Identifying the molecular markers of RAPD type linked to resistance to heat stress and daily body gains in rabbits

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Abstract

Five-years crossing scheme involving the Spanish V-line (V) and Saudi Gabali (S) rabbits was practiced to produce new synthetic lines named Saudi-2 with structure of $((¾V¼S)^2)^2$ to be used as a maternal line. A total of 622 animals from Saudi-2 were used to perform both phenotypic and molecular analysis. From Operon Technologies, Alameda, 40 RAPD primers (Random Amplified Polymorphic DNA) were used to amplify to determine which primers can be used as genetic marker to differentiate between individuals of Saudi-2 and to study the association between these markers and daily gain rate traits and measures of respiration rates and body temperatures as indicator to heat stress resistance (respiration rates at 6 and 12 weeks of age, rectum temperature at 6 and 12 weeks of age, ear temperature at 6 and 12 weeks of age). DNA from grandsons of four sires was used to determine which primers out of 40 primers were amplified. Five markers ($OPA12_{1500}$, $OPA19_{1100}$, $OPA20_{1200}$, $OPF09_{700}$ and $OPF12_{900}$) were used later to search for the presence or absence of these markers in 618 grand progenies from the 5th generation of Saudi-2 line. Data of phenotypic values were analyzed using MTDFREML program to estimate the breeding values (EBV) of each animal for each studied trait. Linkage analyses between EBV and present or absent of markers bands were performed finally using multiple regression analysis. In general, results of molecular analyzes indicate that using RAPD markers was successfully linked to both body temperature and daily gain rate traits.

Keywords: Rabbits, Crossbreeding, Heritabilties, EBV, RAPD marker, Heat stress, Daily gain.

Introduction

The modern molecular techniques such as QTL analysis and single marker analysis for carcass traits can provide us with rapid and accurate genetic results without slaughtering the animal. In the last two decades of the last century, the applications of molecular techniques were reached a far away point in cattle (Bennewitz *et al.*, 2003; Zhang *et al.*, 1998; Zhiliang *et al.*, 2007), in sheep (El-Zarei, 2004) or in pig (Zhiliang *et al.*, 2005). In rabbits, the application of molecular techniques is still far from other species. Some studies were made to study the possibility to use RAPD markers to differentiate between individuals or breeds in rabbits (Yang *et al.*, 2000; Pang, 2000), to find the linkage between quantitative traits and RAPD markers (Ren *et al.*, 2008; Khalil *et al.*, 2008), to build a genetic linkage map depending on AFLP techniques and study QTLs linked to this map (Van Haeringen *et al.*, 2002), to study the linkage between microsatellites markers and QTLs (Van Haeringen *et al.*, 2001) and to build a linkage map and finding the suitable markers such microsatellites (Chantry-Darmon *et al.*, 2006).

As reported by many researchers, RAPD technique is one of the suitable techniques for identifying the markers linked to traits of interest without the necessity for mapping the entire genome (Bardakci, 2001). 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 populations that are poorly known genetically, in introgression studies (McCoy and Echt, 1993), and in parental.

The present study aimed to detect the linkage between single molecular markers (RAPD type) and daily gain rate traits and measures of respiration rates and body temperatures as indicator to heat stress resistance (respiration rates at 6 and 12 weeks of age, rectum temperature at 6 and 12 weeks of age, ear temperature at 6 and 12 weeks of age) using multiple regression analysis.

Materials and methods

Crossbreeding program and design of molecular analysis

Five-years crossbreeding project involving a desert Saudi (S) and a Spanish V line (V) was carried out in the experimental rabbitry, College of Agriculture and Veterinary Medicine, Qassim University in Saudi Arabia. This crossbreeding plan permitted simultaneous production of Saudi-2 with a genetic structure of $((¾V¼S)^2)^2$ (Al-Saef *et al.*, 2008). To perform the genetic analysis, DNA from grandsons of four sires of the parental generation (V-line and Saudi purebreds) were used to determine which genetic markers (from 40 markers used) can be used

to differentiate between individuals. To reach a high accuracy of homogeneity within families, 618 grandsons of Saudi-2 produced in the 5th generation were used in the genetic and statistical analyses. Data used in this study included daily gain rate traits (4-6, 6-8, 8-10 and 10-12 weeks daily gain)and measures of respiration rates and body temperatures as indicator to heat stress resistance (respiration rates at 6 and 12 weeks of age, rectum temperature at 6 and 12 weeks of age, ear temperature at 6 and 12 weeks of age).

