DETECTION OF SOME FECUNDITY GENES IN OSSIMI BREED

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Animal genetic resources are essential components of agriculture development. They have contributing to food and agriculture for more than 12000 years. Sheep is one of the small livestock of choice in Egypt, where sheep meat production is more important than fiber production. Egyptian sheep population increased by 66.7% from 1961 to 2005.

There are three major breeds in Egypt; Rahmani, Ossimi, Barki. Barki ewes are sexually maturer earlier compared with Rahmani and Ossimi ewe lambs.

For increasing sheep meat production, the number of born lambs should be increased. This will be achieved through increasing both ovulation rate and litter size, these traits are genetically controlled. So studying genes affecting ovulation rate and litter size, which are known as fecundity genes, become imperative.

Since 1980, there has been increasing interest in the identification and utilization of major genes for prolificacy in sheep. Mutations that increase ovulation rate have been reported in the BMPR-1B (bone morphogenetic protein 1B receptor), BMP15 (Oocyte-derived bone morphogenetic protein 15) and GDF9 (growth differentiation factor 9) genes, and others are known to exist from the expressed inheritance patterns although the mutations have not been pinpointed.

Concerning the types of Fecundity genes in sheep (*FecB, FecX and FecG*^{H}) the FecB was found to result from a mutation in the BMPR-1B (bone morphogenetic protein 1B receptor) (COGNOSAG, 1989), and is considered to be single mutation, duplication, or deletion event (Montgomery *et al*., 1992) that is expresses in oocytes and granulosa cells. Fec B is a dominant single autosomal gene located in chromosome 6, which has an additive effect on ovulation rate. One copy of FecB increases ovulation rate by about 1.5 fold and two copies of 3.0 fold. These extra ovulations in turn increase litter size by 1.0 and 1.5 fold (Davis, 2004). It is hypothesized that this mutation might reduce the signaling through the receptors of granulosa cells (Wilson *et al*., 2001) . The mutation that cause super prolificacy and the mutant sheep are characterized by precocious differentiation of ovarian follicles, leading to the production of large numbers of ovulatory follicles that are smaller in diameter than wild-type follicles.

The Booroola merino was the first breed of sheep where ovulation rate and litter size were shown to be affected by segregating major gene (Piper *et al*., 1985).

Four different fecundityX-linked gene, mutations have been discovered but each produced the same phenotype with four different alleles ($FecX^I$, $FecX^H$, $FecX^G$, $FecX^B$). These are X-linked genes that increase ovulation rates in heterozygotes and cause infertility which might due to streak ovaries in homozygotes. (Davis, 2004)

All these mutations can be detected directly by a PCR restriction fragment length polymorphism RFLP (Davis *et al*., 2002).

This study aimed to investigate the presence of the *FecB* and $FecX^I$ mutations in the main two Egyptian sheep breeds (Ossimi and Barki) through DNA studies using both the simple sequence repeats and forced restriction fragment length polymorphysim techniques by screening these two breeds for the presence of the Booroola fecundity genes.

MATERIALS AND METHODS

Samples collection

Blood samples from two local Egyptian sheep breeds Barki and Ossimi were collected from two different stations. King-mariout station which belongs to the Desert Research Center and Sids station which belongs to Animal Research Institute, Agriculture Research Center. These samples were taken from thirty individuals (ten males, ten females with low twining rate and ten females with high twining rate) from each breed.

Blood samples were collected from each animal by plastic vacuette tubes (Greiner Labortechnik, Tübingen, Germany), which contain EDTA-Na2 (disodium EDTA) or heparin as anticoagulant reagent. Serum of plasma was obtained by centrifugation at 5000 rpm for 15 minutes at 4°C, and the plasma protein (supernatant) was then transferred to clean plastic vials and stored at –20°C until electrophoretic analyses was done. The pellet was to be immediately stored at –80°C for DNA extraction.

Molecular Markers

DNA extraction

DNA extraction was done according to *Sambrook et al.* (1989).

Amplification of genomic DNA using polymerase chain reaction (PCR).

PCR was performed with a final volume of 25-µl according to Williams *et al.* (1990) Amplification reactions were carried out in Techne, TC-312, with a heated lid to reduce evaporation, and the PCR mixture was prepared according to the pamphlet provided with the Taq polymerase enzyme (GoTaq® Flexi DNA polymerase; Catalog# M8301) Purchased from Promega Corporation distributer, and (SuperHOT Master Mix from BIOMOL).

The PCRs were performed into PCR tubes Containing 12.5 µl of PCR mixture as a total volume of GoTaq and 25 µl of PCR mixture as a total volume of SuperHOT Master Mix.

Simple Sequence Repeats (SSR)

Genomic DNA of twenty sampled individuals from the two breeds under study (ten female samples from high twining rate and other ten samples from low twining rate) were used as templates for PCR which are carried out with twelve primers (From BIOMOL) that are specific for twelve microsatellite loci located in *FecB* region. The codes, sequences and annealing temperatures of these primers are shown in Table (1). Amplification reactions were carried out as mentioned before with an initial strand separation cycle at 94°C for 2 min followed by 35 cycles including a denaturation step at 94°C for 30 sec, an variable annealing temperature for 45 sec and polymerization step at 72°C for 2 min, with a final extension step at 72°C for 5 min.

Forced Restriction Fragment Length Polymorphism-PCR (RFLP-PCR)

PCR was carried out using the forced RFLP method described by Galloway *et al*. (2000), Wilson *et al*. (2001), Kumar *et al*. (2005) and Davis *et al*. (2006).

Forced Restriction Fragment Length Polymorphism-PCR for FecB

The forced RFLP-PCR technique was used to detect the *FecB* mutation in bone morphogenetics protein receptor-1B (BMPR-1B) gene of Barki and Ossimi breeds which might showe point mutation at position of 830 bp (A to G transition), which is translated to a change from glutamine to arginine amino acid at position 249 of the mature protein (GenBank Accession No. AF312016). The 140 bp region of BMPR-1B was amplified using F-12 and R-15 primers from BIOMOL described by Wilson *et al*. (2001) with the following sequences:

F-12: (5' GTCGCTATGGGGAAGTTTGGATG 3')

R-15: (5' CAAGATGTTTTCATGCCTCATCAACACGGTC 3')

About 100ng of template DNA was put in 25µl reaction volume from SuperHOT Master Mix (from BIOMOL) in PCR with the following amplification conditions; initial denaturation at 94°C for 1 min, followed by 94°C for 15 s, 60°C for 30 s, 72°C for 30 s for 35 cycle and final extension at 72°C for 5 min. The products were tested in 3% agarose gel and then 10 µl PCR products was digested with *AvaII* enzyme from Promega Catalog#R6131 with recognition sequence (G/GACC) mixture which was prepared according to the pamphlet at 37°C for 2 h and loaded in 3.6% agarose gel with 5 µl DNA molecular size marker (20 base pair ladder ready-to-use) from jena Bioscience GmbH which yields the following 10 discrete fragments 200, 180, 160, 140, 120, 100, 80, 60, 40 and 20 bp. Wild type Booroola alleles (normal) are (+) and (B)

individual was carried booroola mutation. After digestion, the "BB" individual (homozygous carrier, High ovulation rate phenotype) is supposed to show a 110 bp band, "B+" (heterozygous carrier, Intermediate phenotype) is supposed to show 140 and 110 bp bands and the "++" animals (homozygous non-carrier, Low ovulation rate phenotype) should reveal uncut 140 bp band.

