

RAPD MARKERS LINKED TO LITTER, LACTATION AND GROWTH TRAITS IN RABBITS

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ABSTRACT

A five-years crossing scheme involving the Spanish V-line (V) and Saudi Gabali (S) rabbits was practiced during five generations to produce new synthetic lines: Saudi 2 with structure of $((\frac{3}{4}V\frac{1}{4}S)^2)^2$ to be used as maternal line, and Saudi 3 with structure of $((\frac{3}{4}S\frac{1}{4}V)^2)^2$ to be used as paternal line. To perform the genetic analysis, DNA from four sires of the parental generation (V-line and Saudi Gabali purebreds) were used to determine which genetic markers (from 40 RAPD markers used) can be used to differentiate between individuals. To reach high accuracy of homogeneity within families, 526 grand-daughter progenies from the 5th generation (Saudi2, with the structure of $((\frac{3}{4}V\frac{1}{4}S)^2)^2$) were used to perform the genetic and statistical analyses. Traits under investigation included litter size at birth (LSB) and weaning (LSW), litter weight at birth (LWB), 7 days (LW7), 21 days (LW21) and weaning (LWW), litter gain at days interval of 0-21 (LG021) and 0-28 (LG028), pre-weaning litter mortality (PLM), milk yield at lactation intervals of 0-7 days (MY07), 0-21 days (MY021), 7-21 days (MY721), 21-28 days (MY2128), total 0-28 days (TMY), and body weight at 4 and 8 weeks of age. Application of the RAPD (Random Amplified Polymorphic DNA) technique with 40 primers revealed five polymorphisms between the strains. In single-marker analysis, RAPD marker of OPF09₇₀₀ explained variation ranged from 10 to 14.7% for litter weight (LWB, LW7, LW21 and LWW) and gains (LG021), PLM and milk yield (MY07, MY21). RAPD marker of OPA19₁₁₀₀ explained variations ranging from 10 to 14% for LW7, LW21, LWW, LG021 and PLM, while OPF12₉₀₀ marker explained 14.7 and 16.8% of the variation for body weight at 4 and 8 weeks of age, respectively.

Keywords: Rabbits, Synthetic Lines, Sire-granddaughters design, RAPD, Litter traits, Lactation, Growth.

INTRODUCTION

In Saudi Arabia, rabbit production has recently developed and the targets of research were directed to identify which breeds or lines were convenient for this hot country. For this reason, special emphasis was paid to construct a genetic improvement programme for this hot climate country (Khalil *et al.*, 2002). The rabbits used in this project were V-line and Saudi Gabali. V-line is a maternal rabbit line which has been selected for the number of young weaned per litter for 21 generations (Estany *et al.*, 1989). Recent results obtained for V line in Saudi Arabia have confirmed the good adaptability of line V in heat stress conditions (Khalil *et al.*, 2002). Saudi Gabali is a Saudi purebred breed exhibiting good performances under hot and desert conditions especially in Najd area (Al-Saef *et al.*, 2007) and rabbits of this breed are characterized by litter size of 6-8 young, mature body weight of 3.2-3.8 kg and has the ability to produce and reproduce under hot environment.

RAPD technique is one of the most widely used techniques in applications of molecular biology for identifying the markers linked to traits of interest without the necessity for mapping the entire genome (Bardakci, 2001). In this concept, Williams *et al.* (1990) introduced the DNA polymorphisms amplified by arbitrary primers as genetic markers to be used in genetic maps and the use of this type of markers increased rapidly. Simplicity, applicability and low cost of the RAPD technique gave this technique wide range of applications in many areas of genetics and molecular biology. Also, RAPD

technique provides a useful approach for evaluating genetic differentiation particularly in species that are poorly known genetically (Dawson *et al.*, 1993; Hsiao and Rieseberg, 1994; Nesbitt *et al.*, 1995; Wachira *et al.*, 1995; Sale *et al.*, 1996; Wen and Hsiao, 1999), in genetic identification (Kresovich *et al.*, 1992; Welsh and McClelland, 1990), in introgression studies (McCoy and Echt, 1993), in parental test (Welsh *et al.*, 1991), and also in phylogenetic study (Halward *et al.*, 1992). Bahy (2003) used RAPD technique to detect band variations between normal and abnormal phenotypes of male broiler chickens.

In the present study, RAPD technique was used to study the association between molecular markers and litter size at birth and weaning, litter weight at birth, 7 days, 21 days and weaning, litter gain at days interval of 0-21 and 0-28, pre-weaning litter mortality, milk yield at lactation intervals of 0-7 days, 0-21 days, 7-21 days, 21-28 days, total 0-28 days, and body weight at 4 and 8 weeks of age.

