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Molecular Characterization of Maize (*Zea mays* L.) Genotypes for Iron Content

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ABSTRACT

The present study was conceptualized and executed for screening maize genotypes for iron content and identification of SSR markers closely associated with micronutrient content in maize genotypes. Fourteen maize genotypes were obtained from the Maize Research Centre, ARI, ANGRAU, Hyderabad. The samples analyzed for grain iron content by Atomic Absorption Spectrophotometry, were grouped under high, medium and low categories. The iron content of the maize genotypes ranged from 9.81 to 80.47 mg / kg of grain and four genotypes had high iron content, six genotypes possessed medium iron content and four genotypes exhibited low iron content. A total of eighty SSR markers distributed over the ten chromosomes of maize were used for identifying the primers closely linked with the genomic regions associated with micronutrient content. Among the eighty markers used, only fifty markers showed amplified bands, out of which, the markers UMC1982, UMC1353, UMC1008 and UMC1349 showed polymorphism between four maize genotypes having high iron and the three genotypes with low iron content. These four markers were used to confirm whether polymorphism between fourteen maize genotypes was due to iron content. Definite trend of polymorphism that could be attributable to high and low iron content in the grains was exhibited by the SSR marker, UMC1008, located on chromosome 4.

Key words : Iron content, Maize, Molecular markers, Polymorphism.

Maize (*Zea mays* L.) is a major cereal crop widely consumed in developing countries, which have a high prevalence of anemia due to iron (Fe) deficiency. Iron deficiency may affect as many as 3000 million people in the world (Underwood, 2000).The major cause of iron deficiency in these countries is inadequate intake of bioavailable iron, where poverty is a major factor. Therefore, biofortification of maize by increasing iron concentration and / or bioavailability has great potential to alleviate this deficiency. Biofortification is the process of breeding food crops that are rich in bioavailable micronutrients. These crops fortify themselves by loading high levels of minerals and vitamins in different plant parts. Both conventional and molecular breeding approaches can be used for selection of high micronutrient and low anti-nutrient lines. Parental lines are normally screened for nutrient content and closely linked DNA markers will be identified in marker assisted selection. A better understanding of iron homeostasis, involving knowledge of the basic physiological processes of iron absorption, distribution and storage in plants, can serve as a starting point for the biotechnological manipulation of crops (Grusak, 2002 and Grotz and Guerinot, 2002). Maize is also a model system for genomic research and thus allows the opportunity for gene discovery (Hoekenga *et al.*, 2007). Chauhan (2006) and Sharma and Chauhan (2008) predicted from the available genome sequence data that 33 genes were involved in iron and zinc transport in maize. Simple Sequence Repeats (SSR) as well as Single Nucleotide Polymorphism (SNP) were identified in the candidate genes. These genes were expected to be of potential use in genetic and association mapping, molecular marker-assisted selection and development of transgenic plants for micronutrient enrichment.

The present investigation was designed to screen maize genotypes for iron content and to identify SSR markers closely linked with iron content which may be used for marker assisted breeding for developing lines with high iron content.

MATERIAL AND METHODS

Fourteen popular maize genotypes including seven inbred lines (BML-6, BML-7 and BML-10, CM-118, CM-119, CM-130 and CM-211),

one pop corn (Amber popcorn), two sweet corn (Madhuri and Priya) and four quality protein maize lines (QPM-91-2, QPM-66, QPM-66-2 and QPM-266-2) were collected from Maize Research Centre, Agricultural Research Institute (ARI), ANGRAU, Hyderabad. The iron content of grain samples of these maize genotypes were estimated by Atomic Absorption Spectrophotometer (Varian 240 FS, Australia). Based on grain iron content, the genotypes with less than 20 mg / kg were categorized as low iron containing genotypes, while those with iron content between 20-40 mg / kg were classified as medium and those with more than 40 mg / kg iron content as high iron containing genotypes.

