Genetic factors influencing rabbit breeding. An overview

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Abstract

Rabbit breeding is a branch of animal husbandry, that provides high-quality and dieted meat. In Bulgaria, this sector is slightly developed, regardless of the growing interest in it. Rabbits have excellent biological abilities – early maturity, high growth intensity, high fertility, and good feed absorption. Rabbit meat has excellent taste qualities, low cholesterol content, and a low percentage of bones in the carcass. In Bulgaria, rabbits are raised mainly for meat. Although the industry is well developed, rabbit meat production is in demand on the market due to its dietary properties, such as low fat and high protein content. Most of the rabbit population in Bulgaria has consisted of White New Zealand and California breeds, but the breeds White Giant, Belgian Giant, Chinchilla, the newly created breed group – Veselina, and the hybrid ZIKA are also bred but in smaller populations and mainly in private farms. The rabbit genome consists of 44 chromosomes (2n = 44). The identification of genome regions and genes related to important phenotypic traits allows the selection of the genetic markers linked with the trait of interest. Marker-assisted selection (MAS) contributes to choosing animals at the early stage of breeding by improving and increasing the expected results compared to the standard methods of selection.

Key words: Genetic Variation, Candidate genes, Rabbit breeding, Polymorphism, Gene expression

Introduction

Rabbit breeding is a branch of animal husbandry, that provides high-quality and dieted meat, and furs. In Bulgaria, this sector is slightly developed, regardless of the growing interest in it, most breeders have small farms and grow rabbits for their needs.

Rabbits have excellent biological abilities – early maturity, high growth intensity, high fertility, and good feed absorption. Rabbit meat has excellent taste qualities, low cholesterol content, and a low percentage of bones in the carcass (Esteves et al., 2018).

Rabbits are bred for the food industry, due to their high-quality dietary meat, considered a delicacy, as well as for skins and rabbit wool. They are also used as experimental animals (in vivo and in vitro), with great contributions to modern medicine - in immunology, vaccine development, genetics, in the study of the development and prevention of infectious diseases (Peng et al., 2015).

In Bulgaria, rabbits are raised mainly for meat. Although the industry is well developed, rabbit meat production is in demand on the market due to its dietary properties, such as low fat and high protein content (Sotirov et al., 2009).

Most of the rabbit population in Bulgaria has consisted of White New Zealand and California breeds, but the breeds White Giant, Belgian Giant, Chinchilla, the newly created breed group – Veselina, and the hybrid ZIKA are also bred but in smaller populations and mainly in private farms (Dimitrova et al., 2008).

In the last decade, different studies have been conducted on the association between the genetic variation of candidate genes and phenotypic traits (Zaghloul et al., 2019).

A candidate gene is defined as a gene that is identified either by its protein product that suggests it could be the determinant of a certain trait in question (a biological candidate), or by its position in a chromosomal region that has been linked with the trait (positional candidate) (Litonjua et al., 2006).

The identification of genome regions and genes related to important phenotypic traits allows the selection of the genetic markers linked with the trait of interest. Marker-assisted selection (MAS) contributes to choosing animals at the early stage of breeding by improving and increasing the expected results compared to the standard methods of selection (Moriel et al., 2019).

The rabbit genome consists of 44 chromosomes (2n = 44). By using the classic method of karyotyping (G- and R-banding) and the FISH method (fluorescent "in situ" hybridization) have been established 23 chromosome-specific gene markers (Hayes et al., 2002). The first genetic and cytogenetic map of the rabbit genome was constructed based on microsatellite and AFLP markers and covered a total of 2767 cM (Rogel-Gailland et al., 2009).

Marker-assisted selection

Marker-assisted selection (MAS) is an indirect selection process where a trait of interest is selected not based on the trait itself but on a marker linked to it. Its purpose is to combine all genetic information at markers and QTL with phenotypic information to improve genetic evaluation and selection. The advantage of MAS is that the effect of genes on production is estimated directly on the genotype of the animal rather than the phenotype (Ondruška et al., 2020).