MATERIALS AND METHODS

Crossbreeding program and design of molecular analysis

A five-years crossbreeding project involving a desert Saudi Gabali (S) and a Spanish V-line (V) started in September 2000 in the Experimental Rabbitry, College of Agriculture and Veterinary Medicine, Al-Qassim University in Saudi Arabia. Eighty pedigreed does and sixteen pedigreed bucks of V-line rabbits were imported from Universidad Politécnica de Valencia (Spain) in September 2000. V-line is a maternal rabbit line selected for number of young weaned per litter for 21 generations (Estany *et al.*, 1989), while Saudi Gabali is a Saudi breed raised under the desert conditions, especially in Najd area, and rabbits of this breed are characterized by litter size of 6-8 young, mature body weight of 3.2-3.8 kg and the ability to survive and adapt to produce and reproduce under hot environment. This crossbreeding plan permitted simultaneous production of 14 genetic groups of V, S, $\frac{1}{2}V\frac{1}{2}S$, $\frac{1}{2}S\frac{1}{2}V$, $\frac{3}{4}V\frac{1}{4}S$, $\frac{3}{4}S\frac{1}{4}V$, $(\frac{1}{2}V\frac{1}{2}S)^2$, $(\frac{1}{2}S\frac{1}{2}V)^2$, $(\frac{3}{4}V\frac{1}{4}S)^2$, $(\frac{3}{4}S\frac{1}{4}V)^2$, $((\frac{3}{4}V\frac{1}{4}S)^2)^2$, $((\frac{3}{4}S\frac{1}{4}V)^2)^2$, Saudi 2 [synthetic maternal line with a genetic structure of $((\frac{3}{4}V\frac{1}{4}S)^2)^2$ interse mated] and Saudi 3 [synthetic paternal line with a genetic structure of $((\frac{3}{4}S\frac{1}{4}V)^2)^2$ interse mated]. Details of housing, feeding, procedures and crossbreeding plan used in the project to form new synthetic lines were described by Khalil *et al.* (2007). The bucks were randomly assigned to mate the does naturally with the restriction to avoid the mating of animals with common grandparents.

To perform the genetic analysis, DNA from four sires of the parental generation (V-line and Saudi Gabali purebreds) were used to determine which genetic markers (from 40 markers used) can be used to differentiate between individuals. To reach high accuracy of homogeneity within families, 526 granddaughter progenies from the 5th generation with the structure of $((\frac{3}{4}V\frac{1}{4}S)^2)^2$ were used to perform the genetic and statistical analysis.

Data collected (phenotypic characterization)

Data collected were litter size at birth (LSB) and weaning (LSW), litter weight at birth (LWB), 7 days (LW7), 21 days (LW21) and weaning (LWW), litter gain at days interval of 0-21 (LG021) and 0-28 (LG028), pre-weaning litter mortality (PLM), milk yield at lactation intervals of 0-7 days (MY07), 0-21 days (MY021), 7-21 days (MY721), 21-28 days (MY2128), total 0-28 days (TMY), and body weight at 4 and 8 weeks of age.

DNA isolation and RAPD analysis

DNA was isolated from blood samples using standard salting out procedure described by Miller *et al.* (1988). RAPD polymorphisms were detected using arbitrarily primed PCR as described by Williams *et al.* (1990). Briefly, arbitrary 10-base oligonucleotides (Operon Technologies, Alameda, CA) RAPD 10-Mer kits of A (OPA-01 to OPA-20) and F (OPF-01 to OPF-20) were used alone or in pairs to amplify random sequences from genomic DNA.

RAPD-PCR reaction mixtures consisted of 2.0 mM MgCl₂, 0.1 mM each dNTP (dATP, dCTP, dGTP, and dTTP), arbitrary primers (0.4 μM if a single primer was used; 0.2 μM each if two primers were used), 5 units AmpliTaq Polymerase, 1x PCR AmpliTaq PCR buffer, and 25 ng of genomic DNA. Cycle parameters (Thermolyne Amplitron thermocycler) were: Denaturation at 94° for 2 min, followed by 45 cycles of 1 min at 94°, 1 min at 36°, and 2 min at 72°. Resultant amplification products were run out on 1.5% agarose gels and visualized by ethidium bromide staining. RAPD banding patterns on gels were scrutinized for variation in presence/absence variation of a band at a specific position on the gel, presumed to reflect priming sequence variation or large insertion/deletion variants that preclude successful amplification.