Genomic DNA was isolated from the fresh leaves of 10–12 days old seedlings of fourteen maize genotypes by CTAB method. The purity and concentration of the isolated genomic DNA samples were estimated by UV-VIS absorption spectrophotometer (Thermo UV1) as per the procedure described by Sambrook and Russel (2001). Quantification of DNA was also done by analyzing the purified DNA on 0.8 per cent agarose gel with diluted 100 bp ladder DNA (New England Biolabs) as standard. The genomic DNA was subjected to PCR amplification as per the procedure described by Chen *et al*. (1997) using eighty maize specific SSR primer pairs randomly selected (Table 1) from available SSR database distributed over the ten chromosomes for PCR amplification. The PCR reactions were performed in 10 ml reaction containing 2 ml of template DNA, 1X PCR buffer [10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, (Strata gene) 0.2 mM of each dNTPs, 10 pmol of each primer and 1 U of *Taq polymerase* (1 U/ml; Strata gene)]. Samples were amplified through a thermal profile consisting of 5 min of initial denaturation at 94°C, 35 cycles of 30 sec of denaturation at 94°C, 1 min of annealing at 55°C, 1 min 30 sec of extension at 72°C and 10 min of final extension at 72°C in a Thermal cycler (Eppendorf, Germany). The PCR amplified products were resolved on 3 % agarose (Lonza, Sea kem) gel, stained with ethidium bromide in 50X TAE buffer. Before loading, the PCR amplified products were mixed with 1/6th volume of gel loading dye (40% sucrose, 0.25% brophenol blue) and the samples were run at 80-100V for two hours using Horizontal Electrophoresis Unit (GeNei; Bangalore), and visualized under UV-transilluminator and documented using a gel documentation system (Gene Flash; SYNGENE, U.K). The sizes of amplified fragments were determined by comparing with 50 bp / 100 bp ladder (New England Biolabs).

Table 1: Iron content of maize genotypes

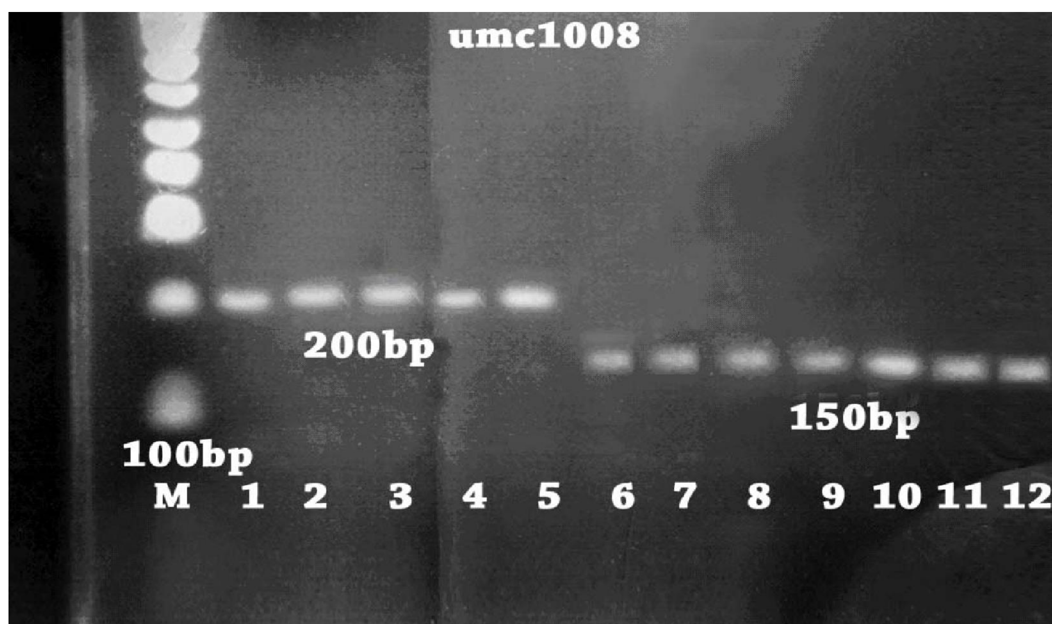
S. No.	Genotypes	Iron Content (mg / kg)
1.	QPM-91-2	80.47
2.	Amber popcorn	46.08
3.	BML-7	45.06
4.	CM-119	42.08
5.	CM -130	26.08
6.	BML-10	26.06
7.	CM-211	24.75
8.	BML-6	24.51
9.	QPM-66	24.04
10.	QPM-66-2	22.01
11.	Priya	19.85
12.	Madhuri	19.12
13.	CM-118	19.05
14.	QPM-266-2	9.81