Forced Restriction Fragment Length Polymorphism-PCR for FecX^I

The forced RFLP-PCR technique was used to detect the *FecX^I* described by Galloway *et al*. (2000). Mutation in Oocyte-derived bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) are key regulators of follicular development.

Genomic DNA was amplified using Primer 12 (5' GAAGTAACCAGTGTTCCCTCCACCCTTTTCT 3') and Primer 13 (5' CATGATTGGGAGAATTGAGACC 3') of BIOMOL and the expected size for PCR product is 154 bp. Primer 12 has been designed to generate a forced *XbaI* restriction enzyme site (T/CTAGA) in PCR products from carriers of the *FecX^I* mutation in the BMP15 gene, whereas products from non-carriers of the mutation lack this site. About 100 ng of template DNA was analyzed in 25 µl reaction volume of SuperHOT Master Mix from BIOMOL in PCR with the following amplification conditions; initial denaturation at 94°C for 1 min, followed by 94°C for 15 s, 62°C for 30 s, 72°C for 30 s for 35 cycle and final extension at 72°C for 5 min. The products was tested in 3% agarose gel and then 10 µl PCR products was digested with *XbaI* enzyme of Promega Catalog#R6181, mixture was prepared according to the pamphlet at 37°C for 2 h and loaded in 3.6% agarose gel with 5 µl DNA molecular size marker (20 base pair ladder ready-to-use) from jena Bioscience GmbH yields the following 10 discrete fragments (in base pairs): 200, 180, 160, 140, 120, 100, 80, 60, 40 and 20 bp. Products containing the FecX mutation yield of 124 bp and 30 bp fragments, non-carrier products remain uncut at 154 bp.

RESULTS AND DISCUSSION

Simple Sequence Repeats (SSR)

To study and detect the fecB mutation in ossimi breed, 12 specefic SSR primers were chosen according to Mulsant *et al*. (2001). All primers succeded in amplifying DNA fragments. Polymorphism levels differed from one primer to the other. The average number of alleles per locus ranged from 1 to 7 according to the involved locus as shown in Figs (1-4). The following is a description of the breeds profile against the used primers which showed differcies between and within breeds which could be used to caculate heterozygosity ratio.

Heterozygosity ratio in Ossimi sheep breed

High twining rate Ossimi females showed more than one allele and gave

differences between homozygote and heterozygote for *FecB*, for primer 6 (Fig. 1 and Table 2) samples No. 2, 6 and 8 gave two different bands with sizes of 149 and 193 bp with heterozygosity ratio of 30% but primer 11 gave a heterozygote bands in samples 3 with molecular sizes of 127 and 136 bp, sample 4 with molecular sizes of 96 and 143 bp, sample 5 with molecular sizes of 103 and 113 bp, sample 6 with molecular sizes of 87 and 103 bp, sample 7 with molecular sizes of 100 and 127 bp, sample 8 with molecular sizes 100 and 127 bp, and sample 9 with molecular sizes of 87 and 103 bp and gave heterozygosity ratio of 70%, therefore the average heterozygosity ratio for Ossimi high twining rate females was approximatly 50%.

Ossimi low twining rate females (Fig. 2 and Table 3) showed heterozygosity in *FecB* locus for four primers (6, 7, 10 and 11). Primer 6 with sample 4 with molecular sizes of 149 and 229 bp, samples 6 and 7 with molecular sizes of 85 and 149 bp and sample 9 with molecular sizes of 69 and 125 bp and with heterozygosity ratio of 40%. Primer 7 in all samples gave bands with molecular sizes of 100, 210 and 870 bp with heterozygosity ratio of 100%. Primer 10 with samples 1, 3, 6, 7 and 8 gave bands with molecular sizes of 137 and 154 bp with heterozygosity ratio of 50%,. Primer 11 with samples 8 and 3 with molecular sizes of 86 and 137 bp, and sample 6 with molecular sizes of 81 and 137 bp and with heterozygosity ratio of 30%, thus the average of heterozygosity ratio for Ossimi low twining rate females was above 55%.

Barki high twining rate females showed more than one locus and gave differences between homozygote and heterozygote for *FecB* (Fig. 3 and Table 4).For primer 6; samples 3 and 4 gave two different heterozygotes with sizes of 145 and 166bp, sample 5 gave bands with molecular sizes of 108, 140 and 156 bp. sample 7 gave bands with molecular sizes of 140 and 156 bp. Sample 8 gave bands with molecular sizes of 126 and 140 bp. Sample 9 gave bands with molecular sizes of 140 and154 bp. The heterozygosity ratio of this primer was 60% but with primer 7 all samples gave bands with molecular sizes of 319 and 202 bp with heterozygosity ratio of 100%. Primer 10 with sample 1 gave two different bands with sizes of 172 and 204 bp and heterozygosity ratio of 10%. On the other hand, primer 11 with samples 1 and 2 gave two different bands with sizes of 104 and 149bp, sample 3 gave bands with molecular sizes of 126 and 169bp, sample 5 gave bands with molecular sizes of 118 and 146 bp, sample 7 gave bands with molecular sizes of 146 and 169 bp, Sample 8 gave bands with molecular sizes of 104 and146bp, and samples 9 and 10 gave bands with molecular sizes of 118 and 146 bp, with heterozygosity ratio of 80%. Thus the average of heterozygosity ratio for Barki high twining rate females was approximetly 62.5%.

Barki low twining rate females (Fig. 4 and Table 5) showed more than one locus and gave differences between homozygote and heterozygote for *FecB*, for primer 7 all samples except samples 3 and 8 gave four different bands with molecular sizes of 107, 139, 173 and 210 bp, with heterozygosity ratio of 80%. but primer 11 with sample 1 gave two different bands with sizes 112 and 175 bp, sample 2 gave bands with molecular sizes of 125 and 175 bp, sample 4 gave bands with molecular sizes of 145 and 183 bp, sample 6 gave bands with molecular sizes of 112 and 183 bp, sample 9 gave bands with molecular sizes of 158 and 191 bp, and samples 10 gave bands with molecular sizes of 145 and 183 bp, the heterozygosity ratio was 60%. Thus the average of heterozygosity ratio for Barki low twining rate females was 70%.

Regarding all the SSR obtained data it could be concluded that lower heterozygosity ratio for females with high twining rate from both breeds under study (Ossimi and Barki) in comparison with females with low twining rate might be due to high inbreeding since the breeder always tends mate to between similar trait in this concern. This was in disagreement with Jandurova *et al*. (2004) who analyzed microsatellite variation in two breeds of goat and calculated heterozygosity (H). They found that both breeds exhibited relatively high level of heterozygosity . However, Sodhi *et al*. (2005) characterized the Nali and Chokla thin tailed, brown-faced sheep breed from arid and semi-arid region of India using 25 specific Microsatellite markers. Their results revealed high level of genetic variability in each of the two investigated breeds. Low level of genetic differentiation between Nali and Chokla sheep was evident from low genetic differentiation estimates. Population inbreeding estimates indicated considerable level of inbreeding and high genetic homogeneity in the investigated sheep populations.