Statistical analysis

For data of single-marker analysis, a total of 526 rabbits of a genetic group of $((\frac{3}{4}V\frac{1}{4}S)^2)^2$ were used in regression analysis to detect the association between markers and traits using program of SAS (1996). Multiple regression analysis was employed to detect the effects of markers linked to the total phenotypic variation for body weights at 4 and 8 weeks of age, litter traits at birth and weaning, and milk yield traits. The model of statistical analysis included the effects of year-season, parity, litter size in which the animal was born in addition to regressions on polymorphic markers. The percentage of the total phenotypic variation explained by the association between each marker of 40 marker used and each trait was estimated and expressed as R^2 value.

RESULTS AND DISCUSSION

Analysis of polymorphism

From a total of 40 markers used, five markers (OPA12, OPA19, OPA20, OPF09, and OPF12) were able to identify five polymorphic fragments at molecular weight of 1500, 1100, 1200, 700 and 900 bp, respectively. These polymorphic markers were further used to check their linkage to phenotypic traits, using progeny of F₅ from a cross obtained from crossing Gabali and V line rabbits. Queney *et al.* (2001) using microsatellites markers found low polymorphism in rabbits. Chantry-Darmon *et al.* (2006) attributed this trend due to the use of inbred rabbit strains for building the reference families.

Single-marker analysis

Results of single-marker analysis given in Table 1 were used to identify the linkage between RAPD markers and variation in phenotypic characters of F₅ progeny. The analysis of variance showed significant associations between phenotypic traits and the three markers of OPF12₉₀₀, OPF09₇₀₀ and OPA19₁₁₀₀ ($P < 0.05$ or $P < 0.01$; Figure 1). The other two markers of OPA12₁₅₀₀ and OPA20₁₂₀₀ were not significantly associated with any of the phenotypic traits. OPF09₇₀₀ marker was linked with LWB, LW7, LW21, LWW, LG021, PLM, MY07 and MY21 accounting for 12.6, 14.7, 12.6, 12.6, 10.0, 12.0, 10.4 and 10.6% of the phenotypic variation, respectively. The RAPD marker of OPA19₁₁₀₀ showed significant linkage with litter weight at days interval of 0-7 and 0-21, litter weight at weaning, litter gain at days interval of 0-21, and pre-weaning litter mortality ($P < 0.05$ or $P < 0.01$) accounting for 14.0, 12.0, 14.0, 10.0 and 12.0% of the phenotypic variation for LW7, LW21, LWW, LG021 and PLM, respectively. The marker of OPF12₉₀₀ showed significant linkage ($P < 0.01$) with weight at 4 and 8 weeks of age accounting for 14.7 and 16.8% of the variation in weight at 4 and 8 weeks of age, respectively.

Findings of the two markers of OPF09₇₀₀ and OPA19₁₁₀₀ showed that associations of these markers with some litter traits such as LW7, LW21, LWW, LG021, and PLM were compromised and not compromised for other traits (LWB, MY07 and MY21). In this concept, Al-Seaf, 2007 reported that their were significant phenotypic correlations among litter and milk traits studied.

Table 1: Significant associations between RAPD markers linked to phenotypic traits measured on the progeny of F₅ obtained from crossing V-line and Saudi Gabali rabbits

Markers and traits	R ² (%)	P-value
OPF09₇₀₀:		
Litter weight at birth (LWB)	12.6	0.01
Litter weight at interval of 0-7 days (LW7)	14.7	0.01
Litter weight at interval of 0-21 days (LW21)	12.6	0.01
Litter weight weaning (LWW)	12.6	0.01
Litter gain at interval of 0-21 days (LG021)	10.0	0.02
Pre-weaning litter mortality (PLM)	12.0	0.01
Milk yield at lactation intervals of 0-7 days (MY07)	10.4	0.01
Milk yield at lactation intervals of 0-21 days (MY21)	10.6	0.02
OPA19₁₁₀₀:		
Litter weight at interval of 0-7 days (LW7)	14.0	0.01
Litter weight at interval of 0-21 days (LW21)	12.0	0.01
Litter weight weaning (LWW)	14.0	0.01
Litter gain at interval of 0-21 days (LG021)	10.0	0.02
Pre-weaning litter mortality (PLM)	12.0	0.01
OPF12₉₀₀:		
4-week weight	14.7	0.01
8-week weight	16.8	0.01