Table 2. Details of SSR markers used for detection of polymorphism among the maize genotypes with diversified levels of grain iron content .

S. No	Marker	Chromosome	Forward primer sequence	Reverse primer sequence
1	UMC1566	1	ATCTCGTCTACCTAACCCACCCTC	CAGGTGAAGAATCTGGTGAGGTC
2	Bnlg2204	1	AGGCGACTTAGCTGCAGAAG	CGACTTTCGGTTTGGAAAAG
3	UMC1126	1	CAACAGGGTGAACCCTCTGTACTT	AATATGGTGTTGTGATTTGCATCG
4	UMC1812	1	TACAAGGAAGGCAAGTTCATCCTC	ATGCAGGTGACATTCATCATCATC
5	UMC1082	1	CCGACCATGCATAAGGTCTAGG	GCCTGCATAGAGAGGTGGTATGAT
6	UMC1353	1	AGACAGGATCATCGAAAACACACA	ACCTCAGCCTCCTCGTCAACTACT
7	UMC1071	1	AGGAAGACACGAGAGACACCGTAG	GTGGTTGTCGAGTTCGTCGTATT
8	UMC1177	1	CGTGTACCGCTCCTCTATAGTCGT	AAGTGGCCGAATTCATCCTTTATT
9	UMC1222	1	CTCAGAACAGAAGCCATCAAAAGC	CGTCTTCGTGAGAGACATCCTGT
10	UMC1269	1	TATATTAGAGGCACCTCCCTCCGT	AGCTGCTTCAGCGACTTTGG
11	UMC1363	1	TGTTTAAGTGTTGGCAGAAAGCAA	TCTCCCTCCCCTGTACATGAATTA
12	UMC1622	2	CTGGATGAGGAGGAAGAATACGAG	CCTCGGATTTTCCAAAACATTTCT
13	UMC1419	2	CTCATCACAACTAGCGCCACTCTA	ATAGTGCAGAGGTCATCGTGGC
14	UMC1776	2	AAGGCTCGTGGCATACCTGTAGT	GCTGTACGTACGGGTGCAATG
15	UMC1026	2	TCGTCGTCTCCAATCATACGTG	GCTACACGATACCATGGCGTTT
16	UMC1736	2	CCATCCACCACTAGAAAGAGAGGA	TTAATCGATCGAGAGGTGCTTTTC
17	UMC2214	2	ACCCCCTGATTCTCTCTTACGTTT	CTGGATGAGGAGGAAGAATACGAG
18	mmc0231	2	GAGCGACTGCGAGACGG	AGATCGCGCCACCGCTC