Forced Restriction Fragment Length Polymorphism-PCR (RFLP-PCR)

The forced RFLP-PCR technique was used to detect the *FecB* mutation in bone morphogenetics protein receptor-1B gene (BMPR-1B) and the $FecX^{I}$ mutation in Oocyte-derived bone morphogenetic protein 15 (BMP15) of Barki and Ossimi breeds.

Forced Restriction Fragment Length Polymorphism-PCR for FecB of Ossimi and Barki breed

Figures (5, 7, 10 and 12) showed the *FecB* genotyping of 10 samples from each of Ossimi high twining rate females, Ossimi low twining rate females, Barki high twining rate females and Barki low twining rate, respectively, before digestion with *AvaII* as a restriction enzyme. The expected product is only one band with molecular size of 140bp and the profile showed that all the studied samples gave this expected result.

However, after digestion with *AvaII* restriction enzyme, the expected results are as follows: “BB” individual

(homozygous) is supposed to show a 110 bp band, "B+" (heterozygous carrier) is supposed to show 140 and 110 bp bands and the "++" animals (homozygous non-carrier) would show uncut 140 bp band. Figure (6) shows the results of Ossimi high twining rate females samples after digestion with *AvaII* which gave only one band at 140bp. Figure (11) shows the results of Barki high twining rate females samples after digestion with *AvaII* which also gave only one band. Thus, the mutation in bone morphogenetics protein receptor-1B gene is not present in either Ossimi or Barki breeds.

Forced Restriction Fragment Length Polymorphism-PCR for FecX^I of Ossimi and Barki breed

Figures (8, 9,13 and 14) showed the *FecX*^{I} genotyping of 10 samples from each of Ossimi high twining rate females, Ossimi low twining rate females, Barki high twining rate females, and Barki low twining rate, respectively. Before digestion with *XbaI* as a restriction enzyme. The expected product is only one band with molecular size of 154 bp. The profile show that all the studied samples gave this expected result.

However, after digestion with *XbaI* the expected result is products containing the *FecX*^{I} mutation yield (124 bp and 30 bp fragment), whilst non-carrier products would remain uncuted (154 bp). Thus, from the results mentioned above, it could be concluded that the mutation in oocyte-derived bone morphogenetic protein 15 (BMP15) of Barki and Ossimi breeds are not found.

Therefore, the results of the present study indicated that both the *FecB* and *FecX*^{I} mutatios did not exist in either Ossimi or Barki Egyptian breeds. The obtained results were as expected ,since we have chosen the high twining rate females of either breed to give birth to two lambs which is available in Egypt. However, the high twining rate breeds should give birth to more than three as usually happens in such studies.

SUMMARY

The present study revealed that the Egyptian sheep breeds represented by the two breeds (Ossimi and Barki) do not contain either the *FecB* or the *FecX*^{I} mutation which are responsible for high fecundity. This would explain the reason to the low twining rate in the Egyptian sheep breeds. These results are in agreement with Davis *et al*. (2002) who reported that the *FecB* mutation was found in the Garole and Javanese sheep but not in Thoka, Woodlands, Olkuska, Lacaune, Belclare, and Cambridge sheep. None of the sheep tested had the *FecX*^{I} mutation. Davis *et al*. (2006) suggested that the presence of *FecB* in Booroola Merino and Javanese sheep probably traced back to the Garole sheep of Bengal, because these breeds share a common ancestor. The absence of the *FecB* and *FecX*^{I} mutations in the other prolific breeds does not preclude major gene effects on prolificacy in some of these breeds, and more extensive scree-

ning is required to test for newly discovered mutations.

Therefore, for the improvement of this reproductive trait, crossing programs with the high prolific breeds as Booroola merino could be effective. However, prospective studies will be needed for the capability of transferring the Booroola gene using the more sophisticated genetic engineering techniques.

REFERENCES

COGNOSAG (1989). Standardized Genetic Nomenclature for Sheep and Goats. Lavoisier, Paris

Davis, G. H., S. M. Galloway, I. K. Ross, S. M. Gregan, J. Ward, B. V. Nimbkar, P. M. Ghalsasi, C. Nimbkar, G. D. Gray, Subandriyo, I. Inounu, B. Tiesnamurti, E. Martyniuk, E. Eythorsdottir, Ph. Mulsant, F. Lecerf, J. P. Hanrahan, G. E. Bradford and T. Wilson (2002). DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction, 66: 1869-1874.

Davis, G. H. (2004). Fecundity genes in sheep. Animal Reproduction Science, 82: 247–253.

Davis, G. H., L. Balakrishnan, I. K. Ross, T. Wilson, S. M. Galloway, B. M. Lumsden, J. P. Hanrahan, M. Mullen, X. Z. Mao, G. L. Wang, Z. S. Zhao, Y. Q. Zeng, J. J. Robinson, A. P. Mavrogenis, C. Papachristoforou, C. Peter, R. Baumung, P. Cardyn, I. Boujenane, N. E. Cockett, E. Eythorsdottir, J. J. Arranz and D. R. Notter (2006). Investigation of the Booroola (*FecB*) and Inverdale (*FecXI*) mutations in 21 prolific breeds and strains of sheep sampled in 13 countries. Animal Reproduction Science, 92: 87-96.

Galloway, S. M., K. P. McNatty, L. M. Cambridge, M. P. E. Laitinen, J. L. Juengel, T. S. Jokiranta, R. J. McLaren, K. Luiro, K. G. Dodds and G. W. Montgomery (2000). Mutation in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. Nat. Genet., 25: 279-283.

Jandurova, O. M., T. Kott, B. Kottova and V. Czernekova (2004). Seven Microsatellite markers useful for determining genetic variability in White and Brown Short-Haired goat breeds. Small Ruminant Research, 52: 271-274.

Kumar, S., A. P. Kolte, A. K. Mishra, A. L. Arora and V. K. Singh (2005). Identification of the *FecB* mutation in Garole×Malpura sheep and its effect on litter size. Small Ruminant Research, 2-6

Montgomery, G. W., K. P. McNatty and G. H. Davis (1992). Physiology and molecular genetics of muta-

tions that increase ovulation rate in sheep. Endocr. Rev., 113: 309-328.

Mulsant, P., F. Lecerf, S. Fabre, L. Schibler, P. Monget, I. Lanneluc, C. Pisselet, J. Riquet, D. Monniaux, I. Callebaut, E. Cribiu, J. Thimonier, J. Teyssier, L. Bodin, Y. Cognie, N. Chitour and J. M. Elsen (2001). Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. Proceeding of the National Academic of Sciences (PNAS), 98: 5104-5109.

Piper, L. R., B. M. Bindon and G. H. Davis (1985). The single gene inheritance of the high litter size of the Booroola Merino. In: Land, R. B., Robinson, D. W., (Eds), Genetics of reproduction in sheep. Butterworths, London, UK, p. 115-125.

Sambrook, J., E. F. Fritsch and T. Maniatis (1989). In Molecular Cloning: a laboratory manual, Cold spring harbor laboratory press, New York.

Sodhi, M., M. Mukesh and S. Bhatia (2005). Characterizing Nali and Chokla sheep differentiation with microsatelitte markers. Small Ruminant Research.

Williams, J. G. K, A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuc. Aci. Res., 18: 6531-6535.

