

Applied Biological Research 22(1): 69-75; (2020) DOI: 10.5958/0974-4517.2020.00010.5

PARENTAL POLYMORPHISM IN IRON- AND ZINC-RICH RICE VARIETIES (SWARNA AND TYPE 3) USING SSR MARKERS

D. Shivani^{1,3}, K. Suman², P. Madhubabu², Ramya Rathod³, C. Cheralu³, V. Gouri Shankar³ and C.N. Neeraja¹*

^{1,2}Indian Institute of Rice Research, Rajendranagar, Hyderabad - 500 030, Telangana (India) ³Department of Genetics & Plant Breeding, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad – 500 030, Telangana (India) *e-mail: cnneeraja@gmail.com

(Received 29 September, 2019; accepted 14 February, 2020)

ABSTRACT

A microsatellite is a specific sequence of DNA bases which contains repeats. In present study, rice microsatellite (RM) markers were used to study the parental polymorphism between two parents. The experiment was conducted at Indian Institute of Rice Research, Rajendranagar Hyderabad (India). 'Swarna' and 'Type 3' rice varieties dense in grain iron and zinc were used to study the parental polymorphism. Out of 171 rice markers used for parental polymorphism, 52 markers showed polymorphism and 119 were monomorphic with expected base pair ranging from 84 to 383 bp. Therefore, these polymorphic rice microsatellite markers can be used in fine mapping of iron- and zinc-rich micronutrient genes to study the mapping populations of crosses obtained from these parents.

Keywords: Biofortification, iron, microsatellite markers, parental polymorphism, rice, zinc.

INTRODUCTION

Rice (*Oryza sativa* L.) is a "Global Grain" widely cultivated across the world. As humankind faces nutrient deficit in cultivated crops, malnutrition has been designed as the most serious challenge to humanity (Copenhagen Consensus, 2008) because two-third of the world's population is at risk of deficiency in one or more essential mineral elements (Cakmak, 2002; White and Broadley, 2009; Stein, 2010). Biofortification is a new approach that aims at biological and genetic enrichment of food stuffs with vital nutrients (vitamins, minerals and proteins) [Phattarakul *et al.*, 2012]. The rapid development of DNA marker technology provides great opportunities to enhance nutritive values of traditionally cultivated crops and grains.

Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes (Rahman *et al.*, 2007). DNA fingerprinting is used to describe the combined use of several single locus detection systems and is used as versatile tools for studying various aspects of plant genomes including the characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, etc. (Bouis, 2000). Molecular data provides a basis for better management and conservation of collection which could be used as reference for its enhanced use in breeding programs (Wang *et al.*, 2005).

D. Shivani et al.

Marker-aided selection (MAS) techniques can increase the selection efficiency to as high as100% and permit simultaneous selection for a number of traits (Xu and Crouch, 2008). Cultivars are needed for developing the techniques that have been identified, but the DNA-level polymorphism between traditional cultivars and other yield improving types will determine the rate of progress. This can be done by microsatellites, or simple sequence repeats (SSRs) (referred to as a variable number of tandem repeats or VNTRs), at polymorphic loci present in nuclear and organellar DNA that consist of repeating units of 1-6 bp in length. They are highly polymorphic molecular markers, which have wide-ranging applications in the field of genetics (Cho *et al.*, 2000).

Improved rice varieties with high nutrients like iron and zinc could also provide better nutrition to even the poor people. By increasing the iron and zinc content of food staples through plant breeding and biotechnology, the entire iron and zinc status distribution curve can be shifted to that smaller group of nutrient deficient persons which could become feasible in the developing countries. In next decade, it is expected that DNA markers will be available for finding micronutrient availability in a less expensive and less time-consuming procedure in rice crops (Chen *et al.*, 1997). In view of above, the present work was undertaken to estimate the parental polymorphism in iron and zinc rich rice varieties Swarna and Type 3 using SSR markers.

MATERIALS AND METHODS

The experimental plant material for the study comprised of a donor parent 'Type 3', which is an aromatic rice variety with high iron content, and a recipient parent 'Swarna' (a high yielding variety adaptable long duration, medium resistant to bacterial leaf blight and sheath blight) and SSR markers were collected from IIRR (Indian Institute Of Rice Research), Hyderabad, India.

Genomic DNA was extracted from both the parents as per the protocol described by Sambrook (1989). The extracted DNA was checked for quality and quantity by spectrophotometer. PCR amplification was performed in 96 well plate for 171 RM markers. The PCR plates were labeled with respective sample number and 4 μ L (i.e. 50-100 ng) of template DNA was added to the respective wells. The master mix consisted of 1.0 μ L forward primer (2.5 pmoles), 1.0 μ L reverse primer (2.5 pmoles), 0.5 μ L dNTP's (2.5 mM), 0.3 μ L Taq DNA polymerase (3U μ L⁻¹; Bangalore Genei Pvt. Ltd.), 1.0 μ L of 10 x PCR buffer (Tris with 1.5 mM MgCl₂, Bangalore Genei Pvt. Ltd.) and 2.2 μ L of sterile distilled water was added to make up the volume to 10 μ L. Then the master mix (6.0 μ L) was dispensed to the PCR plate with template DNA. The PCR plate was covered with a sealing mat. It was then placed in a programmable thermal cycle (M/s Applied Biosystem, USA) for DNA amplification.

PCR amplification

PCR reactions were performed on each DNA sample in a 10 μ L reaction mix containing 1 μ L of 10x AmpliTaq polymerase buffer, 2 μ L of 10 μ M primer, 1 μ L of 250 μ M dNTPs, 1 unit of AmpliTaq DNA polymerase (Bangalore Genei, India) and 75 ng of genomic DNA and a suitable amount of sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mix was preheated at 94°C for 3 min followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 54°C and elongation or extension at 72°C for 2 min. After the last cycle, a final extension of 7 min at 72°C was added.

Agarose gel electrophoresis

Amplified products from each sample were separated electrophoretically on 1.4% agarose gel (Fisher Biotech, USA) containing ethidium bromide in 1XTAE buffer at 120 V for 1½ h. To determine molecular weight a DNA marker (Ø X 174 DNA/Hae III digest and /or 100 bp DNA ladder) was

electrophoresed alongside RAPD products. DNA bands were observed on UV-transilluminator and photographed by a Gel Cam Polaroid camera.

