

Detection of DNA alteration in abnormal phenotype of broiler chicken male by random amplified polymorphic DNA (RAPD)

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RAPD technique was used in this study to detect DNA band variations between both normal and abnormal male of broiler chicken based on RAPD marker. DNA polymorphisms between normal and mutant birds were detected using fifteen oligonucleotide primers. Using these primers, DNA band loss ranged from 25 to 75%. Data demonstrated that RAPD marker could detect DNA alterations.

Keywords: DNA alteration, RAPD, abnormal phenotype, male, broiler chicken.

INTRODUCTION

DNA alterations include DNA adducts formation, breaks, point mutations, large rearrangements, and other changes such as structural distortion induced by chemical or physical agents following direct and/or indirect interaction with the genomic DNA. Such alteration can be detected by the random amplified polymorphic DNA (RAPD). The main advantages of the RAPD method lie in its rapidity and applicability to any organism without prior information of the nucleotide sequence. However, the RAPD assays can only provide qualitative results of the extent of the DNA alterations unless the changes occurring in DNA profiles are specifically analyzed by sequencing. The effects of mutations on the RAPD profiles have been previously studied. RAPD profiles deriving from *Escherichia coli* wild and mutant strains, were compared under the hypothesis that stochastic mutations can accumulate at higher rate in the defective strains during cell replication. The RAPD assay was performed on a given DNA template with a set of primers differing by a single base in comparison to the original primer. The changes occurring in RAPD profiles following genotoxic treatments include variation in band intensity as well as gain or loss of bands (Atienzar et al., 2002a). The AP-PCR method was successfully applied to detect x-ray-induced DNA damage (Shimada and Shima, 1998), genetic alterations in human tumors (Ambas et al., 1997) and to evaluate the involvement of a DNA repair gene following genotoxic treatment (Lopez et al., 1999). In addition, the RAPD assay was also used to detect

genetic instability in tumors (Luceri et al., 2000) as well as detected DNA alterations induced by benzo[a]pyrene (Atienzar et al., 2002b), copper (Atienzar et al., 2001), mitomycin C (Becerril et al., 1999), 4-n-nonylphenol and 17- β estradiol (Atienzar et al., 2002c), chrysotile asbestos (Yoshida et al., 2001), UV or X-rays (Atienzar et al., 2000; Jones and Kortenkamp, 2000), and radionuclide exposure (Theodorakis, 2001).

Becerril et al. (1999) have also studied the capability of the randomly amplified polymorphic DNA technique to show genotoxic effects induced by chemicals in fish cells. The results obtained after optimum conditions were established, showed that this system could be useful for the assessment of DNA alterations in vitro genotoxicity studies.

It has been observed that members of the fibroblast growth factor (FGF) family can induce additional limb formation in the flank of chick embryos. The phenotype of the ectopic limb depends on the somite level at which it forms: limbs in the anterior flank resemble wings, whereas those in the posterior flank resemble legs (Ohuchi et al., 1998). This indicated the effectiveness of RAPD in detecting polymorphism in chicken populations, application in population studies, and establishment of genetic relationships among the chicken populations have been previously reported (Sharma et al., 2001).

In the present study, RAPD-PCR technique has been used to detect band variations between both normal and abnormal phenotypes of broiler chicken male based on RAPD marker

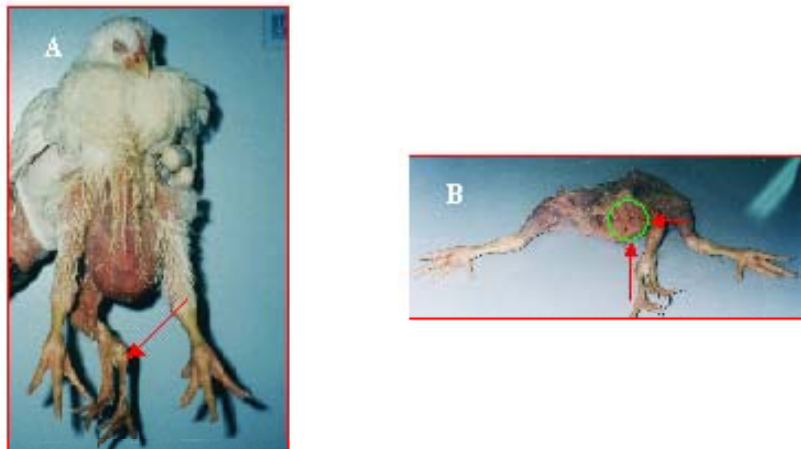


Figure 1. Chicken abnormalities: (A) additional two legs, (B) two cloacal apertures.

Materials and Methods

Birds

Two males (45 days of age) of broiler chicken were obtained from commercial farm in Borg El-Arab, Alexandria, Egypt. The first male was morphologically normal and the second was abnormal, it had two normal and two abnormal legs and two cloacal apertures (Figure 1), and less body weight (624 g) than normal male (1500 g).