A total of 618 rabbits from the database of the project were used to estimate heritabilities using single-trait animal model, using MTDFREMEL program of Boldman *et al*. (1995). The animal model (in matrix notation) used for analyzing body temperatures and daily gain rate traits were:

$$\mathbf{y= Xb + Z_a u_a + Z_c u_c + e..... (Model\ 1)}$$

Where y = vector of for the slaughtered rabbits, b = vector of fixed effects of year-season of birth of the slaughtered rabbits, sex, parity order, and litter size in which the rabbit was weaned; u_a = vector of random additive effect of the individual rabbit, u_c = vector of random effects of the litters in which the animal was born; X, Z_a and Z_c are incidence matrices relating the records to the fixed effects, additive genetic effects, and common litter environmental effects, respectively; and **e** is a vector of random residual effects. Variance components of the random effects were estimated by derivate-free restricted maximum likelihood using MTDFREML (Boldman *et al.*, 1995). The inverse of the numerator relationship matrix (A^{-1}) was used with $Var(u_a)= A\sigma^2_a$, $Var(u_c)= I\sigma^2_c$ and $Var(e) = I\sigma^2_e$ representing variance components for additive genetic, common environmental and error effects, respectively. Heritabilities (h^2) were computed from estimates of variance components as (with A augmented for all animals in the pedigree):

$$h^2 = \frac{\hat{\sigma}^2_a}{\hat{\sigma}^2_a + \hat{\sigma}^2_c + \hat{\sigma}^2_e}$$

For estimating the breeding value of each animal for each trait (**EBV**), data were collected on 622 rabbits and the solutions for equations of animals computed by the package of Boldman *et al* (1995) were used as the the breeding values.

Molecular data and analysis of polymorphism

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 (Table 1).

Table 1. Selected Operon primers and its sequence.

RAPD Primers	Sequence (5'-3')	RAPD Primers	Sequence (5'-3')
OPA-01	5'-CAGGCCCTTC-3'	OPF-01	5'-ACGGATCCTG-3'
OPA-02	5'-TGCCGAGCTG-3'	OPF-02	5'-GAGGATCCCT-3'
OPA-03	5'-AGTCAGCCAC-3'	OPF-03	5'-CCTGATCACC-3'
OPA-04	5'-AATCGGGCTG-3'	OPF-04	5'-GGTGATCAGG-3'
OPA-05	5'-AGGGGTCTTG-3'	OPF-05	5'-CCGAATTCCC-3'
OPA-06	5'-GGTCCCTGAC-3'	OPF-06	5'-GGGAATTCGG-3'
OPA-07	5'-GAAACGGGTG-3'	OPF-07	5'-CCGATATCCC-3'
OPA-08	5'-GTGACGTAGG-3'	OPF-08	5'-GGGATATCGG-3'
OPA-09	5'-GGGTAACGCC-3'	OPF-09	5'-CCAAGCTTCC-3'
OPA-10	5'-GTGATCGCAG-3'	OPF-10	5'-GGAAGCTTGG-3'
OPA-11	5'-CAATCGCCGT-3'	OPF-11	5'-TTGGTACCCC-3'
OPA-12	5'-TCGGCGATAG-3'	OPF-12	5'-ACGGTACCAG-3'
OPA-13	5'-CAGCACCCAC-3'	OPF-13	5'-GGCTGCAGAA-3'
OPA-14	5'-TCTGTGCTGG-3'	OPF-14	5'-TGCTGCAGGT-3'
OPA-15	5'-TTCCGAACCC-3'	OPF-15	5'-CCAGTACTCC-3'
OPA-16	5'-AGCCAGCGAA-3'	OPF-16	5'-GGAGTACTGG-3'
OPA-17	5'-GACCGCTTGT-3'	OPF-17	5'-AACCCGGGAA-3'
OPA-18	5'-AGGTGACCGT-3'	OPF-18	5'-TTCCCGGGTT-3'
OPA-19	5'-CAAACGTCGG-3'	OPF-19	5'-CCTCTAGACC-3'
OPA-20	5'-GTTGCGATCC-3'	OPF-20	5'-GGTCTAGAGG-3'