RESULTS AND DISCUSSION

The parental polymorphism was performed by using 171 rice markers (RM) distributed across the 12 chromosomes. Of these, 119 were monomorphic and 52 SSR markers were found to be polymorphic across the 12 chromosomes as given in Table 2 and Fig. 1. Similar results on parental polymorphism were reported by Kiranmayi *et al.* (2014) using 71 gene specific markers, of these only 13 (18.3%) were polymorphic while Gangaprasad Chowdary *et al.* (2013) used 64 rice SSR markers and of these 52 markers showed polymorphism. Similarly, Shankar Ilango and Sarla (2010) used 112 RM markers and found 33 RM primers to be polymorphic. The distribution of 52 polymorphic markers across 12 chromosomes is given in Table 2. Some of the representative polymorphic markers have been shown in gel pictures (Plate 1 & 2). Among all the chromosomes higher number of polymorphic primers i.e. nine were observed on chromosome 6.

| Chromosome | No. of | Markers names |
|------------|---------|--|
| No. | markers | |
| 1 | 7 | RM11744, RM11743, RM11741, RM10361, RM11307, RM 243 |
| | | and RM10018 |
| 2 | 3 | RM13347, RM14181 and RM3515 |
| 3 | 5 | RM16097, RM15630, RM 282, RM517 and RM232 |
| 4 | 6 | RM348, RM261, RM 17201, RM241, RM413 and RM16427 |
| 5 | 5 | RM 169, RM142, RM3486, RM413 and RM164 |
| 6 | 9 | RM340, RM19341, RM19620, RM3431, RM253, R111, RM276, |
| | | RM402 and RM190 |
| 7 | 2 | RM20844, RM11 |
| 8 | 2 | RM22885, RM22524 |
| 9 | 5 | RM3912, RM257, RM 24035, RM24085 and RM296 |
| 10 | 1 | RM5095 |
| 11 | 3 | RM27289, RM206 and RM27962 |
| 12 | 4 | RM27962, RM 28607, RM235 and RM5313 |