Wilson, T., W. X. Yang, J. L. Jennifer, K. I. Ross, M. L. Joanne, A. E. lord, G. K. Dodds, A. G. Walling, C. J. McEwan, R. A. O'Connell, P. K. McNatty and G. W. Montgomery (2001). Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK_6) that is expressed in both oocytes and granulosa cells, Biology of Reproduction, 64: 1225-1235.

Table (1): Codes, sequences and annealing temperatures of primers used during SSR assays.

code	Name	Type	sequences	Alleles	Tm
1	320R	snp	5' TCACCTTGAATCATTTCTTTAG 3' 5' GTGGTGTTTGGAACTTTGTAA 3'	4	52
2	300R	snp	5' CCAAACATCAGCAAACCATAA 3' 5' CTGTGACCATCTCTCTGTG 3'	3	52
3	OB2	snp	5' AGATTGGAAAAGGTCGCTATG 3' 5' ACCCTGAACATCGCTAATACA 3'	2	57
4	OB1	snp	5' CAGAACGGAATGAATGTAATAA 3' 5' AGAACAGGGTAAGGAAGTAAA 3'	4	52
5	Bulge5	ms	5' GACAAACATTCGGACATGACC 3' 5' TGAGGAGGGCACATCCATTG 3'	6	57
6	300U	ms	5' GGGGGTTCCTTGTAGGTTTGTG 3' 5' GGAAGTGCAGAGAGTCCCATAC 3'	12	57
7	UNC5C (GC101)	ms	5' ATCCTCACCCTTCAAACAG 3' 5' CTGGGGAGTTTTCTCTGAC 3'	7	57
8	471U	ms	5' CTACAATAAATAATGAGGTGAAA 3' 5' TGAGTAGAGACAAAGCTATAAA 3'	3	52
9	ETL1	snp	5' CATCAATGGGCCTGTGAAAT 3' 5' CAAACAATCACCAACTAATCC 3'	2	52
10	BMS2508	ms	5' AGGTTGACTTCTGTGTCTTTT 3' 5' GTTTCTTAGGGGAGTGTTGATT 3'	3	55
11	LSCV043	ms	5' CCAGAATATAGAGTTTTGTCAAG 3' 5' GCCTGATTTGTATTTGTATGA	6	55
12	UNC5C (GC102)	snp	5' TTCCCTCTTGCTGTATCTTCT 3' 5' AAACACTGGGTGACTGACTC 3'	3	57

Table (2): SSR banding patterns of Ossimi high twining rate females produced by primers from 1 to 12.

Primer No.	MS (bp)	1	2	3	4	5	6	7	8	9	10
1	233	+	+	+	+	+	+	+	+	+	+
2	315	+	+	+	+	+	-	-	+	-	-
	350	-	-	-	-	-	+	+	-	+	+
3	163	-	-	-	-	-	-	+	+	+	+
	179	+	+	+	+	+	+	-	-	-	-
4	580	+	+	+	+	+	+	+	+	+	+
5	134	+	-	-	+	-	+	+	+	+	+
	142	-	+	+	-	-	-	-	-	-	-
	157	-	-	-	-	+	-	-	-	-	-
6	143	-	-	-	+	-	-	-	-	-	-
	149	-	+	-	-	+	+	+	+	-	-
	158	+	-	+	+	-	-	-	-	-	+
	163	-	-	-	-	+	+	-	-	-	-
	193	-	+	-	-	-	-	-	-	-	-
7	220	+	+	+	+	+	+	+	+	+	+
8	188	+	+	+	+	+	+	+	+	+	+
9	286	+	+	+	+	+	+	+	+	+	+
10	158	-	+	+	+	-	-	-	-	-	+
	161	-	-	-	-	-	-	+	+	-	-
	177	-	+	+	+	+	+	-	-	+	+
11	87	-	-	-	-	-	+	-	-	+	-
	96	-	-	-	+	-	-	-	-	-	-
	100	-	-	-	-	-	-	+	+	-	-
	103	-	-	-	-	+	+	-	-	+	-
	113	-	-	-	-	+	-	-	-	-	+
	127	+	+	+	-	-	-	+	+	-	-
	136	-	-	+	-	-	-	-	-	-	-
	143	-	-	-	+	-	-	-	-	-	-
12	320	+	+	+	+	+	+	+	+	+	+

Table (3): SSR banding patterns of Ossimi low twining rate females produced by primers from 1 to 12.

Primer No.	MS (bp)	1	2	3	4	5	6	7	8	9	10
1	237	+	+	+	+	+	+	+	+	+	+
2	242	+	+	+	+	+	+	+	+	+	+
3	185	+	+	+	+	+	+	+	+	+	+
4	283	+	+	+	+	+	+	+	+	+	+
5	170	+	+	+	+	+	+	+	+	+	+
6	69	-	-	-	-	-	-	-	-	+	-
	85	-	-	-	-	-	+	+	-	-	-
	125	-	-	-	-	-	-	-	+	+	+
	149	-	-	-	+	+	+	+	-	-	-
	165	-	-	+	-	-	-	-	-	-	-
	173	+	-	-	-	-	-	-	-	-	-
	187	-	+	-	-	-	-	-	-	-	-
	229	-	-	-	+	-	-	-	-	-	-
7	100	+	+	+	+	+	+	+	+	+	+
	210	+	+	+	+	+	+	+	+	+	+
	870	+	+	+	+	+	+	+	+	+	+
8	185	+	+	+	+	+	+	+	+	+	+
9	300	+	+	+	+	+	+	+	+	+	+
10	137	+	-	+	+	-	+	+	+	+	+
	154	+	+	+	-	-	+	+	+	-	-
11	46	-	-	-	-	-	-	-	-	-	+
	81	-	-	-	-	-	+	+	-	-	-
	86	-	-	+	-	-	-	-	+	-	-
	100	-	-	-	-	-	-	-	-	+	-
	113	-	-	-	-	+	-	-	-	-	-
	137	-	+	+	+	-	+	-	+	-	+
12	310	+	+	+	+	+	+	+	+	+	+

Table (4): SSR banding patterns of Barki high twining rate females produced by primers from 1 to 12.

Primers No.	MS(bp)	1	2	3	4	5	6	7	8	9	10
1	246	+	+	+	+	+	+	+	+	+	+
2	330	+	+	+	+	+	+	+	+	+	+
3	190	+	+	+	+	+	+	+	+	+	+
4	300	+	+	+	+	+	+	+	+	+	+
5	134	+	-	-	+	-	+	+	+	+	+
	142	-	+	+	-	-	-	-	-	-	-
	157	-	-	-	-	+	-	-	-	-	-
6	108	-	-	-	-	+	-	-	-	-	-
	126	-	-	-	-	-	-	-	+	-	-
	140	-	-	-	-	+	+	+	+	+	-
	145	-	-	+	+	-	-	-	-	-	-
	149	+	+	-	-	-	-	-	-	-	-
	154	-	-	-	-	-	-	-	-	+	+
	156	-	-	-	-	+	-	+	-	-	-
	166	-	-	+	+	-	-	-	-	-	-
7	319	+	+	+	+	+	+	+	+	+	+
	202	+	+	+	+	+	+	+	+	+	+
8	190	+	+	+	+	+	+	+	+	+	+
9	290	+	+	+	+	+	+	+	+	+	+
10	157	-	-	-	-	-	+	-	+	-	-
	166	-	-	-	-	-	-	-	-	+	+
	172	+	-	-	-	-	-	-	-	-	-
	178	-	-	-	-	-	-	+	-	-	-
	182	-	+	+	+	-	-	-	-	-	-
	188	-	-	-	-	+	-	-	-	-	-
	204	+	-	-	-	-	-	-	-	-	-
11	104	+	+	-	-	-	-	-	+	-	-
	118	-	-	-	-	+	-	-	-	+	+
	126	-	-	+	-	-	-	-	-	-	-
	146	-	-	-	+	+	-	+	+	+	+
	149	+	+	-	-	-	-	-	-	-	-
	160	-	-	-	-	-	+	-	-	-	-
	169	-	-	+	-	-	-	+	-	-	-
12	310	+	+	+	+	+	+	+	+	+	+

Table (5): SSR banding patterns of Barki low twining rate females produced by primers from 1 to 12.