RAPD-PCR reaction mixtures consisted of 2.0 mM $MgCl_2$, 0.1 mM each dNTP (dATP, dCTP, dGTP, and dTTP), arbitrary primers (0.4 µM of single primer was 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 analyses of molecular data

A total of 618 rabbits of a genetic group of $((¾V¼S)^2)^2$ were used in single-marker analyses applying multiple regression analyses to detect the association of the markers to the total phenotypic variation (the model included the 5 markers used) for studied traits using the following model:

$Y = a + b_n x_n + e$..... (Model 2)

Where Y = observed values of the trait; a = intercept; b = Partial regression coefficients of the marker on the trait x, n = number of marker (1….5); e = Random error.

Results and discussion

Description of phenotypic data

Means, standard deviation, minimum and maximum values for different traits were shown in Table 2. The average daily gain in weight of rabbits ranged from 23.40 to 31.75 for DG10-12 and DG4-6, respectively. Respiration rate seem to be the same during the studied periods from 6 to 12 weeks of age (36.38) while ear temperature decrease4 from 37.95 to 37.87. Rectum temperature increasing from 123.72 to 124.65 at the same periods. These results were in agreement with that found by Khalil *et al.*, 2002, whose indicate that in six genetic groups VxV, GxG, GxV, VxG, ¾G¼V and ¾V¼G (V: V-Line; G: Saudi gabali), physiological parameters of rectal and ear temperatures and respiration rates were similar in both strains of the study and no significant differences were observed between the six genetic groups regarding rectal and ear temperatures and respiration rates. Ear temperatures were 38.2, 38.3, 38.3, 38.4, 38.2 and 38.2 ^oC for the same respective groups. Rectal temperatures in different genetic groups were nearly similar and being 36.7, 36.6, 36.7, 36.7, 36.6 and 36.4 ^oC for the same respective groups. The respiration rates were also nearly similar and being 126.4, 125.9, 125.9, 125.5, 128.1 and 124.6 breath/min., respectively. Heritability estimates (Table 2) for respiration rates and body Temperatures traits were low and were mostly moderate for daily gain rates at different studied periods, which make the use of molecular tools very useful to improve such traits.

Table 2. Description of phenotypic data and heritability for different studied trait.

Variable	Mean	Std Dev	Minimum	Maximum	h^2
Daily gain between 4 and 6 weeks of age	31.75	10.06	3.57	50.36	0.31 ± 0.06
Daily gain between 6 and 8 weeks of age	26.85	8.99	2.86	57.86	0.11 ± 0.01
Daily gain between 8 and 10 weeks of age	26.18	10.40	1.43	52.14	0.09 ± 0.01
Daily gain between 10 and 12 weeks of age	23.40	11.02	1.07	46.43	0.11 ± 0.01
Respiration rate at 6 weeks of age	36.38	0.71	34.20	38.40	0.01 ± 0.00
Ear temperature at 6 weeks of age	37.95	0.82	36.40	39.30	0.02 ± 0.00
Rectum temperature at 6 weeks of age	123.72	14.02	80.00	144.00	0.02 ± 0.00
Respiration rate at 12 weeks of age	36.28	0.63	34.20	38.70	0.09 ± 0.00
Ear temperature at 12 weeks of age	37.87	0.82	36.40	39.30	0.05 ± 0.00
Rectum temperature at 12 weeks of age	124.65	13.59	80.00	144.00	0.00 ± 0.00

Analysis of polymorphism

From a total of 40 primers used, five primers (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 primers were further used as genetic markers to check their linkage to the phenotypic traits, using progeny of F_5 from Saudi 2.