Table 1: Distribution of 52 polymorphic markers across 12 chromosomes



Fig. 1: Parental polymorphic SSR markers across 12 chromosomes

 Table 2: Details of parental polymorphic SSR markers

| S. Markers Chr Position Forward sequence Reverse sequence 1 RM11744 1 33,162,055 CCACCCGTATAGGACCAGTCG TAGAGTCTCCAGGCAGTCTCACC 3 31,162,203 33047896 AAGGTCAAGGAAACAGGGACTGG AGCCACGAATTCCACTTTCACC 3 RM11741 1 33,165,203 TGCAGGTAGAAACAGGGACTGG AGCCACGAATGCCAGGGAGTGTAGGAGGAGGG 4 RM 10361 1 6155081 TGCAGGTCTGCAACCTCAACG GCCCTCAGAGCAGTAAGGAGAGG 5 RM10018 1 272,515- ACTAGTACACCTCCAACTTCACTCC CCTTTAGTTGCTGGACC 272,662 7 RM243 1 7970,356 AAGGTCTGCAAGCATCATCAGG GAAAGCTGCAACGAACAGTGC 8 RM14181 2 35160202- AGTACCACCACCACTTCTCTGCAAGC CGATTGGGCATGAGAGTGC 9 RM3315 2 24016552- GGAAAGAGATATGCCATGC AGAGGCGAGAGCATGGAGGACGC 10 RM3515 2 24016552- GGAAGGGATCTCGCCCCAACC ACCCACCTCTCTCCTTCCATCTGC 12 RM16097 3 33589253- CGCCTCGTAAGGTTGGCACC CAGTCCTGTGTTTTCCATCTGC 12 RM15630 2 26073,971- ACTCGTGTCCTTCCTTGCGATAGGATCG ACGCACCACCACCTCCTCCACCC 12 RM16097 3 33589253- CGCCTCGTGACTCGCCCCACC <th>~</th> <th></th> <th>C</th> <th></th> <th></th> <th>D</th> | ~ | | C | | | D |
|--|-----|-------------------|-----|------------------------|--|----------------------------------|
| $ \begin{array}{c c c c c c c c c c c c c c c c c c c $ | S. | Markers | Chr | Position | Forward sequence | Reverse sequence |
| 1RM1174133,162,005CCACCCGTATAGGACCAGTCGTAGAGTCTCCAGGCAGTCTCACC2RM 11741133047896AAGGTCAAAGGAAACAGGGACTGGAGCCACGAATTCCACTTCAGC3RM 11741133047896CCTTGATCGGAAGTAGAACTTGAAGCAGTGGATGTTAGGTGTAACAGG4RM 1036116155081CCTTGATCGGAAGTAGCTCAACGGCCCTCAGAGCAGTAAGGAAGAGG5RM10018127,2515ACTAGTACACCTCAACTTCACTCCCCTTTAGTTTGCTTGTGACC6RM11307123,292,502AAAGCTCTGCAGTTGCACGATACTACGGAAAGCTGCAACAACAGTGC7RM24317,970,722CAGACCCACCACTTCTCTCGCAAGCGAAAGCTGCAACGAACGATGTGTCC7RM1347235160205AGTACCACCACCACTTCTCTGCAAGCGAAAGGCGCAAGGAAGGCATGGAGTGC9RM1347219374071TCCTTCTCTTGAACAGCGACAGCAGAGGGCAAGGAATCAGAAACACCAAC10RM3515224016,552-GGAAAGAAGATATGCCATGCAGAGAGAATCAGAAACACCAAC224016,552-CGGCTCGTAAGGTTGAGATCGTGCCCTGTTCTTTCCATCTTGC12RM1609733589300CCGCTGTTCCGTAAGGTGGACCACCCACCCCCCTCCTCACGCTGTACG14RM282312,407,382-CTGTGTCGGAAGGCTGCACCCGGATCTTGCCCGTGACGAAGGCTTGCAGC14RM282312,407,382-CTGTGTCCCTTTCCTTCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG14RM282312,407,382-CTGTGTGCGCTTCCATGGCGCACCCCGACTTTTCCTCGCGAGAAACCAGC16RM26146,574,518CTACTTCCTCCCTTGTGTCGTGAGATCACCACCGCGAAATCAGG17 | No. | | No. | (bp) | | |
| 33,162,203 33,162,203AAGGTCAAGGAAACAGGGACTGGAGCCACGAATTCCACTTTCAGC 330479232RM 11741132981599 32981624TGCAGGTAGAAACTGGAAGCAGTGGATGTAGGTGTAAGAGGAGAGG GCCTCAGAGCAGTAGGAGAGGG4RM 1036116155048 6155081CCTTGATCGGAAGTAGCTCAACGGCCCTCAGAGCAGTAGGAGAGGG5RM100181272,515 23929,520 23929,520ACTAGTACACCTCAACTTCACTCCCCTTTAGTTGCTTGTGACC 23,929,7277RM24317970,220 23160225AGACCACCACCACTATCTCTGCAAGCGAAAGCTGCAACGATGTGTGCC 7970,8368RM14181235160225 35160225AGTACCACCACCACCATTCTCTGCAAGCTCGATTGGCCATGAGTTCTCG 3351602259RM1337219374071- 10TCCTCTCTCTTGCAAGCGACAGCAGAGGCGAGAGGCATGGAGTGC AGAGGCGAGAGGCATGGAGTGC10RM3515224,016,552- 24,016,578GGAAAGAAGATATGCCATGC 24,016,748AGCCCCGGTATCGGCCCAACC ACCCACCTCTCCAGCCTGTCCTGC12RM1609733589253- 3589300CGCCTCGTAAGGATCGCCCCAACC ACCCACCTCTCCCAGCCTGTGCGCCCAACC 26,074,256ACCCACCTCTCCCAGCCTGTGCGCCCAACC ACCCACCTCTCCCAGCCTGTGCGCCCAAGG14RM222312,407,350 24,977,382CTACTTCCCCCTTGCTGCG CTACTTCCCCTTGCGTGCGTGACATCGCCGAGAATCAAGG AGTGTTTCACCTGGAAAGCCTGGACCATGG CCGGTATTCACCGTGCCGCAACG15RM51736,165,992- 6,166,181CTACTTCCCCCTTGCTGCGGCGCACCT CTACTTCCCCCTTGCGGGCGATTGTGCGAGTGTTTCACCTTGGCCGCAGTTGTAGG GCGAGTTAGGAGGGTTGGAGG16RM26146,574,396- 6,166,181GTCCTCTCTCCCATGCGGCCCTCCCCGAGATTAGGAGGGTTGGAGG <br< td=""><td>1</td><td>RM11744</td><td>1</td><td>33,162,055-</td><td>CCACCCGTATAGGACCAGTCG</td><td>TAGAGTCTCCAGGCAGTCTCACC</td></br<> | 1 | RM11744 | 1 | 33,162,055- | CCACCCGTATAGGACCAGTCG | TAGAGTCTCCAGGCAGTCTCACC |
| 2RM 11743133047896 33047923AAGGTCAAGGAAACAGGGAACTGG AGGCACGAATTCCACTTTCAGC3RM 11741132981599TGCAGGTAGAAACTTGAAGC 32981624AGTGGATGTTAGGTGTAAGAGGAGAGG GCCTCAGAGCAGTAAGGAAGGAGG5RM1001812155081 23,929,580ACTAGTACACCTCAACTTCACTCC 23,929,580CCTTGATCGGAAGTAGCTCAACG GAATACGACATCAGAACAGTGC 23,929,7777RM24317,970,722 2,903,860CAAGCTCACCACCACCATCTCTCTCC AGTACCACCACCACCACTCTTCTCTGCAAGC AGTACCACCACCACCACTTCTCTGCAAGCGAAAGCTGCAACGATGTTGTCC GAATACGACATCAGAACAGTGC 351602228RM14181235160202 3516022AGTACCACCACCACCACTTCTCTGCAAGC AGTACCACCACCACCACCACTTCTCTGCAAGC AGAAGGCGAGAGGCATGGAGTGCTCGATTGGCCATGGAGTGCC AGAGGCGAGAGGCATGGAGTGC10RM351524,016,552 2,016,748GGAAAGAAGATATGCCATGC 24,016,748AGCCACCTCGTAGGTGTAGCATGC AGAGAGAATCAGAAACACCAAC 24,016,748ACCCGGCTCGTTAGGATCG TGCCTGTTTCCATCTGC 33589253CGCCTCGTAAGGATCGGCCCAACC AGCCACCTCCTCCAACC 26,073,971- AACTCGAAGGATCTCGCCCAACC ACCCACCTCCTCCAACC ACCCACCTCCTCCAACC ACCCACCTCCTCCAACC ACCCACCTCCTCCAACG AGAGCTCAGCCAAGGAAATCAAGG AGAGCTCTGCGCAACA AGATCTGCCCCCTGGATGCACC CCGACTTTCCTCCCCTTGGTGTG CCGACTTTCCCCCTTGGACCCAACC CCAGTCTTCCCCCTTGGTGCG AGTACCATCGCCAAAATCAAGG AGTAGCCATGGCCAAAATCAAGG AGTAGCCATGGCCAATCTCC AGATAGCCATGGCCAATCTCC CCAATCTTGCTTCCCACTTCGCACTCCCCCTTGCATTCCCTTGCCACTCTCTGCCACTCTCTGCCACCCCACCC AGATAGCCATGGCGATTCTTGG AGATAGCCATGGCGATTCTTGG CCAATCTTGCTCCCCCTTCCATTCCC AGATAGCCATGGCGATTCTTGCCACCC AGATAGCCATGGCGATTCTTGCCACCC AGATAGCCATGGGCATTCTGG12RM1622,5,908,305GCAGGTCGGCACGTAGGAGGAGGAGGGGG | | | | 33,162,203 | | |