Primer No.	MS(bp)	1	2	3	4	5	6	7	8	9	10
1	140	+	+	+	+	+	+	+	+	+	+
2	290	+	+	+	+	+	+	+	+	+	+
3	190	+	+	+	+	+	+	+	+	+	+
4	140	+	+	+	+	+	+	+	+	+	+
5	150	+	+	+	+	+	+	+	+	+	+
6	128	-	-	-	-	-	+	+	-	-	+
	132	-	-	-	-	+	-	-	-	-	-
	149	+	+	+	-	-	-	-	+	+	-
	162	-	-	-	+	-	-	-	-	-	-
7	107	+	+	-	+	+	+	+	-	+	+
	139	+	+	-	+	+	+	+	-	+	+
	173	+	+	-	+	+	+	+	-	+	+
	210	+	+	+	+	+	+	+	+	+	+
8	190	+	+	+	+	+	+	+	+	+	+
9	290	+	+	+	+	+	+	+	+	+	+
10	190	-	+	+	+	+	+	+	+	+	+
11	112	+	-	+	-	-	+	-	-	-	-
	125	-	+	-	-	+	-	-	-	-	-
	145	-	-	-	+	-	-	+	+	-	+
	158	-	-	-	-	-	-	-	-	+	-
	175	+	+	-	-	-	-	-	-	-	-
	183	-	-	-	+	-	+	-	-	-	+
	191	-	-	-	-	-	-	-	-	+	-
12	295	-	+	+	+	+	+	+	+	+	+
	300	+	-	-	-	-	-	-	-	-	-

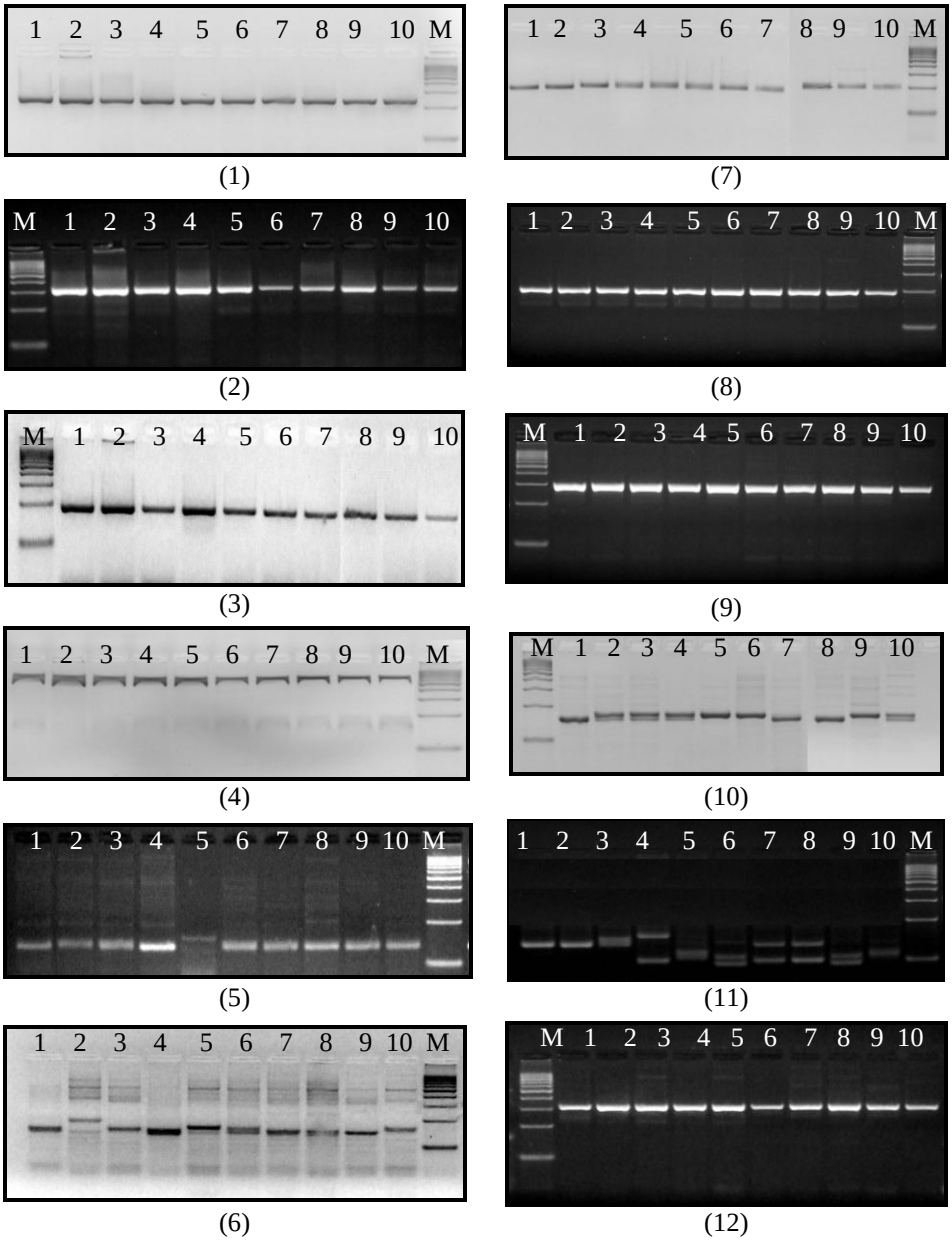

Fig. (1): Electrophoretic patterns of Ossimi (high twining rate female) against twelve primers. M= molecular weight marker (100 bp ladder).