DNA extraction

DNA extraction was carried out by method of Sharma et al. (2000) as follows. Venous blood samples were mixed with EDTA as anticoagulant, and stored at -20°C . To an aliquot of 50 μl blood (after thawing), 700 μl of lysis buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0, 0.5% SDS) and 60 μg of proteinase K (20 mg/ml) were added. The mixture was vortexed and incubated at 37°C overnight. DNA was extracted with equal volumes of phenol, phenol-chloroform (1:1) and chloroform-isoamylalcohol (24:1). DNA was precipitated by adding 0.1 by volume of 3 M sodium acetate and 2 volumes of chilled ethanol. The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA). The concentration of DNA and its relative purity were determined using a spectrophotometer based on absorbance at 260 and 280 nm respectively.

RAPD analysis

Random Amplified Polymorphic DNA (RAPD) was carried out using the following random primers (Table 1).

Table 1. The sequences of the fifteen primers used to amplify the chicken DNA.

Primer	Sequence 5'- 3'
1	GGC ACT GAG G
2	CGC TGT CGC C
3	AGT CCT CGC C
4	TGG TGG ACC A
5	GAA TGC GAC G
6	ATG ACG TTG G
7	CTG AGG AGT G
8	GGG CTA GGG T
9	ACC GGG AAC G
10	AGC AGG TGG A
11	AGG CCC CTG T
12	ATG CCC CTG T
13	AGC CAG CGA A
14	AAA GCT GCG G
15	ACC GCC GAA G

The polymerase chain reaction mixture (25 μ L) consisted of 0.8 U of Taq DNA polymerase (Fanzyme), 25 pmol dNTPs, 25 pmol of random primer, and 40 ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycler (Perkin Elmer 9700). The PCR programme included an initial denaturation step at 94°C for 2 min followed by 45 cycles with 94°C for 30 s for DNA denaturation, annealing step at 36°C for 30 s with all 15 primers, extension at 72°C for 30 s and final extension at 72 °C for 10 min were carried out. The amplified DNA fragments were separated on 2.5% agarose gel and stained with ethidium bromide. The amplified pattern was visualized on a UV transilluminator and photographed.

RESULTS AND DISCUSSION

Data presented in Table 2 and Figure 2 show the total number of bands, number of polymorphic bands and percent polymorphism between normal and abnormal male using different random primers. It can be observed that all fifteen primers produced polymorphic bands except two (1 and 15). Polymorphism ranged from 9.09 to 100%. The variation of amplified bands for both normal and abnormal birds. With respect to abnormal male, the loss of bands ranged from 25 to 75%. These results demonstrate that abnormal bird phenotype can be discriminated by RAPD profiles.

Table 2. The total number of bands, polymorphic bands and polymorphism between normal and abnormal male using different random primers.

Primers	Total Bands	Polymorphic Bands	Polymorphism %
1	6	0.0	0.00
2	6	4	66.66
3	5	5	100.00
4	3	1	33.33
5	10	4	40.00
6	8	4	50.00
7	10	2	20.00
8	9	7	77.77
9	13	5	38.46
10	6	2	33.33
11	7	1	14.28
12	8	4	50.00
13	2	2	100.00
14	11	1	9.09
15	6	0.0	0.00

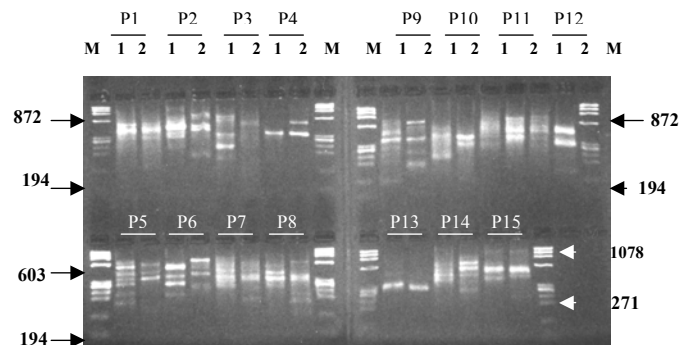


Figure 2. RAPD amplification products generated by different random primers (P1 to P15) Lane M: Φ X174 DNA marker (bp). The two lanes of each primers are: normal male (lane 1) and abnormal male (lane 2).

Data obtained from this study also show that RAPD is a good tool to detect genomic DNA alterations resulting from different environmental and mutagenic agents. The results from this study are in agreement with findings of several researchers who studied capability of RAPD to detect X-ray-induced DNA damage (Shimada and Shima, 1998), and genetic alterations in human tumors (Ambas et al., 1997). In addition, RAPD assay have also used to detect genetic instability in tumors (Luceri et al., 2000) and DNA alterations induced by benzo[a]pyrene (Atienzar et al., 1999; 2002b), copper (Atienzar et al., 2001), mitomycin C (Becerril et al., 1999), chrysotile asbestos (Yoshida et al., 2001), UV or X-rays (Atienzar et al., 2000; Jones and Kortenkamp, 2000), and radionuclide exposure (Theodorakis, 2001). Sharma et al. (2001) have earlier established the effectiveness of RAPD in detecting polymorphism between chicken populations and their applicability in population studies and in the establishment of genetic relationships among the chicken populations. It is important to mention that the results obtained in this study can be extended to further dissect the traits in a more refine way to exactly know which gene (s) pathways are affected. There is also the need to study the sequences of polymorphic bands, to determine the genes detected by the RAPD experiment.

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