| 33047923TGCAGGTAGAAACTTGAAGCAGTGGATGTTAGGTGTAACAGG32981624RM1036116155081GCCTGAGAGAGAGC4RM103611212,515ACTAGTACACCTCAACTCACTCCCCTTTAGTTGCTGTGGACC272,662272,662CAAGCTCTGCAATCTTCTCTCCGAATACGACATCAGAACAGTGC23,929,580AAAGCTCTGCAATCTGCAGATACTACGGAAAGCTGCAACGAGATGTTGTCC7RM24317,970,722CAGACTGCAGTTGCACGATACTACGGAAAGCTGCAACGATGTTGTCC7RM14181235160225AGTACCACCACCACCATCTCTCTGCAAGCTCGATTGGCCATGAGTTCTCG9RM13147219374071-TCCTTCTCCTTGAACAGCGACAGCAGAGGGCGAGAGGCATGGAGGC10RM3515224,016,572GGAAAGAAGATATGCCATGCAGAGAGAAATCAGAAACACCAAC24,016,748126,073,971-AACTCGAAGGATCTGGCCCAACCACCCACCTCTCACGCTGTACG12RM15609336,073,971-AACTCGAAGGATCTGGCCCAACCACCCACCTCCTCAGGCTGTACG13RM282312,407,310-CCGGTATCCTTCGATATTGCCCGACTTTTCCTCTGAGCAGG14RM232376,720,300-CCGGTATCCTTCGATATTGCCCGACTTTCCCTGGACG15RM134824165,992-CAACTCTGCTGCTTCTATCGTGCTGTGTACCATCGCCAAAGG16RM26146,574,398-CTACTTCTCCCCTTGGTGCGTGTACCATCGCCAAAGCATCAGG18RM134824172000000CCCTGCTGATGCACTCAGGGAGTGTTTCACCTTGGAGCATTGGGG18RM134824172000000CCCTGCTGCATCTCCGGATCTTCCAGATAGCCATGGCGATTGTGGGG19RM2614< | 2 | RM 11743 | 1 | 33047896- | AAGGTCAAGGAAACAGGGACTGG | AGCCACGAATTCCACTTTCAGC |
| 3RM 11741132981599TGCAGGTAGAAACTIGAAGCAGTGGATGTTAGGTGTAACAGG4RM 1036116155048CCTTGATCGGAAGTAGCTCAACGGCCCTCAGAGCAGTAAGGAGAGG5RM100181272,515ACTAGTACACCTCAACTTCACTCCCCTTTAGTTGCTGTGGACC7RM23317,970,722CAGACTGCAGTGCACGATCTTCTCCCGAAAGCTGCAACGATGTGTCC7RM24317,970,722CAGACTGCAGTTGCACGATACTACGGAAAGCTGCAAGAACAGTGC8RM14181235160205AGTACCACCACCACCATTCTCTGCAAGCTCGATTGGCCATGAGTTGTCC9RM3517219374071TCCTTCTCTCTGTGAACAGCGACAGCAGAGGCGAGAGGCATGGAGTGC10RM3515224,016,552GGAAAGAAGAAGATATGCCATGCAGAGAGAAACAGCAAC11RM1609733589300CGCCTCGTAAGGTTGAGATCGTGCCCTGTTCTTTCCATCTGC12RM15630326,073,971-AACTCGAAGGATCGGCACACACCCACCTCCTCACGCTGTACG14RM232312,407,382CTGTGTGCGAAAGGCTGCACCCGACTTTTCCTCTGACG15RM51736,165,992-CAGCTCTCTCTATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG16RM124425274570CCCATCTTCTCCTTGCGATCTTCCTGTACCATCGCCAAACTCTCC18RM154824172000000CCCTGCTGATGCTGCTTCCAATGGGGGCGGATTAGGAGCGTTTGTAGG19RM241426,857,374-GTCATTTCCTGCGATCTTGCAGATAGCCATGGGCATTCTTGC20RM413422,12,376-CCAATCTTGTCTCCATTGCAGAAGACCAGGGATCTTGCC20RM414425,90 | | | | 33047923 | | |
| 32981624 (21)CCTTGATCGGAAGTAGCTCAACG (21)GCCTTGATCGGAAGTAGCTCAACG (21)GCCTTGATCGGAAGGAGAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG | 3 | RM 11741 | 1 | 32981599 - | TGCAGGTAGAAACTTGAAGC | AGTGGATGTTAGGTGTAACAGG |
| 4RM 1056116155081CCTTGATCGGAAGTAGCTCAACGGCCCTCAGAGCAGTAAGGAGGAGG5RM100181272,515ACTAGTACACCTCAACTTCACTCCCCTTTAGTTGCTGTGGACC23,929,7277RM24317.970,722CAGACTGCAGTGCAGTACTACGGAAAGCTGCAACGAACAGTGCC7RM24317.970,722CAGACTGCAGTGCACGATACTACGGAAAGCTGGCAACGAACGAGGTGTCC8RM14181235160202AGTACCACCACCACTTCTCTGCAAGCTCGATTGGCCATGAAGTGCGGC9RM1347219374071-TCCTTCTCTCTGTGAACAGCGACAGCAGAGGGGAGAGGCATGGAGGCC10RM3515224,016,552-GGAAAGAAGATATGCCATGCAGAGAGAAATCAGAAACACCAAC24,016,748333589300CGCCTCGTAAGGTTGAGATCGTGCCCTGTTCTTTCCATCTGC12RM1609733,12,407,382-CTGTGTGCGAAAGGCTGCACCAGTCCTGTGTTGCAGCAAG14RM22312,407,382-CTGTGTCGTCGTACGTCCCCCGACTTTTCCTCTGACG16RM26146,5714,396-CTACTTCTCCCTTGTGTCGTCAGATCTAGCCGAGAAATCAAGG16RM26146,574,396-CTACTTCTCCCTTGTGTCGTGTACCATCGCCAAACTCC18RM134824172000000CCCTGCTGATGCTGCTTCCAATGGGGGCGGATTAGGAGCGTTTGTAGG19RM21426,857,374-GTTCATTCGTGCTCCTGCGGTGTTCTGCAGATAGCCATGGGGGATTCTTGG18RM14824220,018,690-CCCTCCTCCTGCGGTATCTTGCAGATAGCCATGGGGGATTCTTGC18RM1482412000000CCCTGCTGTGTCTCCGGATCTTGCAGATAGCCATGGGGATTCTGCG18RM1482 <td< td=""><td></td><td>D) (100 (1</td><td></td><td>32981624</td><td></td><td></td></td<> | | D) (100 (1 | | 32981624 | | |
| 6155081 $15272,515$ $272,662ACTAGTACACCTCAACTTCACTCCCCTTTAGTTGCTTGTGACC6RM11307123,929,82023,929,727AAAGCTCTGCAATCTTCTCCCGAATACGACATCAGAACAGTGC23,929,7277RM24317,970,72225160202-35160202-35160202-4074011-AGACTGCAACCACCACCACCATTCTCTGCAAGCGAAAGCTGCAACGATGAGTTCTCGTCGATTGGCCATGAGTTCTCG35160202-193741039RM1347219374071-19374071-TCCTTCTCTTGAACAGCGACAGCAGAGGCGAGAGGCATGGAGTGC1937410310RM3515224,016,552-24,016,748GGAAAGAAGATATGCCATGCAGAGAGAAACACCAAC24,016,74811RM16097333589300CGCCTCGTAAGGTTGAGAATCGTGCCCTGTTCTTTCCATCTGC24,016,74812RM15630226,074,25612,407,510CTGTGTCGAAAGGCTGCACACCCACCTCCTCAGCGTGTACG12,407,51014RM222376,720,300-12,407,510CCGGTATCCTTCGATATTGCTCAGATCTAGCCGAAGAAATCAAGG12,407,51016RM26146,574,396-6,574,518CTACTTCTCCCCTTGTGTCGTCAGATCTAGCCGAAAATCAAGG12,27457018RM1342417200000CCCTGCTGATGCAACTACGGGCGGATTAGGAGGCGTTTGTAGG22,212,314-19RM14342,212,736-2,212,314-CCCATCTTCCACTTCCATTCCGGATCTTGC20RM41342,212,736-2,212,736-CCAATCTTGCCGTGATCTTGG2,212,736-CCAATCTTGCCCACTTCCATTCC21RM1642744,355,105CCAATCTTGCCGGATCTTCCAGAAGACCCAGGGATTCTTGCG2,212,316-$ | 4 | RM 10361 | 1 | 6155048- | CCTTGATCGGAAGTAGCTCAACG | GCCCTCAGAGCAGTAAGGAGAGG |