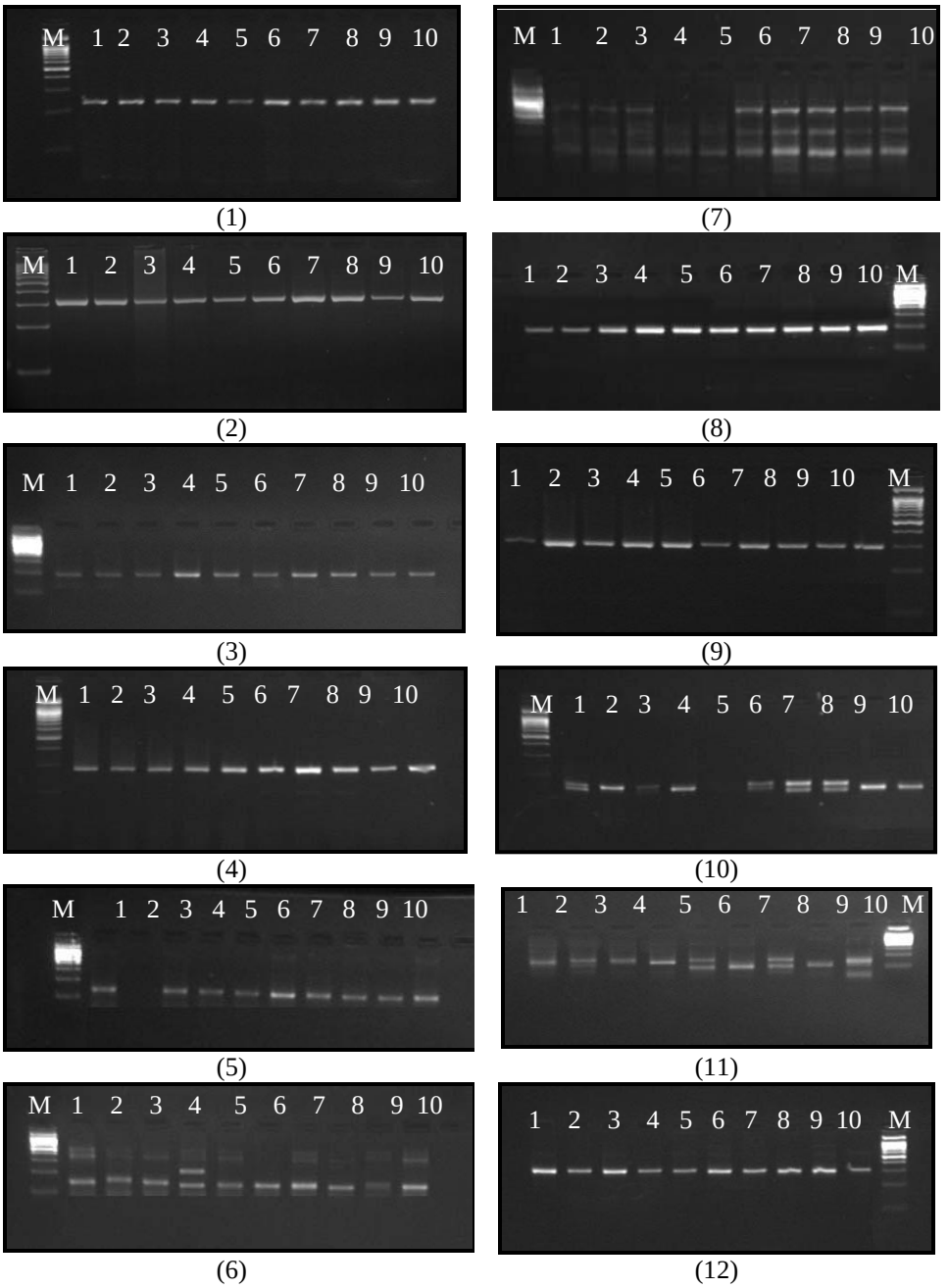

Fig. (2): Electrophoretic patterns of Ossimi (low twining rate female) against twelve primers. M= molecular weight marker (100 bp ladder).

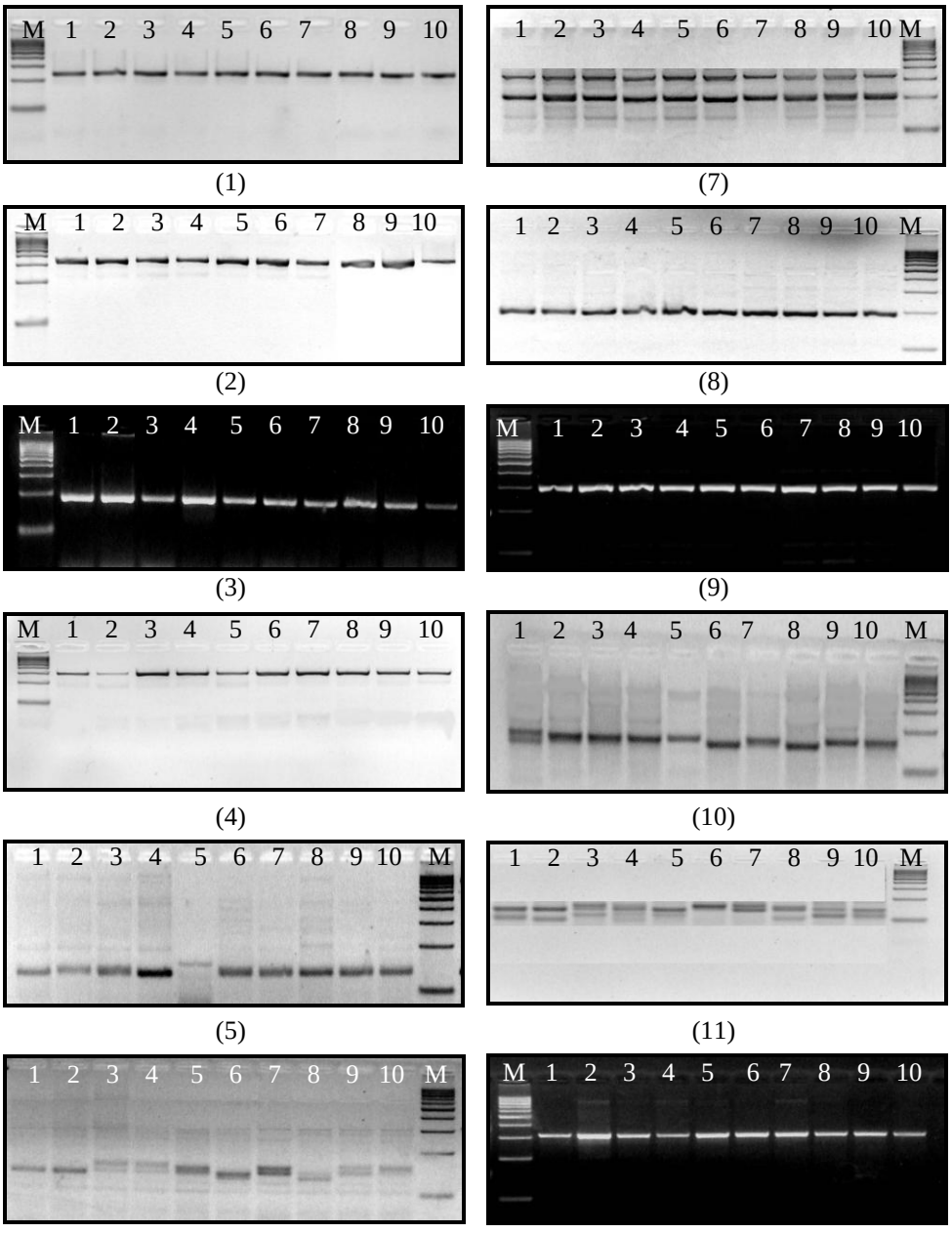

Fig. (3): Specefic SSR primers electrophoretic patterns of Barki (high twining rate females) against twelve primers. M= molecular size marker (100 bp ladder).