Traits not included in this Table are not linked significantly with the markers
R² = Percentage of phenotypic variance explained by the RAPD marker

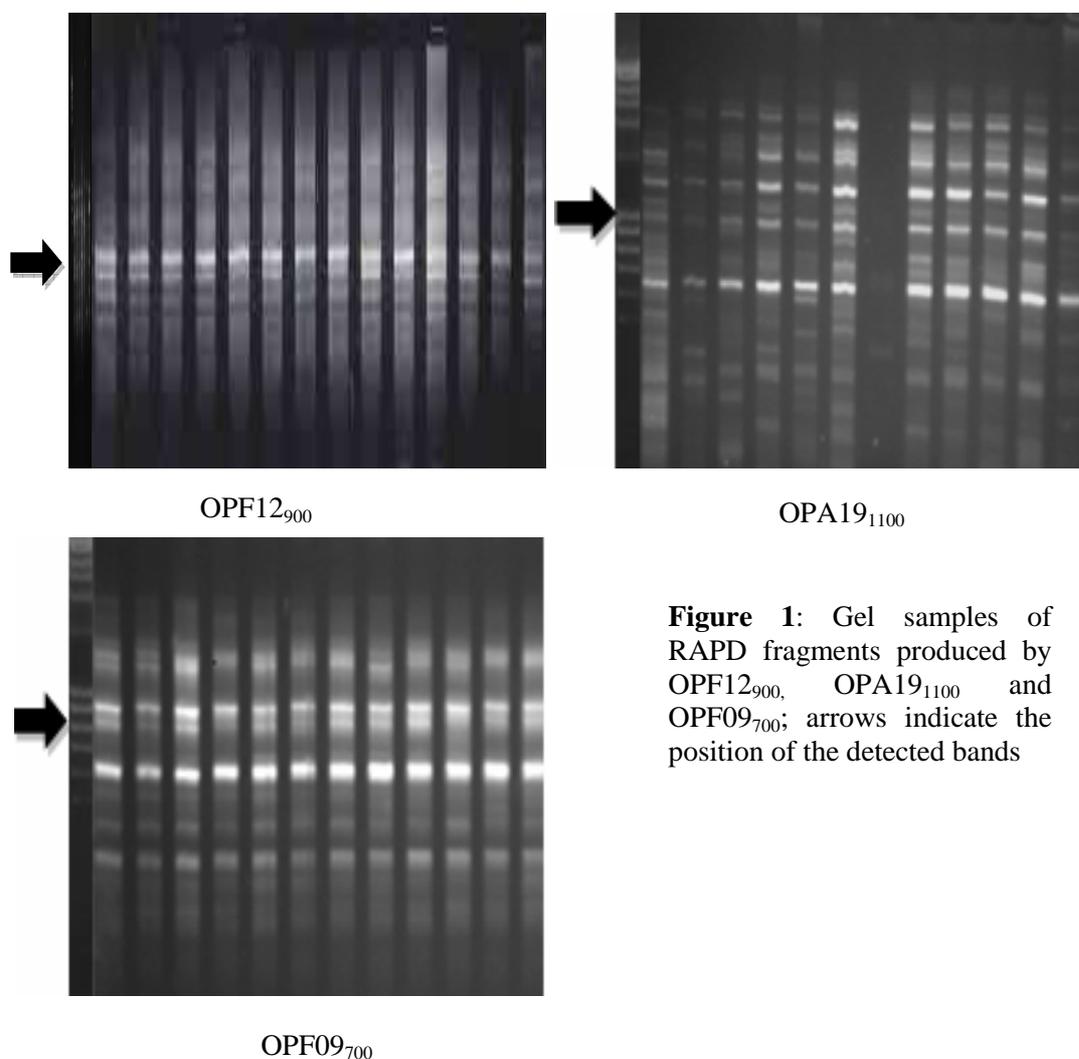


Figure 1: Gel samples of RAPD fragments produced by OPF12₉₀₀, OPA19₁₁₀₀ and OPF09₇₀₀; arrows indicate the position of the detected bands

CONCLUSIONS

Three markers out of 40 markers used in this study (OPA19, OPF09 and OPF12) showed significant linkages with body weight at 4 and 8 weeks of ages, litter weight and gain traits, milk yield at lactation intervals of 0-7 and 0-21 days and pre-weaning litter mortality. RAPD markers can be used in differentiation between animals of Saudi 2 line, and as a marker linked to quantitative traits. Further studies must be continued to get more suitable markers explaining the majority of the variation in different productive traits in rabbits.

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