| 5RM100181272,515- 272,562ACTAGTACACCTCAACTTCACTCCCCTTTAGTTTGCTTGTGACC6RM11307123,929,580- 23,929,727AAAGCTCTGCAATCTTCTCTCCGAATACGACATCAGAACAGTGC7RM24317,970,722- 7970,836CAGACTGCAGCACCACCACCACCATCTCTCTGCAAGCGAAAGCTGCAACGATGTTGTCC8RM14181235160202- 9974071- 127101AGTACCACCACCACCACCACCACTCTCTGCAAGCTCGATTGGCCATGAGTTCTCG9RM13347219374103TCCTTCTCTTGCAACGCGCAAGCAGAGGAGAACACCAAC10RM3515224,016,572- 33589350GGCAAAGAAGAATATGCCATGCAGAGGAGAATCAGAAACACCAAC11RM160973326,073,971- 3,3589253-CGCCTCGTAAGGTTGAGATCGTGCCCTGTTCTTCCATCTTGC12RM15630326,073,971- 2,407,510ACTCGAAAGGATGCGCCCAACCACCCACCTCCTCAGGCTGAAGG14RM232376,720,300- 76,721,100CCGGTATCCTTCGATATTGCCCGACTTTGCCGGAGAAATCAAGG 76,721,10015RM51736,165,992- 6,574,518CAGCTCCTTCCTATCCGTCCC 76,721,100TCAGATCTAGCCGAGAAAACACCAAGG 76,721,10015RM1482417200000CCCTGCTGATGCAACTACGG 25274574GATCGTTGCTGCGCTTTCAATGAGG GCCATGCTGCTGCTCCCTGGGCACTTGCGGGCACTTGCGGGCACTTGCGGGCACTTGCGGG 225274574AGATAGCCATGGGGCACTTGCGGGACCTTGCG 22,12,81417RM113442,212,736- 2,212,814CCCATCTCTCCCGGATCTTGC 2,212,814AGAAGCTCGGGACTTCTGCG CCAATCTTGCTCCCACTTCCATTCC 2,212,81420RM41342,212,736- 2,212,814CCCCTCTCTGCTGCCTCC 2,212,8 | | | | 6155081 | | |
| 272,6626RM11307123,929,580AAAGCTCTGCAATCTTCTCTCCGAATACGACATCAGAACAGTGC7RM24317,970,722CAGACTGCAGTTGCACGATACTACGGAAAGCTGCAACGATGTTGTCC7 $7,970,722$ CAGACTGCACGATTCTCTGCAAGCGAAAGCTGCAACGATGTTGTCC7 $7,970,722$ CAGACTGCACCACCACCATTCTCTGCAAGCTCGATTGGCCATGAGTTCTCG8RM14181235160225TCCTTCTCTTGAACAGCGACAGCAGAGGCGAGAGGCATGGAGTGC9RM13347219374103TCCTTCTCTTGCAAGGTGCAGCAGCAGAGGCGAGAGGCATGGAGTGC10RM3515224,016,552GGAAAGAAGATATGCCATGCAGAGAGAAACACCAAC24,016,748224,016,748CGCCTCGTAAGGTTGAGATCGTGCCCTGTTCTTTCCATCTGC11RM16097333589253CGCCTCGTAAGGTGCACCACCCACCTCCTCACGCTGTACG12RM1563032,6073,971- 2,6074,256CTGTGTGCGAAAGGCTGCACCAGTCCTGTGTTGCAGCAAG13RM282312,407,510CCGGTATCCTCGGATATTGCCCGACTTTTCCTCTGACG14RM23237,6720,300-CCGGTATCCTCGGTGTCGTGTACCATCGCGAGAAATCAAGG15RM51736,165,992- 6,166,181CTACTTCTCCCCTTGTGTGCGTGTACCATCGCCAAAATCAGG16RM26146,574,396-CTACTTCTCCCCTTGTGTGCGTGTACCATCGCCAAAATCAGG18RM13482417200000CCCTGCTGATGCAACTAGGGGCGAGTTAGGAGGGTTTGTAGG20RM41342,212,736-CCAATCTTGCTCCGGATCTTGCAGAAGCCATGGGGATTCTTGCG21RM1642744,358,8 | 5 | RM10018 | 1 | 272,515- | ACTAGTACACCTCAACTTCACTCC | CCTTTAGTTTGCTTGTGACC |
| 6RMI1307123.929.580- 23.929.727AAAGCTCTGCAATCTTCTCTCCGAATACGACATCAGAACAGTGC7RM24317,970,722- 25.02CAGACTGCAGTTGCACGATACTACGGAAAGCTGCAACGATGTTGTCC8RM14181235160202- 35160202-AGTACCACCACCACCATTCTCTGCAAGCTCGATTGGCCATGAGGTTCTCG9RM13347219374010- 19374010-TCCTTCTCTCTGAACAGCGACAGCAGAGGGCGAGAGGCATGGAGTGC10RM3515224.016,552- 24.016,548GGAAAGAAGATATGCCATGCAGAGAGAAACACACAAC11RM1609733589253-CGCCTCGTAAGGTTGAGATCGTGCCCTGTTCTTTCCATCTTGC12RM1563026.073.971- 2.0674.256AAACTGGAAAGGATCCGCCCAACCACCCACCTCCTCACGCTGTACG13RM282312.407.382- 2.67721.100CTGTGTCGAAAGGCTGCACCAGTCCTGTGTTGCAGCAAG14RM232376.720.300- 6.166.181CCGGTATCCTTCCATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG16RM26146.574.396- 6.574.518CTACTTCTCCCTTGTGTCGTGTACCATCGCCAAATCCTCC 6.574.51817RM117201425274544- 25274544GATCGTTGCTGCTTTCAATGAGGAGGTGTTTCACCTTGGAGCCCATGC 25274547018RM1342417200000CCCTGCTGATGCTGCTGCA 2.212.814AGATAGCCATGGGCGATTCTTGG 2.212.814CCCAATCTTGTCTCCGGATCTTGC 2.212.81420RM41342.212.736- 2.212.814CCAATCTTGTCCCACTTCCATTTCC 2.212.814AGAAGCCATGGGCACTTCCCC 2.212.81421RM1642744.354.814- 4.355.105CTCTCCTCTCCATTCCATTTCC 2.212.814< | | | | 272,662 | | |
| 23,929,7277RM24317,970,722CAGACTGCAGTTGCACGATACTACGGAAAGCTGCAACGATGTTGTCC7,970,8367,970,836TCGATTGGCCATGAGTTGTCCTCGATTGGCCATGAGTTCTCG8RM14181235160225GGAAAGAAGACACCACCACCATTCTCTGCAAGCAGAGGGCGAGAGGCATGGAGTGCC9RM1347219374071-TCCTTCTCTCTGCAAGGCACGCAGAGGGCGAAGGAAGGCATGGAGTGC10RM3515224,016,552-GGAAAGAAGATATGCCATGCAGAGGAGAAACACCAAC24,016,74824,016,748ACTCGAAGGATCTGGCCCAACCAGAGGAGAATCAGAAACACCAAC11RM16097333589233-CGCCTCGTAAGGTTGAGATCGTGCCCTGTTCTTTCCATCTTGC12RM15630326,073,971-AACTCGAAGGATCTCGCCCAACCACCCACCTCCTCACGCTGTACG14RM232376,720,300-CCGGTATCCTTCGATATTGCCCGACTTTTCCTCTGACG15RM51736,155,992-CAGCTCCTTCCTATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG16RM26146,574,396-CTACTTCTCCCCTTGTGTCGTGTACCATCGCCAAATCTCC18RM13482417200000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGAAGG19RM241426,857,637CCAATCTTGCTGCGTGATCTTGCAGATAGCCATGGGCGATCTTGCG20RM41342,212,736-CCAATCTTGTCTCCGATTCTGGAGATAGCCATGGGGGATCTTCACC212, RM14252,018,899-TCTTCCTCTCCACTTCCATTTCCAGAAGACCCATGGGGGATCTTCACC22RM14352,212,736-CCAATCTTGTCCCACTTCCATTTCCAGAAGACCCATGGGCGATCTTCCGCCATGG24RM34 | 6 | RM11307 | 1 | 23,929,580- | AAAGCTCTGCAATCTTCTCTCC | GAATACGACATCAGAACAGTGC |