19	UMC1185	2	AGTAAAAGAGGCAAGGACTACGGC	GCGGCGATATATACGAGGTTGT
20	UMC1265	2	GCCTAGTCGCCTACCCTACCAAT	TGTGTTCTTGATTGGGTGAGACAT
21	UMC2118	3	CGTCTCCGTCTGCAGTCACTATTA	TATGGTCCTCGGAGTTTGTTTGTT
22	UMC1780	3	CTGTCCCCAGGTTGCTGTAGTAGT	CATGATGTACCCGCAACAAATG
23	UMC2276	3	CTAGGTAGCCAGCTAGGTACGGGT	AGTGGAGCTTCTTCATCCTACCG
24	UMC2048	3	GCTGAAGTCCCAACCACCAC	TTGACATGTTCTACCATCTCACCAA
25	UMC2256	3	GGTCCTAGTCGTTAATTCTTTTAGCG	GGTCAAGGACTCTTCTTCCTCCTT
26	UMC2377	3	CCTTCAAACCAAATGTACAGCAGC	CTCCTCAACGACAGCGTGTACC
27	UMC1886	3	GTTTGACAGCACAAGTGCAAGAAA	GAGGTGGACATTGGACAACACC
28	Bnlg1647	3	CGTCGTCTGTGGACGTACTG	AGAAGCTCACAAGCCTGCTC
29	UMC2259	3	GGCTCGACTTCGAGGACACC	GAGGAGGAGAGGGACAGGGAAG
30	UMC1968	3	CTTCCCCTCCGCTACTGCTC	GTACTTGGTGTTGTCGCTCTTCTTC
31	UMC1773	3	GGATCACACTATCGAGTCAGCGAT	CAAGGTAGCGTCGTCCTCCTC
32	UMC1501	3	CCACATTTGGCTGAATTTGTTGTA	CTTGTTGGCTAGAAATTTGCCTTG
33	UMC1539	3	GAGTCCAGGCAGCACGCTAGT	GAGCAGCACACGAGGACCAG
34	UMC1008	4	TCTAGCTTGTGGTGGTGGTTGA	ACATGAGCACAAAGACTGACGC
35	UMC2279	4	TGTCTCCTCCCAGGTCGTAGTGT	CAGAAGAGTAACCACACTGAACACACA
36	UMC1902	4	CCTCATCTCTCATGGGATGGATA	TTCAGCATGACATATCATACAGTAGCA
37	Bnlg2291	4	CCTCTCGATGTTCTGAAGCC	GTCATAACCTTGCCTCCCAA
38	Bnlg1890	4	ACCGGAACAGACGAGCTCTA	GTCCTGCAAAGCAACCTAGC
39	Bnlg1126	4	GAGATCGAAGGTCATGGCAC	GAGATCGAAGGTCATGGCAC
40	UMC2309	4	CATCTCCTACCAGCTCACCCC	CATCTCCTACCAGCTCACCCC