Quantitative trait loci (QTL) mapping and genomic research will lead to the precise and efficient selection. MAS allows the detection of individuals who do not express a certain trait (milk production and egg laying in males) (Wakchaure et al., 2015).

Candidate genes for meat and carcass traits

Improving meat quality is beneficial to the rabbit industry and private rabbit farms. For example, the color, intramuscular fat (IMF) content, and pH value are all typical meat quality parameters. Also, the genetic effect is one of the important factors when trying to improve meat quality (Wang et al., 2015).

MSTN (GDF8) gene, as a key candidate gene affecting muscle development in different species, is associated with different myostatin variants with several carcass composition traits in rabbits (Sternstein et al., 2015). It acts as a regulator of muscle size and growth. MSTN-deficient animals show an increase in skeletal muscle mass known as double muscling (Navrátilová et al., 2018). Myostatin is expressed in many tissues (including the mammary gland) but most prominently in skeletal muscles. This gene has been highly conserved throughout evolution and comprises three exons and two introns (Navrátilová et al., 2018). The activation of the MSTN receptor also inhibits *Akt* (protein kinase B) activity, a major determinant in muscle protein synthesis and cell proliferation. Enlargement of muscle fiber size, a process called fiber hypertrophy is in large part controlled by *Akt* activity (Navrátilová et al., 2018).

In the study of Sternstein et al. (2015), out of three SNPs in the MSTN gene, only SNPc.373 + 234G > A [GenBank: NM_001109821] showed a significant association, while the SNPs c. -125T > C and c.747 + 34C > T was not significant, according to their results. They registered a highly significant QTL (genome-wide significance $p < 0.01$) for different carcass weights on chromosome 7 with a peak position at 91 cM (157 Mb), a significant QTL ($p < 0.05$) for bone mass on chromosome 9 at 61 cM (65 Mb), and another one for drip loss on chromosome 12 at 94 cM (128 Mb) (Sternstein et al., 2015).

In the experiment of Navrátilová et al. for the rabbit myostatin gene polymorphism, 70 rabbits for SNP c.747 + 34C > T and 60 rabbits for SNP c.*194A > G were genotyped. With the use of PCR-RFLP, two restriction endonucleases AluI (c.747 + 34C > T) and TaaI (c.*194A > G) both studied alleles of SNPs were detected. Single nucleotide polymorphisms in the myostatin gene of the rabbit (c.747 + 34C > T and c.*194A > G) were significantly associated with a body weight of rabbits and carcass yield ($p < 0.05$). The observed animals with genotypes TT and GG showed higher values in all measured parameters compared to CC and CT, resp. AA and AG genotypes (El-Aksher et al., 2016).

PGAM2 (Phosphoglycerate mutase) is the catalyst of the conversion of 3-phosphoglycerate into 2-phosphoglycerate and releases energy during glycolysis in muscle tissues. PGAM2 has been considered a candidate gene to influence growth, development, and carcass traits in livestock (Wu et al., 2015). It is also associated with drip loss, meat color, fat deposition, lean content, muscle fiber diameter, and carcass traits (Nahácky et al., 2018).

PGAM2 also has a function in controlling the contraction of muscles (Helal et al., 2021). Its expression is involved in two form of development – brain form PGAM1 and PGAM2 – muscle form (Helal et al., 2021).

Nahácky et al., 2018 genotyped by PCR-RFLP in a total of 44 rabbits for PGAM2 (195C > T) with three genotypes AA, AG, and GG. Rabbits with genotype TT (PGAM2) had a higher level of all observed parameters compared to CC and CT genotypes for live weight, body weight, carcass weight, the weight of skin, and weight of the thigh.