| 7RM24317,970,722CAGACIGCAGTIGCACGATACTACGGAAAGCTGCAACGATGTTGTCC7 $790,836$ 35160202AGTACCACCACCATCTCTCTGCAAGCTCGATTGGCCATGAGTTCTCG9RM1347219374071TCCTTCTCTCTGTGAACAGCGACAGCAGAGGGCGAGAGGGCATGGAGTGC10RM3515224,016,552GGAAAGAAGAAGATATGCCATGCAGAGAGAAACACCAAC24,016,74833589253CGCCTCGTAAGGTTGAGATCGTGCCTGTTCTTCTCACTTGC11RM16097333589253CGCCTCGTAAGGTTGAGATCGTGCCCTGTTCTTCCACGTGTACG12RM15630326,073,971AACTCGAAGGATCTCGCCCAACCACCCACCTCCTCACGCTGTACG26,074,256CTGTGTCGAAAGGCTGCACCCGGTCTTGTGTGCAGCAAG12,407,31014RM232376,720,300CCGGTATCCTTCGATATTGCCCGACTTTTCCTCTGGACG15RM51736,165,992CAGCTCCTTCCTATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG16RM26146,574,518GATCGTTGCTGCTTCCAATGAGGAGTGTTTCACCTTGGACCCATGC18RM13482417200000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG19RM21126,857,374GTCCATTGCTGCTGCTTCCAAGGAGGCAGATTTACAGGTTGCTAGG19RM134842,212,736CCAATCTTGTCTCCGGATCTTGCAGATAGCCATGGGCGATCTTGC20RM41342,212,736CCAATCTTGTCCTGCATTCCTGGATCCTTGGAGATAGCCATGGGGATCTTCACC21RM14252,598,899TCTTCCTCTCCCATTCCATTCCAAGAAGCTCGGGATCTTCACC22RM438652,5908,3660GGAGGTCGCACGTAGTAGAGGG | _ | | | 23,929,727 | | |
| 7.970,836 8 RMI4181 2 3516022- 35160225 AGTACCACCACCATTCTCTGCAAGC TCGATTGGCCATGAGTTCTCG AGAGGCGAGAGGCATGGAGTGC IP374103 9 RM13347 2 19374071- 19374103 TCCTTCTCCTTGAACAGCGACAGC IP374103 AGAGGCGAGAGGCATGGAGTGC AGAGAGAATCAGAAACACCAAC 10 RM3515 2 24,016,52- 24,016,748 GGAAAGAAGATATGCCATGC 24,016,748 AGAGAGAATCAGAAACACCAAC 11 RM16097 3 3589253- 3589300 CGCCTCGTAAGGTTGAGATCG 26,074,256 TGCCCTGTTCTTCCACGCTGTACG 26,074,256 12 RM15630 2 26,073,971- 24,07,510 AACTCGAAAGGCTGCAC ACCCACCTCTCACGCTGTACG 26,074,256 13 RM282 3 12,407,382- 76,721,100 CTGTGTCGAAAGGCTGCAC CCGACTTTTCCTCCTGAGCAAG 76,721,100 14 RM221 3 76,720,300- 6,166,181 CTGCTTCTTCCTATCCGTCTCC TCAGATCAGCCAAGGAAATCAAGG 6,574,518 16 RM170 4 25274544- 25274570 CAGCTCTTCTTCCAATGCGGG AGTGTTTCACCTTGGACCATGC 25274570 18 RM13482 4 17200000 CCCTGTCTGTGTGTGTGATCTTGGAC 26,857,637 CTCTCATTGTCTTCCGGATCTTGC 26,26,857,637 AGATAGCCATGGGCGATTCTTGG 22,212,814 <t< td=""><td>7</td><td>RM243</td><td>1</td><td>7,970,722-</td><td>CAGACTGCAGTTGCACGATACTACG</td><td>GAAAGCTGCAACGATGTTGTCC</td></t<> | 7 | RM243 | 1 | 7,970,722- | CAGACTGCAGTTGCACGATACTACG | GAAAGCTGCAACGATGTTGTCC |
| 8RMI4I81235160222 35160225AGTACCACCACCATTCTCTGCAAGC 35160225TCGATTGGCCATGAGTTCTCG 351602259RM13347219374071- 19374103TCCTTCTCTCTGAACAGCGACAGC 19374103AGAGGCGAGAGGCATGGAGTGC AGAGAGAATATGCCATGC10RM3515224,016,552- 24,016,748GGAAAGAAGATATGCCATGC AGGAGGAGACCCAACCAGAGAGAATCAGAAACACCAAC AGAGAGAATCAGAAACACCAAC11RM16097333589253- 26,074,256CGCCTCGTAAGGATCGGCCCAACC 26,074,256ACCCACCTCCTCACGCTGTACG ACCCACCTCCTCCACGCTGTACG 26,074,25612RM282312,407,382- 12,407,510CTGTGTCGAAAGGCTGCAC CCGGTATCCTTCGATATTGC CCGGCTTTCCATCCGTCCCCAGTCCTGTGTGCAGCAAG CCGACTTTTCCTCCTGACG CCGACTTTTCCTCCTGACG CCGACTTTCCTCCTGACG CCGACTTTCCCCCTGGAGCAAATCAAGG 6,166,18116RM26146,574,396- 6,574,518CTACTTCTCCCCTTGTGTCG CCCAGTCTGCGCTGCTCCCAACTGC CCGAGATTAGGAGCGTTTGTAGG CCCAATCTCCAGTAGCCATGGGCGAATCAGG CGGAATTACAGGTTGCTAGG GCGAATTACAGGTTTGCAAGG18RM134824172000000CCCTGCTGTGTGCTGCTTCCAATGAGG CCCAATCTTGTCTTCCGGATCTTGC 2,212,814AGATAGCCATGGGCGATTCTGG CCGAGATTACAGGTTGCTAGG CCGAGATTACCCTCTGCCGCGATCTTGC AGATAGCCATGGGCGATCTTGCC AGATAGCCATGGGCGATCTTGCC 2,212,81420RM4132,212,736- 2,212,736-CCAATCTTGTCTCCATTCCCATTCC CCAATCTTGCTCCCACTTCCATTCCC 2,0,519,086AGATAGCCATGGGCATCTTCCC AGATAGCCATGGGCATCTTCACC 20,519,08623RM3486525,908,305- 2,212,736-GGAGGTCGCCACGTAGTAGAGG CCAATCTTGCTCCCATTCCCGCCGAGTCTTCCCCCCCGCACTGC AGATAGCCATGGGCATCTTCCC24RM4 | _ | | _ | 7,970,836 | | |
| 35160225 9 RM13347 2 19374071- 19374103 TCCTTCTCCTTGAACAGCGACAGC AGAGGCGAGAGGCATGGAGTGC 10 RM3515 2 24,016,552- 24,016,748 GGAAAGAAGATATGCCATGC AGAGAGAAATCAGAAACACCAAC 11 RM16097 3 3589253- 26,074,256 CGCCTCGTAAGGTTGAGATCG TGCCCTGTTCTTCCATCTTGC ACCACCTCTCACGCTGACG 12 RM15630 3 26,073,971- 26,074,256 AACTCGAAAGGATCTCGCCCAACC ACCCACCTCTCACGCTGTACG ACCCACTCTGTGTGCAGCAAG 14 RM282 3 12,407,382- 12,407,510 CTGTGTCCTATCCGTCCTCC 76,721,100 CCGGTATCCTTCCAATTGC CCGACTTTTCCTCTGACG ACCACTCGCCAAAATCAAGG 15 RM517 3 6,165,992- 6,166,181 CAGCTCCTTCCTATCCGTCTCC 6,574,518 TGACATCGCCAAAATCCACGC 16 RM261 4 6,574,396- 722,74570 CTACTTCTCCCCTTGTGTGG TGTACCATCGCCAAATCTCC 6,2687,637 18 RM13482 4 172000000 CCCTGCTGATGCAACTACGG GCGGATTAGGAGCGTTTGTAGG 18 RM13482 4 26,857,374- 22,122,736- 22,122,814 GTCCTTCTCTCTCCATTCCGATCTGC AGATAGCCATGGGCGATTCTTGG 20 RM413 4 2,21 | 8 | RM14181 | 2 | 35160202- | AGTACCACCACCATTCTCTGCAAGC | TCGATTGGCCATGAGTTCTCG |