S. No	Marker	Chromosome	Forward primer sequence	Reverse primer sequence
41	UMC1490	4	GCCCTAGCTTGCTAATTAACTAACA	GCCCTAGCTTGCTAATTAACTAACA
42	UMC1964	4	CTTCTCACTGTCGCAGAACAAGAG	CTTCTCACTGTCGCAGAACAAGAG
43	UMC1702	4	ACGAGGCTCTTCCGAGTTCC	ACGAGGCTCTTCCGAGTTCC
44	dupssr	4	TTCTTTAACTATTGGAAGCCCA	TTCTTTAACTATTGGAAGCCCA
45	Bnlg1444	4	GCATGGATGGAGAAAGAGGA	GCATGGATGGAGAAAGAGGA
46	UMC2291	5	CTCGACGAGTTCAAGCGCTAC	AACTTCTCCTGGCGAGCATCT
47	Bnlg1879	5	TGCTCTCACAAGATGGTGGA	CCACAGGATAAAATCGGCTG
48	UMC1349	5	ACGACCAGTGCTTCGCTCAC	AGTTTCCATCGTATGATGTCGAGG
49	Bnlg389	5	GGTCACCCTCCCTTTGCAG	ATTGCCTACACAGTTTGATTGG
50	UMC1153	5	CAGCATCTATAGCTTGCTTGCATT	TGGGTTTTGTTTGTTTGTTTGTTG
51	UMC1097	5	CTCGTCAACGTCAACCCAAGTAAG	CTGTTAGATGTGCGACAACAGAGC
52	Bnlg565	5	TAAGAACGACGAACGGTAACTG	GCTCACTGCACGCCAACAC
53	mmc0351	5	GCAGTGCATGTATCTGATCTAC	AGGCTCTCTTGATCCTTCA
54	UMC1429	5	GGGCCCTGTTAATCCTCATCTG	TCCTCCTTTCTCTCATGTTTCTCG
55	UMC1221	5	GCAACAGCAACTGGCAACAG	AAACAGGCACAAAGCATGGATAG
56	Bnlg118	5	CTTCCAGCCGCAACCCTC	CCAACAACGCGGACGTGA
57	UMC1375	5	AGTTCGACTTCGACCCGGAC	GTCAGGCTTCTTCTCGACACACC
58	UMC1002	6	AGCTAGCTATACACCGCCAGG	TCAGTTTGGAACAGGGAAAAGTA
59	UMC1257	6	CAACGGAAGTGGCTGTAGAGTTTT	ACAGAGCATGTCAGGTATTTGCAG
60	UMC1314	6	ACAAACTAAACTGCATGTACCCCC	CACAAATGATGTGGTGGCAATATC
61	UMC1520	6	AGCAAATATATGAGCAATTAAGAACAGG	GTGTCGCCACCTATAATTTGATGA
62	Bnlg1136	6	TAACCGGATGAGCATCTTCC	CATCAGCTTCAACGAGTTCG
63	UMC2324	6	GATCCTCTGTCGCCAAACACTAAG	AGATGGTGACGATGAGTGATGAAC
64	UMC1753	6	AAGATCTTGCTCCGTTTCCTCTCT	TTCAGATGCAAATCTCTTTTCGCT
65	Bnlg1174	6	CGCATTCCAAGAACAATGAA	TTCGATTGGTGGGAAGATTC
66	UMC2177	7	ACCATGCATGTCTCACGTCACT	GGGTACGTGCTGTGGAGGAC
67	UMC1241	7	TGAAGCAAGTCACTGGTAAGAGCA	TGACACACCCATACTTCCAACAAG
68	UMC1401	7	CTCTGGTCCATCCTCATCGACT	TCTCTTGATCACATATCGATCCCA
69	phi091	7	ATCTTGCTTCCATAAGATGCACTGCTCT	CTCAGCTTCGGTTCCTACACAGT
70	UMC1543	7	TTCCTCTACCAGCTGCCCGT	GTGAGCAGAAGCTTGAGGCG
71	UMC2333	7	GAATTGGATGTTATTCGGATCGTC	TGCCTCCTTTTTCTGATCTACACC
72	phi116	7	GCATACGGCCATGGATGGGA	TCCCTGCCGGGACTCCTG
73	Bnlg1131	8	TTAGTTGGGTAAACGTGCAC	GCATCAGGGGGTAGTTGAGA
74	UMC2154	8	GCTAGTAGTAGTTCCAACGAAGCAACA	GTCACCATCTCCAGGTGCAAGT
75	UMC1957	9	CATGATCGCCGGGATTAATACTAC	GTCCAAGGACGACGATTACGAC
76	UMC1370	9	GGGAGCACACACAGTAGTACTCGAT	AGAGGCTCTCCTCCTTCAAGCTC
77	UMC1366	9	GTCACTCGTCCGCATCGTCT	CCTAACTCTGCAAAGACTGCATGA
78	UMC1137	9	ATCAGTCACTCTTCTGCCTCCACT	GGCTGGATAATGTTGTAGCTGGTC
79	UMC1982	9	TTCATCTTCCTAGTCTCGTCTCCG	AATCGTACTTGGAGGAGGCGTT
80	Bnlg1547	10	TTGGATCAACTTACCCAGGC	ACATGCGTGCTACCCATACA