These results are in agreement with that found by Queney *et al.* (2001) which determined low polymorphism in rabbits by using microsatellites markers. Chantry-Darmon *et al.* (2006) attributed this trend due to the use of inbred rabbit strains for building the reference families.

Single-marker analyses

Results of single-marker analyses were used to identify the linkage between RAPD markers and variation in phenotypic characters of F_5 progeny. The analysis of variance given in Tables 3 showed significant associations between phenotypic traits and the five markers used ($OPA12_{1500}$, $OPA19_{1100}$, $OPA20_{1200}$, $OPF09_{700}$ and $OPF12_{900}$; $P<0.05$, $P<0.01$ or $P<0.001$).

As shown in Tables 2, $OPA12_{1500}$, $OPA19_{1100}$, $OPA20_{1200}$ markers were found to be linked with respiration rates, ear and rectum temperatures at 6 weeks of age. And $OPF09_{700}$ found to be linked with respiration rates, ear and rectum temperatures at 6 and weeks of age; while $OPF12_{900}$ found to be linked with Body Ear and Rectum Temperatures at 12 weeks of age.

By the other way $OPA12_{1500}$, $OPA20_{1200}$, $OPF09_{700}$ and $OPF12_{900}$ markers found to be linked to daily gain rate (4-6, 10-12; 4-6, 6-8; 6-8, 8-10; and 8-10, 10-12 weeks for the fourth markers respectively). These results are in agreement with that found by El-Zarei 2010, who indicate that using RAPD markers was successfully linked to nine traits out of 16 (pre-slaughter weight, hot carcass weight, offal weight, head weight, fur weight, meat to bone ratio, dry matter %, crude protein %, ether extract % and ash %) by using regression analysis method.

Table 3. Associations between RAPD markers and studied phenotypic traits.

Marker	Variable	P-value	R^2
$OPA12_{1500}$	Daily gain between 4 and 6 weeks of age	0.001	0.14
	Daily gain between 10 and 12 weeks of age	0.01	0.15
	Respiration rate at 6 weeks of age	0.000	0.26
	Ear temperature at 6 weeks of age	0.000	0.23
	Rectum temperature at 6 weeks of age	0.000	0.23
$OPA19_{1100}$	Respiration rate at 6 weeks of age	0.004	0.26
	Ear temperature at 6 weeks of age	0.002	0.23
	Rectum temperature at 6 weeks of age	0.002	0.23
$OPA20_{1200}$	Daily gain between 4 and 6 weeks of age	0.000	0.14
	Daily gain between 6 and 8 weeks of age	0.000	0.34
	Respiration rate at 6 weeks of age	0.004	0.26
	Ear temperature at 6 weeks of age	0.002	0.23
	Rectum temperature at 6 weeks of age	0.002	0.23
$OPF09_{700}$	Daily gain rate between 6 and 8 weeks of age	0.000	0.34
	Daily gain between 8 and 10 weeks of age	0.03	0.27
	Respiration at 6 weeks of age	0.000	0.26
	Ear temperature at 6 weeks of age	0.000	0.23
	Rectum temperature at 6 weeks of age	0.000	0.23
	Respiration rate at 12 weeks of age	0.000	0.12
	Ear temperature at 12 weeks of age	0.000	0.12
	Rectum temperature at 12 weeks of age	0.000	0.12
$OPF12_{900}$	Daily gain between 8 and 10 weeks of age	0.000	0.27
	Daily gain between 10 and 12 weeks of age	0.000	0.15
	Respiration rate at 12 weeks of age	0.04	0.12
	Ear temperature at 12 weeks of age	0.04	0.12
	Rectum temperature at 12 weeks of age	0.04	0.12

R^2 = Percentage of phenotypic variance explained by the RAPD marker

Conclusion

Five primers out of 40 were used in this study (OPA12, OPA19, OPA20, OPF09, and OPF12) and these primers could be used as markers in differentiating between animals of Saudi-2 line since these markers showed significant linkages with respiration rates, body temperatures and daily gain rate traits. Further studies must be continued to get more suitable markers explaining the majority of variations in different productive traits in rabbits.

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