Wu et al., 2015, identified three single nucleotide polymorphisms (SNPs) identified by direct sequencing of 20 random individuals of three breeds, incl. c. -10C > T, c.195C > T, and c.414 + 17C > T. c.195C > T was genotyped by PCR-RFLP in a total of 222 rabbits of three breeds (Tianfu black, 53 individuals; Ira, 91 individuals; Champagne, 78 individuals).

Exonic SNP c.195C > T was found to be significantly associated with growth traits, with no consistent association between PGAM2 SNPs carcass and carcass traits, which could be due to differences between species, population size, and specific breed conditions (Wu et al., 2015).

POU1F1 (also named PIT-1) is the first pituitary-specific transcription factor to be identified in human and mouse subjects. As a member of the POU-domain family gene, POU1F1 is a positive regulator for growth hormone (GH), prolactin (PRL), and thyroid stimulating hormone β (TSHβ) by binding to target DNA promoters as a dimer in mammalian animals (Wang et al., 2015).

Meat pH affects many meat quality properties, including protein structure and meat color in rabbits. The decrease in pH between 0 h and 24 h after slaughtering was related to increases in certain values of biceps femoris meat. The higher pH in fresh rabbit meat may imply a higher level of muscle glycogen reserves (Wang et al., 2015).

The rabbit's POU1F1 gene is mapped to chromosome 14 and it contains seven exons and six introns (Ensembl accession NO. ENSOCUG00000017779) (Wang et al., 2015).

By studying a total of 372 rabbits, males (n = 190) and females (n = 182) – 137 Hyla (HY), 144 Champagne (AC), and 91 Tianfu Black (TB) Wang et al. (2015), used PCR primers to amplify a 798 bp fragment of intron 5 in the POU1F1 gene and then the PCR products were sequenced. They revealed one single nucleotide polymorphism which was located at 536 bp in intron 5 of the POU1F1 gene (Wang et al., 2015).

Wang et al., 2015 identified three genotypes (CC, TT, and CT). The genotype frequency of CC was higher than TT and CT. The C allele frequency (0.5604 to 0.6806) was also much higher than T. Their results showed that the three rabbit populations had intermediate levels of genetic diversity. This suggested that there was sufficient genetic diversity for selection to be effective in improving meat quality traits in these three rabbit populations.

By discovering one SNP with 2 alleles (C and T) in intron 5 of the POU1F1 gene Wang et al. (2015) found that the T allele had the highest frequency in the three rabbit breeds. The SNP

was significantly related to pH in the biceps femoris muscle and intramuscular fat in the longissimus dorsi and biceps femoris muscles. Thus, this POU1F1 SNP is of potential use in marker-assisted selection for meat quality traits in rabbits. This assumes that this SNP is closely associated with QTL affecting meat quality traits.

Calpastatin (CAST) was first identified in 1978. It is found commonly in muscle cells. Calpastatins are rich in proline and glutamate, but poor in aromatic amino acids. Calpastatin is also an endogenous inhibitor that controls the activity of calpains in the presence of Ca^{2+} (Wang et al., 2016). It is discovered that the variation in CAST abundance influences postmortem aging rates in different muscles which is involved in protein degradation and meat quality (Wang et al., 2017). Calpastatin gene (CAST, ENSOCUG00000007802) is located at the 11th chromosome (16,359,315 - 16,482,673 F) of the rabbit genome and it has 10 transcripts. The CAST gene is responsible for producing calpastatin, which inhibits the activity of calpain (calcium-dependent cysteine protease) by adding Ca2þ. Calpastatins include high levels of proline, which affect meat quality. Calpastatin gene polymorphisms were investigated in different animal species such as cattle, pigs, sheep, and chickens (Helal et al., 2021).

Studying the single nucleotide polymorphism (SNP) in 372 animals from three rabbit breeds (Hyla, Champagne, and Tianfu Black) for meat quality traits Wang et al. (2017), detected one single nucleotide polymorphism (g.16441502 C > T) located at 67 bp in intron 3 of the CAST gene in Chromosome 11.