| 9 RMI3347 2 19374103 AGAGGCGAGAGGCATGGAGGCATGGAGTGC 10 RM3515 2 24,016,552- 24,016,748 GGAAAGAAGATATGCCATGC AGAGAGAAACACCAAC 11 RM16097 3 33589253- 3589300 CGCCTCGTAAGGTTGAGGTCAACC ACCCACCTCCTCACGCTGTACG 12 RM15630 3 26,073,971- 26,074,256 AACTCGAAAGGCTGCAC ACCCACCTCCTCACGCTGTACG 13 RM282 3 12,407,510 ACCCGGTATCCTTCGATATTGC CCGACTTTTCCTCTGACG 14 RM232 3 76,720,300- 76,721,100 CCGGTATCCTTCGATATTGC CCGACTTTTCCTCTGACG 15 RM517 3 6,156,192- 6,574,518 CAGCTCTTCTATCCGTCTCC TCAGATCTAGCCGAGAAAACACCAGG 16 RM261 4 6,574,396- 6,574,518 CTACTTCTCCCCTTGTGTGC TGTACCATCGCCAAAGCCCATGC 17 RM 17201 4 25274544- 25274570 GTCATTTCGTGATGCTACTGGG AGGTGTTTCACCTTGGACCATGC 18 RM13482 4 172000000 CCCTGCTGATGCAACTACGG GCGGATTAGGAGCGTTTGTAGG 19 RM413 4 26,857,637 GTCATTTCTGCGCTGATCTTGC AGATAGCCATGGGCGATTCTTGG 20 RM413 | 0 | D) (100 / F | | 35160225 | | |
| $ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$ | 9 | RM13347 | 2 | 193/40/1- | TCCTTCTCCTTGAACAGCGACAGC | AGAGGCGAGAGGCATGGAGTGC |
| $ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$ | 10 | D) (2515 | 2 | 193/4103 | | |
| 24,016,748 11 RM16097 3 33589253- 33589300 CGCCTCGTAAGGTTGAGATCG TGCCCTGTTCTTCCATCTTGC 12 RM15630 3 26,073,971- 26,074,256 AACTCGAAGGATCTCGCCCAACC ACCCACCTCCTCACGCTGTACG 13 RM282 3 12,407,382- 12,407,510 CTGTGTCGAAAGGCTGCAC CAGTCCTGTGTTGCAGCAAGG 14 RM232 3 76,720,300- 76,721,100 CCGGTATCCTTCGATATTGC CCGACTTTTCCTCCTGACG 15 RM517 3 6,165,992- 6,166,181 CAGCTCTTCTATCCGTCTCC TCAGATCTAGCCGAGAAATCAAGG 16 RM261 4 6,574,396- 6,574,518 CTACTTCTCCCCTTGTGTCG TGTACCATCGCCAAATCTCC 17 RM17201 4 25274544- 25274570 GATCGTTGCTGCTGCTTCCAATGAGG AGTGTTTCACCTTGGACCCATGC 18 RM13482 4 172000000 CCCTGCTGATGCAACTACGG GCGGATTAGGAGCGTTTGTAGG 19 RM241 4 26,857,637 GTCCATTTCTCCCACTTCCAATTCCG AGATAGCCATGGGCGATTCTTGG 20 RM413 4 2,212,736- 2,212,814 CTCCTCATGTCGCTGATTCTTGG AGATAGCCATGGGCGATTCTTGG 21 RM16427 4 4,354,814- 4,355,105 CTCTCTCTCCACTTCCATTTCC AA | 10 | RM3515 | 2 | 24,016,552- | GGAAAGAAGAIAIGCCAIGC | AGAGAGAATCAGAAACACCAAC |
| 11RM16097333589255CGCCTCGTAAGGTTGAGGATCGTGCCCTGTTTCTTCCACGCTGTTGC12RM15630326,073,971- 26,074,256AACTCGAAGGATCTCGCCCAACCACCCACCTCCTCACGCTGTACG 26,074,25613RM282312,407,382- 12,407,510CTGTGTCGAAAGGCTGCACCAGTCCTGTGTTGCAGCAAG14RM232376,720,300- 76,721,100CCGGGTATCCTTCGATATTGCCCGACTTTTCCTCCTGACG CGACTCTTCCTATCCGTCTCC15RM51736,165,992- 6,574,518CAGCTCCTTCCTATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG 6,166,18116RM26146,574,396- 6,574,518CTACTTCTCCCCTTGTGTCGTGTACCATCGCCAAATCTCC 6,574,51817RM 17201425274570CAGCTCGTGCTGCTTTCAATGAGG 25274570AGTGTTTCACCTTGGACCCATGC18RM134824172000000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG 26,887,63720RM41342,212,736- 2,212,814CCAATCTTGTCTTCCGGATCTTGC 2,212,814AGATAGCCATGGGCGATTCTTGG 2,212,81421RM1642744,354,814- 4,355,105CTCTCTCTCCACTTCCATTTCC 20,519,086AGAGGTCGGCACGTAGTAGAGG 25,908,06023RM3486525,908,305- 25,908,060GGAGGTCGGCACGTAGTAGAGG 25,908,660GCAATCTTGTCTCCCGGATCTTGC24RM41352,212,736- 2,212,736-CCAATCTTGTCTCCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 1.1 | DN (1 6007 | 2 | 24,016,748 | | |
| 12RM15630326,073,971- 26,074,256AACTCGAAGGATCTCGCCCAACCACCCACCTCCTCACGCTGTACG13RM282312,407,382- (12,407,510)CTGTGTCGAAAGGCTGCACCAGTCCTGTGTTGCAGCAAGG14RM232376,720,300- (76,721,100)CCGGTATCCTTCGATATTGCCCGACTTTTCCTCCTGACG15RM51736,165,992- (6,166,181)CAGCTCCTTCCTATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG (6,166,181)16RM26146,574,396- (5,74,518)CTACTTCTCCCCTTGTGTCGTGTACCATCGCCAAATCTCC (76,721,100)17RM 17201425274544- (25274570)GATCGTTGCTGCTTTCAATGAGGAGTGTTTCACCTTGGACCCATGC18RM134824172000000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG19RM241426,857,374- (20,857,637)GTTCATTTCGTGATCTCTGAGCGCAGATTACAGGTTGCTAGG (22,12,814)20RM41342,212,736- (20,519,086)CCAATCTTGTCGCTGATTCTTGGCCGAGATCTACCTCTGCTGTCC (25,908,060)23RM3486525,908,305- (25,908,060)GGAGGTCGGCACGTAGTAGGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTCCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 11 | RM16097 | 3 | 33589253- | CGCCICGIAAGGIIGAGAICG | IGCCCIGITCITICCATCITGC |
| 12RM13650320,073,971-AACTCGAAGGATCTCGCCCAACCACCCACCTCCTCACGCTGTACG13RM282312,407,510CTGTGTCGAAAGGCTGCACCAGTCCTGTGTTGCAGCAAG14RM232376,720,300- 76,721,100CCGGTATCCTTCGATATTGCCCGACTTTTCCTCCTGACG15RM51736,165,992- 6,166,181CAGCTCCTTCCTATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG 6,566,166,18116RM26146,574,396- 6,574,518CTACTTCTCCCCTTGTGTCGTGTACCATCGCCAAATCTCC 6,574,51817RM 17201425274544- 25274570GATCGTTGCTGCTTTCAATGAGGAGTGTTTCACCTTGGACCCATGC 2527457018RM134824172000000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG GCAGATTACAGGTTGCTAGG19RM241426,857,374- 20, 212,814GTTCATTTCGTGATCTTCGGATCTTGC 2,212,814AGATAGCCATGGGCGATTCTTGG20RM41342,2,12,736- 2,212,814CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC CGAGATCTACCTCTGCGGATCTTCC21RM1642744,354,814- 4,355,105CTCTTCCTCTCCACTTCCATTTCC 20,519,086AGAGGTCGGCACGTAGTAGAGG GTCGGTACTATTCCTGCCATCG23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGG 25,908,660GCGAATCTTCCGGATCTTGC24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 10 | DM15(20 | 2 | 33589300 | | |