Fig 1. Amplification pattern of 12 maize (5 with high iron and 7 with low grain iron content) genotypes using SSR marker, UMC1008.



200 bp:	Lane 1 – QPM-91-2	Lane 2 – Amber popcorn	Lane 3 – BML-7
	Lane 4 – CM- 9	Lane 5 – CM-130	
150 bp:	Lane 6 – BML-10	Lane 7 – CM-211	Lane 8 – QPM-66
	Lane 9 – QPM-66-2	Lane 10 – Priya	Lane 11 – Madhuri
	Lane 12 – CM-118		
	Lane M – 100 bp DNA ladder		

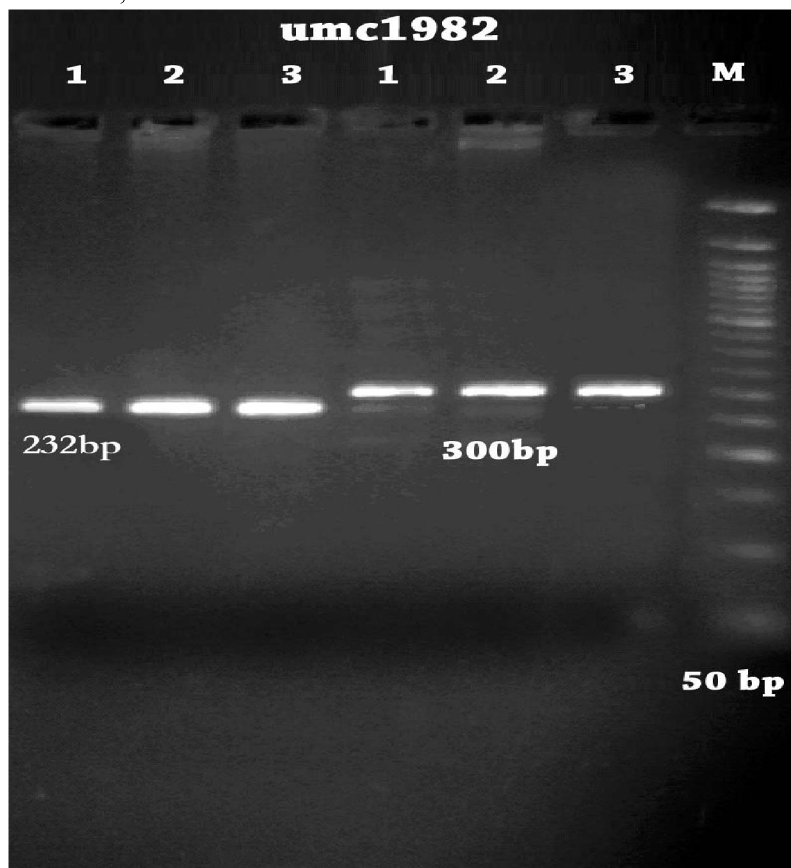
RESULTS AND DISCUSSION

The iron content of different maize genotypes ranged from 9.81 to 80.47 mg / kg (Table 2). Four genotypes had high iron content ranging from 42.08 to 80.47 mg / kg, while six genotypes showed medium iron content ranging from 22.01 to 26.08 mg / kg and the remaining four genotypes exhibited low iron content ranging from 9.81 to 19.85 mg / kg.

Detection of polymorphism for grain iron content through SSR markers

Of the eighty SSR primer pairs used (Table 1), only fifty primer pairs showed amplification. The primer pair UMC 1008 located on chromosome 4 showed polymorphism between eight low and high iron containing maize genotypes. A band of lower size (around 150 bp) was amplified in the four low genotypes with low iron content *viz.,* Priya, Madhuri, CM-118 and QPM-266-2. The other four high iron containing genotypes, QPM-91-2, Amber popcorn, BML-7 and CM-119, showed 200 bp band. The primer pair, UMC1008, was used for amplification of DNA of all the fourteen maize genotypes to assess whether the polymorphism observed could be attributable to high and low iron contents. A 100 bp ladder was used as a standard to assess the size of the bands. The amplified DNA fragments showed polymorphism between high and low iron containing genotypes on the agarose gel (Fig 1). This marker was found to contain by EST p-MEST5-A12 sequence, which detected *mtl1 metallothionein1* (Gene; detected via SSR PCR) (de Framond, 1991). This is a differentially expressed maize gene which has been cloned and sequenced. Transcriptional and translational start sites have been mapped and 2.5 kb of 5' flanking DNA were sequenced. The 8 kDa protein encoded by this gene shows striking similarity to the metallothionein-like proteins described in *Pisum sativum* and *Mimulus guttatus*. The maize *mtl1* gene message is very abundant in roots without exposure to high levels of metals, present at lower concentration in leaves and pith, and at very low concentration in seed.