According to Migdal et al. (2018), the T allele showed a higher prevalence in the studied breeds. They discovered that rabbits with the CC genotype had higher IMF (intramuscular fat) than rabbits with CT and TT genotypes in both longissimus dorsi and biceps femoris muscles among these three rabbit breeds. The studied rabbits with the TT genotype had a significantly greater yellowness at 0 and 24 h postmortem than those with the CC genotype ($p < .05$) in the longissimus dorsi muscle.

Investigating single nucleotide polymorphisms in the calpastatin (CAST) gene, Wang et. al. (2017), studying Hyla, Champagne, and Tianfu Black rabbit breeds detected one single nucleotide polymorphism (g.16441502 C > T) located at 67 bp in intron 3 of the CAST gene in Chromosome 11. According to their study, rabbits with the TT genotype had a significantly greater yellowness at 0 and 24 h postmortem than those with the CC genotype ($p < .05$) in the longissimus dorsi muscle. The CC genotype had higher intramuscular fat content than those with CT and CC genotypes in both longissimus dorsi and biceps femoris muscles in the three breeds ($p < .05$).

Myogenic factor 5 (Myf5) is part of the family of muscle regulatory factors (MRFs) that is involved in the formation of muscle fibers and the transcription of specific muscle genes and has effects on meat deposition capacity and the level of intramuscular fat. According to NCBI Reference Sequence NC_013672.1, the Myf5 gene in rabbits is 2702 bp long and it is consisting of three exons and two introns. The lengths of exons 1, 2, and 3 are 501, 76, and 191 bp, respectively, whereas intron 1 is 773 bp and intron 2 is 418 bp long. Wang et al. (2017) investigated 188 rabbits from two breeds on a single farm in China: Ira (n = 106) and Tianfu Black (n = 82). All rabbits were slaughtered together at 70 days of age. Carcasses were stored at 4 °C for 24 h. The studied meat quality traits were pH, color (L^*, a^*, b^*), and intramuscular fat (IMF). These traits were measured in the *longissimus dorsi* and *biceps femoris* muscles. They extracted genomic DNA from ear tissue and sequenced the samples. The research team found six SNPs and their genotypes in Ira rabbits, but only SNP 1 and its three genotypes were found in Tianfu Black rabbits. Interestingly, for Ira, SNPs 2 and 3, also SNPs 4, 5, and 6 were in complete linkage disequilibrium. For SNP 1, genotype AA was more frequent than genotypes GG and AG, and allele A was predominant in the two breeds. For SNPs 2 and 3, only Ira had three genotypes. For SNPs 4, 5, and 6, Ira rabbits had two genotypes and the CC/C was the prevalent genotype/allele. Genotype distributions for SNP1 and SNPs 4, 5, and

6 deviated from Hardy-Weinberg Equilibrium ($p < 0.05$) (Wang et al., 2017).

QTL and meat quality traits

The genome-wide scan for meat quality traits identified a significant QTL on OCU12 affecting drip loss of the whole carcass. The peak QTL position is located at the end of the q-arm at 94 cM (127.58 Mb) near the marker INRACCDDV0176. The QTL accounted for 4.78% of the phenotypic F2 variance. The GG QTL allele had negative dominance effects. Another QTL for drip loss that tuned out to be significant was mapped on OCU18 (Sternstein et al., 2015).

Sternstein et al. (2015) analyzed QTL for meat quality traits in two rabbit breeds (New Zealand White and Giant Grey identified one genome-wide ($p < 0.05$) significant QTL on OCU12. Additionally, 13 suggestive QTL at the chromosome-wise significance threshold of $p < 0.05$ were identified on chromosomes 1, 2, 5, 8, 9, 11, 16, 17, and 18.

The improvement of existing linkage maps and the mapping of quantitative trait loci (QTL) for carcass and meat quality traits are still limited compared to many other livestock species (Sternstein et al., 2015). Creating a genetic map of valuable QTL is an important step in quantitative trait gene identification which support accurate mapping in Genome-Wide Association Studies (GWAS) (Sternstein et al., 2015).