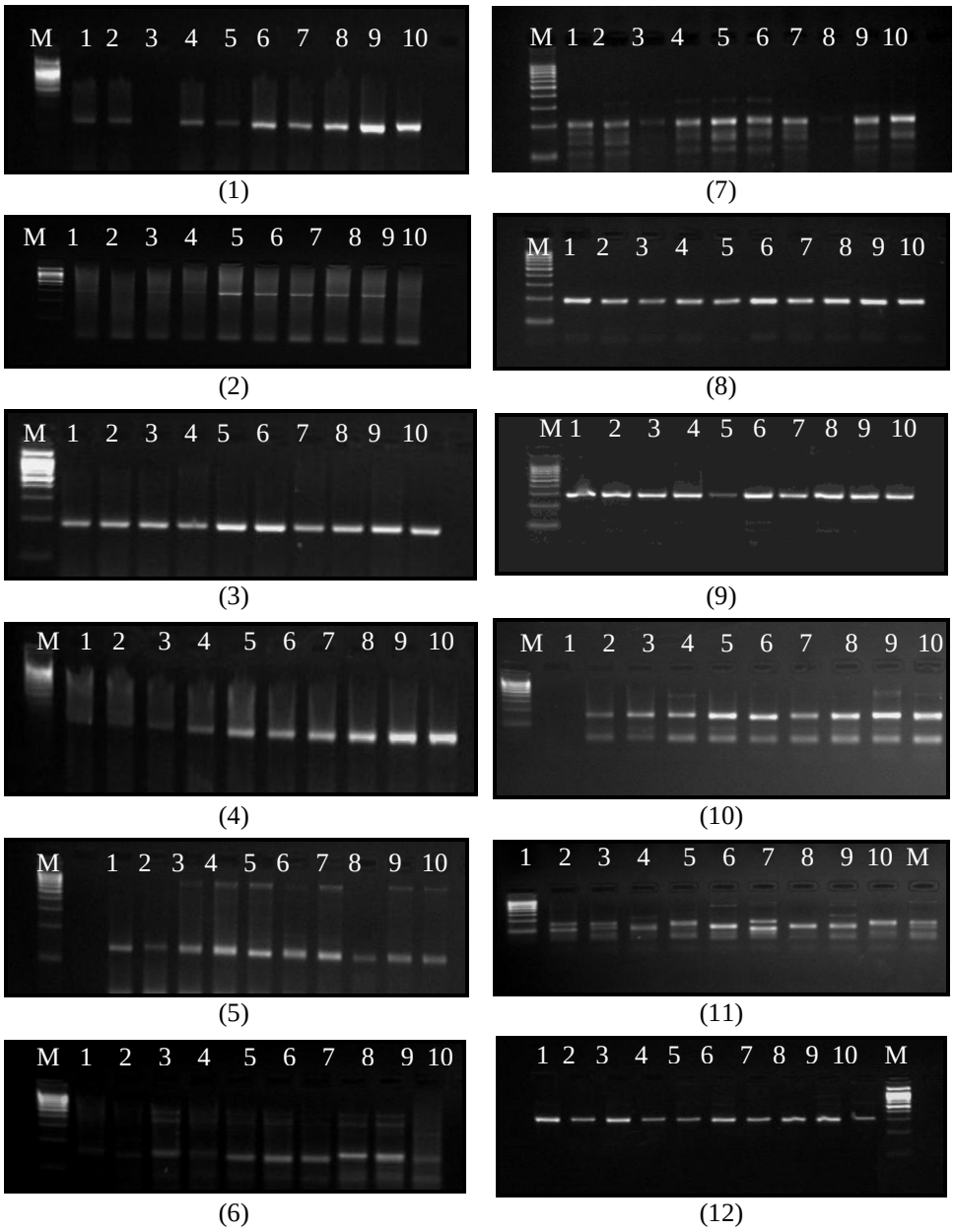

Fig. (4): Specefic SSR primers electrophoretic patterns of Barki (low twining rate females) against twelve primers. M= molecular size marker (100 bp ladder).

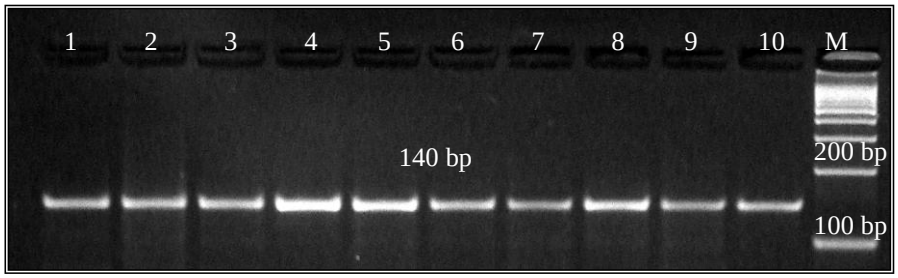

Fig. (5): Detection of *FecB* mutation by Forced-RFLP test. Amplification of genomic DNA from Ossimi breed (high twining rate females) before digestion with *AvaII*. M= molecular size marker (100 bp ladder)

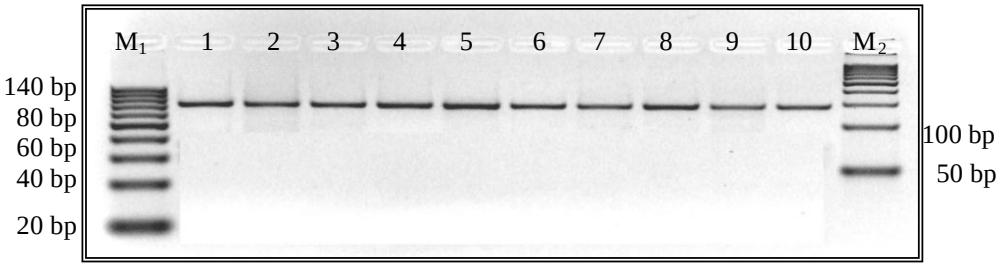

Fig. (6): Detection of *FecB* mutation by Forced-RFLP test. Amplification of genomic DNA from Ossimi breed (high twining rate females) after digestion with *AvaII*. M1= molecular size marker (20 bp ladder), M2 = molecular size marker (50 bp ladder).

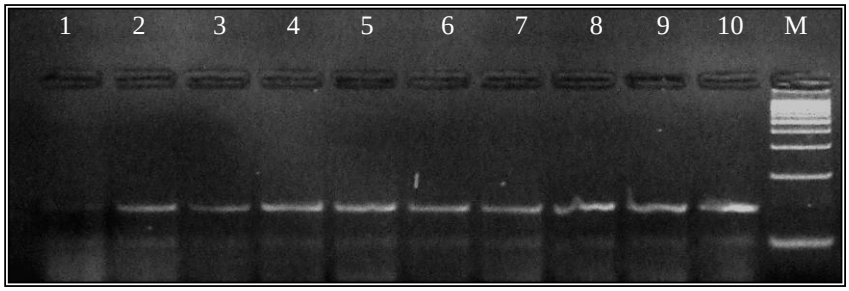

Fig. (7): Detection of FecB mutation by Forced-RFLP test. Amplification of genomic DNA from Ossimi breed (low twining rate females) M= molecular size marker (100 bp ladder).

