide, selection against the *RYR1* gene mutation has decreased its frequency in some breeds (Kamiński *et al*., 2002). The presence of the mutant allele n of PSS was identified in the different breeds included in the present study. The frequencies of this allele found in the Duroc (n: 0.06), Landrace (n: 0.04), Yorkshire (n: 0.03), and Hampshire (n: 0.19) breeds in our study are similar to those reported by Fujii *et al*. (1991) in Yorkshire pigs (n: 0.08); those reported by Houde *et al*. (1993) in Duroc (n: 0.03), Landrace (n: 0.15), and Yorkshire (n: 0.1) pigs, and those reported by O'Brien (1993) in Duroc (n: 0.07), Landrace (n: 0.18), Yorkshire (n: 0.09), and Hampshire (n: 0.07) pigs. The boars of the Piétrain breed in our study presented the highest frequency of the mutant allele (n: 0.45). Among the boars of this breed, four pigs were found to be homozygous n/n. These results are consistent with those reported by O'Brien *et al*. (1993), who reported a frequency of 0.70 in Piétrain pigs from the USA. Reports of the frequency of this mutation in Mexico are scarce; Riojas-Valdés *et al*. (2005) and Davalos-Aranda *et al*. (2010) reported frequencies of n: 0.29 and 0.13, respectively, in hybrid pigs from different farms in northern Mexico. The high frequency of the allele found in the Piétrain pigs in our study may be due to the origin of the mutation, which was detected for the first time in Piétrain pigs, since breeds with outstanding characteristics tend to have a higher incidence of carriers via a greater demand for the production of lean meat without considering its quality (Monin *et al*., 1981). The presence of the mutant allele in the other breeds in our study, even those classified as maternal lines, is possible because it is known from genotypic analysis that the mutation arose from a single founder animal and has been previously identified in breeds such as: Landrace, Yorkshire, Duroc, Poland China (Fujii *et al*., 1991). The results of this study confirm that all breeds of boars have the potential to carry the mutation causing PSS and generate pigs with PSE meat, so genotyping could be a useful test before introducing any boar or its genetics to a new population.

Conclusions

In the present study, the polymorphisms of three genes associated with traits of economic importance were genotyped in boars from the state of Jalisco, Mexico. Two alleles of the *ESR1* gene were identified in all six breeds, but only in Yorkshire pigs and crosses were BB homozygous pigs identified. Alleles A and B of the *PRLR* gene were identified in all the studied breeds, and owing to the variability in allelic frequency and the diversity of the effects previously described for the three genotypes of this gene, we suggest that when this polymorphism is selected, the association of each genotype with the prolificacy parameters that are desired to be improved should be evaluated, and therefore, the relevant favorable genotype should be determined. For the *RYR1* gene, which causes PSS, the results of this study indicate the presence of the mutant allele in all the breeds studied. The Piétrain breed has a higher frequency than other breeds, although their mutation frequency is low, we would suggest that the selection of animals to be used as breeders includes the identification of carrier and affected pigs, with the aim of eradicating this disease from the swine population in Mexico.

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