A candidate gene for growth and body weight

The Leptin gene in rabbits is located at chromosome 7 (16,079,679- 16,081,684) and has one transcript with 2 exons (Helal et al., 2021). The expression of the leptin gene produces the leptin hormone, which is involved in the regulation of body weight by regulating the energy balance. Leptin hormone (16-kDa polypeptide hormone) releases from white adipocytes (adipose cells), and the amount released is in proportion to the cell's size. It has different functions, including binding to LPT receptors in the hypothalamus, which results in a cascade of chemical signals that affect hunger and fullness in the body. It is also positively associated with obesity and body fat mass. The leptin hormone acts as a growth factor, and its concentration level is controlled by GH and IGF1 levels (Helal et al., 2021). Leptin transcripts in rabbits have been described as being encoded by only two exons rather than three as in other species. Leptin also influences the development of the mammary gland (Koch et al., 2013).

In the study of Migdal et al. (2018), 320 crossbred animals of New Zealand white and Belgian Grey were genotyped for the identification of polymorphisms within exon 2—g.16081633T > C, intron 1_2—g.16081420C > T, and within UTR—g.16079636C > G. Identified polymorphisms within rabbit's leptin gene revealed significant differences in dissectible fat percentage in the carcass and dissectible fat weight in the intermediate part (Migdal et al., 2018). Their study concludes that polymorphism in the rabbit leptin gene influences important carcass and meat traits of NZW × BGG crossbreeds.

In the experiment of Luo et al. (2020) with the Tianfu black rabbit breed, the correlation coefficient between the expression levels of the leptin gene in perineal fat and intramuscular fat content in 84-day-old male rabbits was measured. They detected with the use of real-time fluorescence quantitative PCR a value of 0.73 ($p < 0.05$). Expression levels of leptin gene in left biceps femoris and intramuscular fat and 24-hour pH in 84-day-old male rabbits results was 0.95 ($p < 0.01$) and 0.85 ($p < 0.05$).

Fatty acids binding proteins 3 and 4 (FABP3 and FABP4) are related to fatness traits. SNP (single nucleotide polymorphism) within those candidate genes for growth rate and fatness traits were described by Migdal et al. (2017). They announced SNPs associated with intramuscular fat content (IMF) and dissectible fat weight. All three SNPs (g.16081633T > C; g.16079636C > G, g.16081420C > T) were associated with IMF and missense mutation g.16081633T > C and affected dissectible fat percentage in the carcass and dissectible fat weight in intermediate part (loin).

In the FABP4 gene, there were identified several mutations – g.97156738 G > A; g.97156696 A > G; g.97156168 G > A and g.97156084 G > A. Statistical analysis reveals an association of

g.97156025 G > A for L* values obtained 45 minutes after slaughter and after 24 hours of chilling for the longissimus lumborum muscle. Statistically significant differences were found for meat weight of loin, IMF, and shear force for g.97156692 C > A polymorphism (Migdal et al., 2017).

Melanocortin 4 Receptor (MC4R) gene is linked with rabbit body weight gain. The gene encodes MC4 protein, a G protein-coupled receptor that binds Alpha-melanocytes stimulating hormone (Osaiyuwu et al., 2020).

The melanocortin 4 receptor gene (MC4R, ENSOCU G00000025457) is located on chromosome 9 (100,687,330-100,688,331) in rabbits and has a single exon transcript. The MC4R gene is expressed in the hypothalamus and is very important for the regulation of energy homeostasis. In addition, the MC4R gene controls food intake and body weight, and fat deposition. Mutations in the MC4R gene are associated with obesity. The MC4R gene also controls glucose homeostasis and insulin sensitivity (Helal et al., 2021).

Polymorphisms in the melanocortin 4 receptor (MC4R) gene have been already associated with growth performance in different species. MC4R is mainly expressed in the hypothalamus, which plays a key role in controlling energy homeostasis and food intake with effects on body weight and fat deposition (Fontanesi et al., 2013).