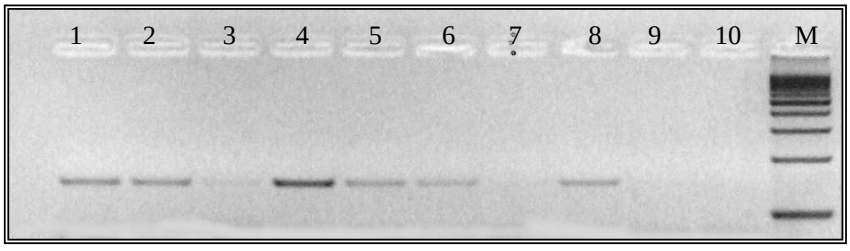

Fig. (8): Detection of $FecX^{I}$ mutation by Forced-RFLP test. Amplification of genomic DNA from Ossimi breed (high twining rate females) M= molecular size marker (100 bp ladder).

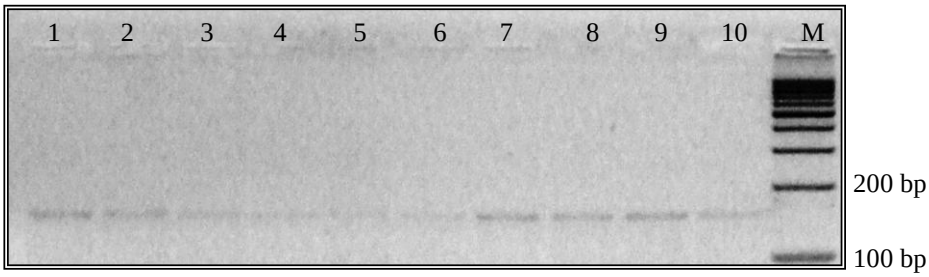

Fig. (9): Detection of *FecX*^{I} mutation by Forced-RFLP test. Amplification of genomic DNA from Ossimi breed (low twining rate females) M= molecular size marker (100 bp ladder).

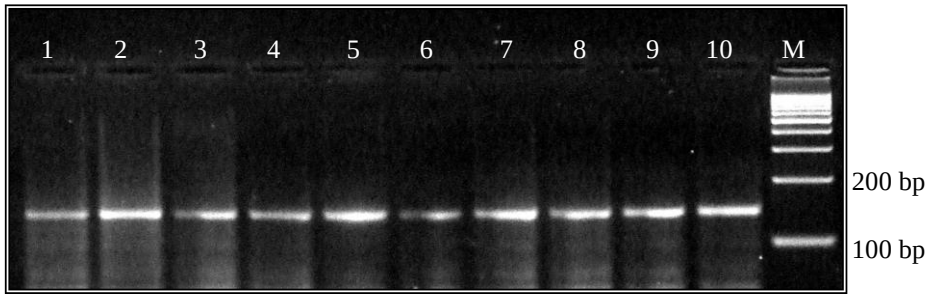

Fig. (10): Detection of *FecB* mutation by Forced-RFLP test. Amplification of genomic DNA from Barki breed (high twining rate females) before digestion with *AvaII*, M= molecular size marker (100bp ladder).

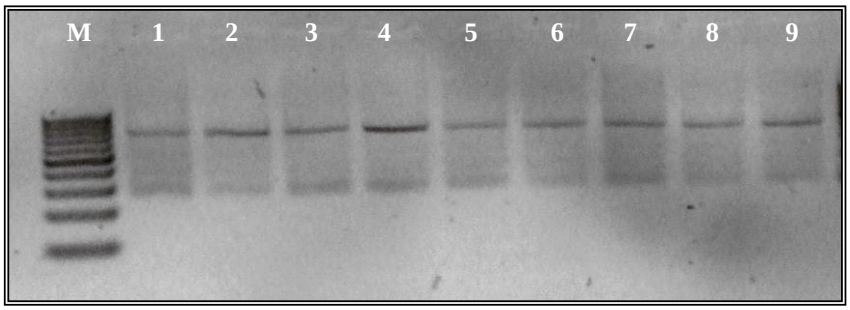

Fig. (11): Detection of *FecB* mutation by Forced-RFLP test. Amplification of genomic DNA from Barki breed (high twining rate females) after digestion with *AvaII*. M= molecular weight marker (20bp ladder).

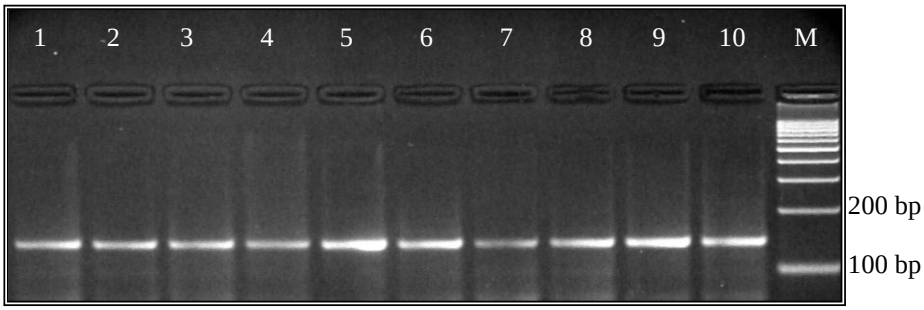

Fig. (12): Detection of *FecB* mutation by Forced-RFLP test. Amplification of genomic DNA from Barki breed (low twining rate females) M= molecular size marker (100 bp ladder).

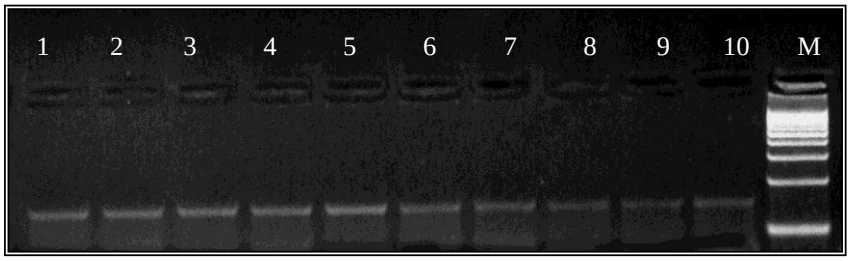

Fig. (13): Detection of *FecX*^{I} mutation by Forced-RFLP test. Amplification of genomic DNA from Barki breed (high twining rate females) M= molecular size marker (100 bp ladder).

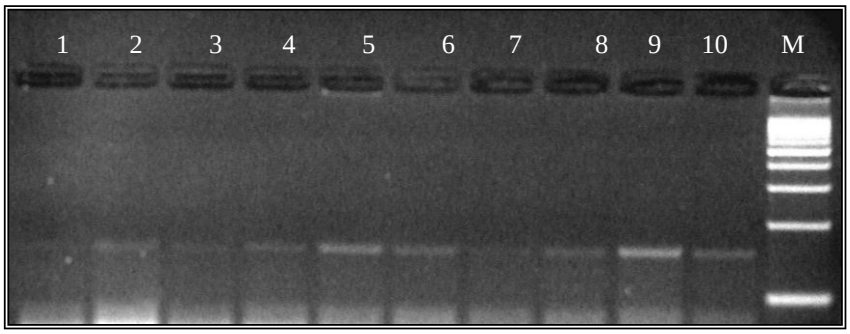

Fig. (14): Detection of *FecX*^{I} mutation by Forced-RFLP test. Amplification of genomic DNA from Barki breed (low twining rate females) M= molecular size marker (100 bp ladder).