Fig 2. Amplification pattern of 8 maize (3 with high iron and 3 with low grain iron content) genotypes using SSR marker, UMC1982.



232 bp:	Lane 1 – QPM-91-2	Lane 2 – Amber popcorn	Lane 3 – BML-7
300 bp:	Lane 1 – Priya	Lane 2 – Madhuri	Lane 3 – CM-118
	Lane M – 50 bp DNA ladder		

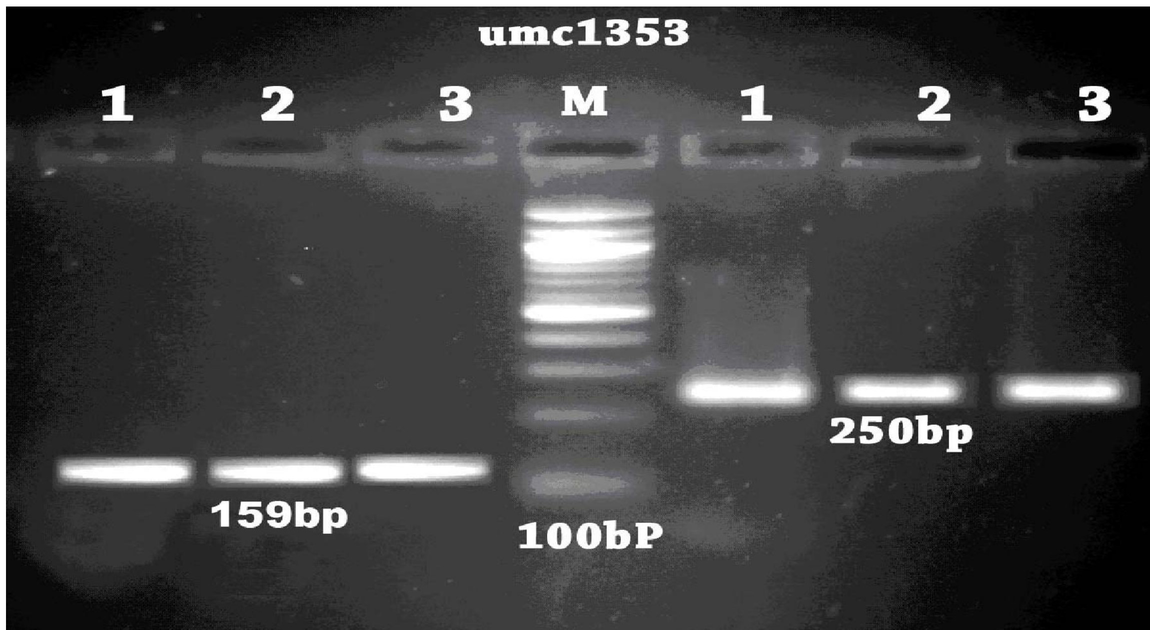
The primer pair UMC 1982 located on chromosome 9 showed polymorphism between six high and low iron containing maize genotypes. A band of smaller size, around 232 bp was obtained, for the three high iron containing genotypes, QPM-91-2, Amber popcorn and BML-7. The band that was amplified in the three genotypes with low iron content, Priya, Madhuri and CM-118, showed a slightly higher size, around 300 bp (Fig 2).

The primer pair UMC 1353 located on chromosome 1 exhibited polymorphism between six high and low iron containing maize genotypes. A band of smaller size (around 159 bp) was observed in three high iron containing genotypes, QPM-91-2, Amber popcorn and BML-7. The band amplified in the three low iron containing genotypes, Priya, Madhuri and CM-118, showed a slightly higher size, around 250 bp (Fig 3).