Osaiyuwu et al. (2020) using 6 different rabbit breeds (20 Fauve de Bourgogne, 26 Chinchilla, 10 New Zealand white, 11 Dutch, 4 English Spot, and 3 Californian) studying the polymorphism of those breeds, detected SNP at c.101G > A, which produced three genotypes (AA AG and GG), linked with body weight in rabbits. The highest allele frequency was observed in the AG genotype (0.69). According to their study, the genotype AA may have the potentials to be associated with higher body weight values than genotypes AG and GG.

Resequencing 1729 bp of the rabbit melanocortin 4 receptor (MC4R) gene, Fontanesi et al. (2013), identified 10 polymorphisms from the sequences – one indel and 9 SNPs. They discovered 3 polymorphisms in the genotype (p.G34D) of MC4R c.101G > A substitution.

The Growth hormone (**GH1**) GH1 is significant for postnatal growth performance, regulation of multiple biological and metabolic functions related to or involved in muscle mass accretion, lipid metabolism, and bone growth, among others. The GH gene alters the metabolism of carbohydrates, proteins, and lipids and promotes postnatal growth of mammals exerting direct or indirect effects on numerous tissues. The growth hormone gene is located on chromosome 19 and has three transcripts. Its role includes regulation of the development and growth pathways such as muscle mass accretion, lipid metabolism and bone growth, among others. The GH1 gene changes the metabolism of carbohydrates, proteins, and lipids, it also stimulates postnatal growth in mammals (Helal et al., 2021). The GH1 must bind with the growth hormone receptor (GHR) to activate the JAK-STAT pathway, then the expression of insulin-like growth factor 1 (IGF1) and other genes will be activated (Helal et al., 2021).

Hristova et al. (2018) observed and reported the effect of the **Growth hormone** in three groups of rabbits using PCR-RFLP for the presence of SNP associated with the GH gene and the impact of crossing close siblings – 86 New Zealand White (NZW) outbred rabbits; first-generation inbred rabbits (F1) and second-generation inbred rabbits (F2). They detected 3 genotypes in the studied rabbit populations: CC, CT, and TT in the amplified 231 bp fragment.

The growth hormone receptor gene plays a vital role in mediating the action of the growth hormone by tyrosinase kinase activation and insulin-like growth factor 1 (IGF1) induction (Helal et al., 2021).

The GHR is composed of three domains: an extracellular domain binding the growth hormone, a transmembrane helix domain responsible for the development of the constitutive GHR dimer; and an intracellular domain interacting with the tyrosinase kinase JAK2 (Helal et al., 2021).

A study by Helal (2019), for the association of the SNP c.106C > G, located in exon 3 of the growth hormone receptor (**GHR**) gene with body weight in rabbits, resulted in the alloca-

tion of three genotypes (CC, GC, GG). This is responsible for certain body phenotypic parameters, expressed by measuring the weight of the sampled individuals at 6, 8, 10, and 12 weeks of age. His results of sampling NZW rabbits and Baladi Red (local breed) concluded the association of body weight for this candidate gene in favor of the exotic New Zealand White breed by calculating the allele frequencies.

Gencheva et al. (2022) studied the influence of the genotypes of SNP c.106C > G in the growth hormone receptor gene (GHR) on individual body weight (IBW) during the growing period at 35, 70 and 90 d of age on a total of 107 weaned Californian breed rabbits. The results revealed that 52.3% of the rabbits carrying c.106C > G SNP were heterozygous. Significant differences were observed between individuals with homozygous c.106C > G CC genotype and those with heterozygous CG genotype. The highest IBW (2462.0 ± 198.3 g) was observed in rabbits carrying the c.106C > G CC genotype and detected individuals were significantly affected by the additive effect. In conclusion the authors suggested that c.78C > T of GH gene and c.106C > G of GHR gene could be useful candidate genes to improve growth performance in Californian rabbits with potential application in rabbit breeding programs.

The insulin-like growth factor (IGF) is an essential regulator affecting growth, mammary gland development, lactation, and fertility (Abdel-Kafy et al., 2015). Insulin-like growth factor 2 (IGF2) plays an important role in the control of reproduction processes such as ovarian folliculogenesis, ovarian oogenesis, and corpus luteum function (Hull and Harvey, 2002). Therefore, IGF2 was suggested as a candidate gene and molecular marker associated with growth, litter size, and milk yield traits in rabbits and other farm animals. Ramadan et al. (2020) evaluated the polymorphism of insulin-like growth factor 2 (IGF2) in Sinai Gabali rabbits. They used the PCR-RFLP method and the HpyF31 restriction enzyme. They announced that the Del/Del genotype of the IGF2 gene showed superiority over the other genotypes for BW at 4 weeks (507.17 ± 8.87 g), 8 weeks (1239.39 ± 14.0 g), and 12 weeks of age (1950.15 ± 18.1 g), as well as for daily weight gain from 4 to 8 weeks (26.05 ± 0.37 g/d), and from 8 to 12 weeks of age (25.48 ± 0.56 g/d).

Candidate genes for reproductive traits in rabbits

Progesterone receptor gene (PRG) is a candidate gene associated with milk production and other traits such as litter size, uterine capacity, ovulation, implantation, and pregnancy Progesterone has two isoforms – PR-A and PR-B. The secretion of progesterone and estrogen takes place mainly in the ovaries, but also in the placenta of pregnant females. This contributes to the maintenance of pregnancy and the development of the mammary glands (Ondruška et al., 2020).

The progesterone receptor gene (PGR) codes a protein that interacts with the progesterone hormone in the establishment and maintenance of pregnancy. The PGR gene is located on chromosome 1: 115,601,359-115,672,617 forward strand (Source: UniProtKB/Swiss Prot; Acc:P06186). Progesterone contributes to the release of mature oocytes, implantation, and maintenance of pregnancy by suppressing of myometrial contractility and promoting uterine growth (Peiró et al., 2010). Progesterone plays a vital role in the mammogenesis and lactogenesis process. It increases both the size and number of milk ducts (Husvéth, 2011). The promoter region of the PGR gene includes an SNP G > A2464 that showed that there were some differences in the early embryo survival and development at 3rd d of gestation between two rabbit lines selected by uterine capacity. Therefore, PGR was considered a candidate gene for the identification of molecular markers associated with litter size and milk yield traits in livestock animals (Peiró et al., 2008; Argente et al., 2010).

In a study by El-Aksher et al. (2016), they detected polymorphisms in the promoter region of the PGR gene by PCR using restriction enzymes. 100 animals belonging to four rabbit populations were studied – the Egyptian Synthetic Rabbit Line called the Moshtohor (M-line) line and their parents from the Spanish V-line and the Si-

nai Gabali rabbit breed. The restriction analysis was conducted with endonuclease Eco31I. They revealed three genotypes – GG, AA, and GA and genotype GG, showing two bands of 416 and 142 SNP, genotype AA, giving one band of 558 bp, and heterozygous genotype GA, giving all three bands of 558, 416, and 142 SNP. The genotype frequency of GA ranges from 0.680 in the V-line to 0.880 in FGP and is higher than the genotypes of AA and GG in all populations studied. The frequency of the A allele is higher than the G allele in all populations, except for the M-line, where the G allele has the highest allele frequency of 0.540. The highest effective number of alleles (Ne) for SNP of the PGR gene was recorded for the M-line (1,987), followed by FGP (1,972), while the lowest number was recorded for the V-line (1,891). The four populations studied were not in Hardy-Weinberg equilibrium ($p < 0.05$ or $P < 0.001$). The observed (Ho), expected (He) heterozygosity and polymorphic information content (PIC) are on average 1,943, 0,485 and 0,367, i.e. the four rabbit populations show intermediate levels of genetic diversity.