The primer pair UMC 1349 located on chromosome 5 showed polymorphism between eight high and low iron containing maize genotypes. Four high iron containing genotypes, QPM-91-2, Amber popcorn, BML-7 and CM-119, exhibited a band of 80 bp size, while four low iron containing genotypes, Priya, Madhuri, CM-118 and QPM-266-2, exhibited a band of slightly higher size, around 150 bp (Fig 4). None of the other markers used in the present study showed any polymorphism between high and low iron containing genotypes.

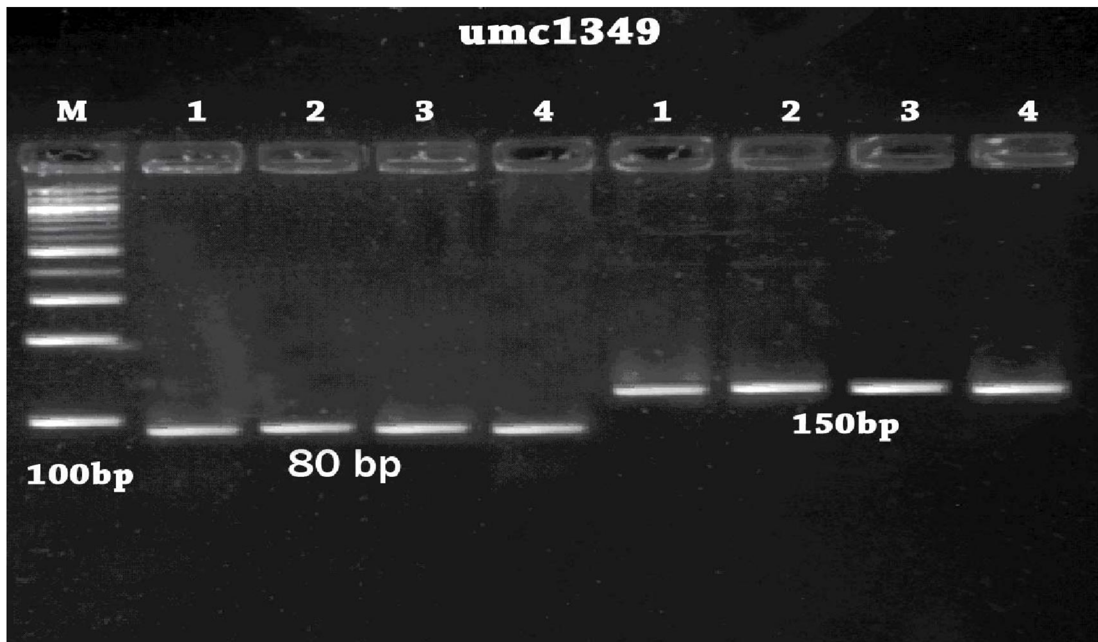
The markers identified in the present study could be used as a tool for identifying and mapping of new genes for high and low iron contents. Additional markers may be surveyed in the targeted region in an attempt to find markers closely flanking the genes governing high iron and low iron contents in the maize grains.

Fig 3. Amplification pattern of 6 maize (3 with high iron and 3 with low grain iron content) genotypes using SSR marker, UMC1353.



159 bp: Lane 1 – QPM-91-2 Lane 2 – Amber popcorn Lane 3 – ML 7
250 bp: Lane 1 – Priya Lane 2 – Madhuri Lane 3 – CM-118
Lane M – 100 bp Ladder

Fig 4. Amplification pattern of 8 maize (4 with high iron and 4 with low grain iron content) genotypes using SSR marker, UMC1349.



80 bp: Lane 1 – QPM-91-2 Lane 2 – Amber popcorn Lane 3 – BML-7
Lane 4 – CM-119
150 bp: Lane 1 – Priya Lane 2 – Madhuri Lane 3 – CM-118
Lane 4 – QPM-266-2
Lane M – 100 bp Ladder

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