Genetic methods for identification of polymorphism in rabbit genome

PCR-RFLP

RFLP markers use cDNA (Complementary DNA (cDNA) is a DNA copy of a messenger RNA (mRNA) molecule produced by reverse transcriptase, a DNA polymerase that can use either DNA or RNA as a template), cloned DNA elements or synthetic oligonucleotides as probes that are labeled with a radioisotope or with conjugated enzymes that catalyze a color reaction, for DNA hybridization. DNA is cleaved with restriction enzymes or amplified by PCR. RFLP is representative of this type of technology and was first developed in 1980 (Botstein et al., 1980) to visualize differences in DNA structure based on the use of bacterial restriction enzymes that cut DNA at sites with specific nucleotide sequences (Mburu and Hannote, 2005). RFLPs are based on the analysis of patterns obtained from a DNA sequence digested with known restriction enzymes. Differences are apparent when the fragment lengths are different, suggesting that the restriction enzyme is cutting the DNA at unrelated sites. Restriction polymorphism occurs when mutations remove an existing restriction site or create a new restriction site. The choice of DNA probe is critical in RFLP analysis (Malekifard et al., 2018)

Direct sequencing

The most used sequencing techniques include sequencing a representative number of subclones of the PCR product or direct PCR sequencing by running independent sequencing reactions for cytosine and thymine. Direct sequencing on a standard sequencer is used to achieve the required throughput in a low-cost way (Qiu et al., 2003).

PCR products can be sequenced using either the dideoxy (Sanger) or chemical (Maxam-Gilbert) approaches. In the dideoxy methods, the target sequence is amplified and an excess of one strand of the target sequence is generated by ”asymmetric PCR”, where one primer is present in vast excess over the other. This single-stranded product serves as the template for conventional dideoxy sequencing methods. Another procedure prepares PCR products for use as templates for characterizing unlabeled products by genomic sequencing and chemical sequencing of end-labeled products (Dorit et al., 2001).

Next-Generation sequencing

Next-generation sequencing is a method that saves time and cost and increases the sequence output compared with Sanger sequencing. Sanger sequencing can produce a sequence for one template per reaction, whereas NGS can perform millions to billions of individual sequencing reactions simultaneously (parallel sequencing). In NGS the DNA molecule is fragmented into usable sizes providing a portion of the nucleotide sequence known as a read. NGS generates large amounts of genomic data that can be used to detect genetic variants related to different functional mutations. Using NGS provides a clear advantage over SNP so the NGS data gives better result prediction. (Kumar et al., 2019).

Vecere et al. (2022) used the advantage of NGS-based methods for the accurate identification and quantification of microbial populations in diagnosing rabbit ear infections can provide

more specific information for the treatment of clinical cases.

Bai et al. (2021) improved the genome assembly of rabbits with long-read sequencing. The current reference genome of European Rabbit (Oryctolagus cuniculus) OryCun2.0 established with whole-genome shotgun sequencing was quite fragmented and had not been updated for ten years. In this work, we provided a new rabbit genome assembly UM_NZW_1.0 to improve OryCun2.0 by leveraging the contig lengths based on long-read sequencing and a wealth of available Illumina paired-end sequence data. UM_NZW_1.0 showed a remarkable continuity increase compared with OryCun2.0, with 5 times longer contig N50 and approximately 75% closed gaps. Many of the closed gaps overlapped with protein-coding genes or transcriptional features, resulting in an enhancement of gene annotations. UM_NZW_1.0 presented a more complete landscape of the MHC region and the IGH locus, therefore providing a valuable resource for future research on rabbits.

Conclusion

The genetic potential of animals is important for the management of selection and breeding programs. The study of candidate genes is essential for improving and increasing the productive traits of individuals. The investigation of genetic variations related to a particular gene is significant for the implementation and examination of common variations across the entire genome and for detecting new regions of interest that is in or near potential candidate genes.

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