| 13RM282312,407,382- 12,407,510CTGTGTCGAAAGGCTGCACCAGTCCTGTGTTGCAGCAAG14RM232376,720,300- 76,721,100CCGGTATCCTTCGATATTGCCCGACTTTTCCTCCTGACG15RM51736,165,992- 6,166,181CAGCTCCTTCCTATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG16RM26146,574,396- 6,574,518CTACTTCTCCCCTTGTGTCGTGTACCATCGCCAAATCTCC17RM 17201425274544- 25274544GATCGTTGCTGCTTTCAATGAGGAGTGTTTCACCTTGGACCCATGC18RM134824172000000CCCTGCTGATGCAACTACGGGCCGGATTAGGAGCGTTTGTAGG19RM241426,857,374- 2,212,736-GTTCATTTCGTGATCTTGGAGCGCAGATTTACAGGTTGCTAGG20RM41342,212,736- 2,212,814CTCCTCATGTCGCTGATTCTTGGAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCTCCTCTCCCACTTCCATTTCCAAGAAGCTCGGGATCTTGCC 4,355,10522RM142520,518,899- 2,5908,860TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC 20,519,08624RM41352,212,736- 2,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 12 | RM15630 | 3 | 26,073,971- | AACICGAAGGAICICGCCCAACC | ACCCACCICCICACGCIGIACG |
| 13 RM282 3 12,407,582 CONTRECOMAGONACIOCAC CAGRECTIGETIGETIGETIGETIGETIGETIGETIGETIGETIGE | 12 | DM202 | 2 | 20,074,230 | | |
| 14RM232376,720,300 76,721,100CCGGTATCCTTCGATATTGCCCGACTTTTCCTCCTGACG15RM51736,165,992- 6,166,181CAGCTCCTTCCTATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG G,166,18116RM26146,574,396- 6,574,518CTACTTCTCCCCTTGTGTCGTGTACCATCGCCAAATCTCC17RM 17201425274544- 25274570GATCGTTGCTGCTGCTTTCAATGAGGAGTGTTTCACCTTGGACCCATGC 2527457018RM13482417200000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG19RM241426,857,374- 26,857,637GTTCATTTCGTGATCTCTGAGC 26,857,637GCGAATTACAGGTTGCTAGG20RM41342,212,736- 21,21,814CTCCTCATGTCGCTGATTCTTGG 20,519,086AGATAGCCATGGGCGATCTTGC 20,519,086AGAAGCTCGGGATCTTCCCATTTCCCATTTCC 20,519,08623RM348652,212,736- 25,908,600CCAATCTTGTCTTCCGGATCTTGC 24AGATAGCCATGGGCGATTCTTGG 24, RM41352,212,736- 2,212,736-CCAATCTTGTCTTCCGGATCTTGC 20,212,736-AGATAGCCATGGGCGATTCTTGG | 15 | KW1282 | 3 | 12,407,582- | CIGIGICGAAAGGCIGCAC | CAUTCIDIOTIOCAUCAAU |
| 14RM232376,721,100CCGGTATCCTTCCTATCCGTCTCCCCGGACTTTCCTGACG15RM51736,165,992- 6,166,181CAGCTCCTTCCTATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG16RM26146,574,396- 6,574,518CTACTTCTCCCCTTGTGTGCGTGTACCATCGCCAAATCTCC17RM 17201425274544- 25274570GATCGTTGCTGCTGCTTTCAATGAGGAGTGTTTCACCTTGGACCCATGC18RM134824172000000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG19RM241426,857,374- 26,857,637GTTCATTTCGTGATCTCTGAGCGCAGATTTACAGGTTTGCTAGG20RM41342,212,736- 2,212,814CCCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATGCAACTACGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 14 | DM222 | 2 | 76 720 200 | CCCCTATCCTTCCATATTCC | CCGACTTTTCCTCCTCACC |
| 15RM51736,165,992- 6,166,181CAGCTCCTTCCTATCCGTCTCCTCAGATCTAGCCGAGAAATCAAGG16RM26146,574,396- 6,574,518CTACTTCTCCCCTTGTGTCGTGTACCATCGCCAAATCTCC17RM 17201425274544- 25274570GATCGTTGCTGCTTTCAATGAGGAGTGTTTCACCTTGGACCCATGC18RM13482417200000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG19RM241426,857,637GTTCATTTCGTGATCTCTGAGCGCAGATTTACAGGTTTGCTAGG20RM41342,212,736- 2,212,814CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 2,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 2,519,086GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 14 | KW1232 | 5 | 76,720,300- | COUTAICCITCOATAITOC | CEGACITTICETECTOACO |
| 16RM31736,165,18116RM26146,574,396- 6,574,518CTACTTCTCCCCTTGTGTCGTGTACCATCGCCAAATCTCC17RM 17201425274544- 25274570GATCGTTGCTGCTGCTTTCAATGAGGAGTGTTTCACCTTGGACCCATGC18RM13482417200000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG19RM241426,857,374- 26,857,637GTTCATTTCGTGATCTCTGAGCGCAGATTTACAGGTTTGCTAGG20RM41342,212,736- 2,212,814CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 15 | RM517 | 3 | 6 165 992 | CAGCTCCTTCCTATCCGTCTCC | ΤΓΔGΔΤΓΤΔGCCGΔGΔΔΔΤΓΔΔGG |
| 16RM26146,574,396- 6,574,518CTACTTCTCCCCTTGTGTCGTGTACCATCGCCAAATCTCC17RM 17201425274544- 25274570GATCGTTGCTGCTGCTTTCAATGAGGAGTGTTTCACCTTGGACCCATGC18RM134824172000000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG19RM241426,857,374- 26,857,637GTTCATTTCGTGATCTCTGAGCGCAGATTTACAGGTTTGCTAGG20RM41342,212,736- 2,212,814CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 15 | KWI517 | 5 | 6 166 181 | experienteenneedneed | Телбитетибесбиблицтенноб |
| 10RML0146,574,518 6,574,518CERTETTETEEEETTGTGTGCTGCTTGTGTGCGTETREETTEGEETTGTGCGCTGTGTTGTGTGC17RM 17201425274544- 25274570GATCGTTGCTGCTGCTGCTGATGAGGGAGTGTTTCACCTTGGACCCATGC18RM134824172000000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG19RM241426,857,374- 26,857,637GTTCATTTCGTGATCTCTGAGCGCAGATTTACAGGTTTGCTAGG20RM41342,212,736- 2,212,814CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 16 | RM261 | 4 | 6 574 396- | CTACTTCTCCCCTTGTGTCG | TGTACCATCGCCAAATCTCC |
| 17RM 17201425274544- 25274570GATCGTTGCTGCTGCTGCTTTCAATGAGGAGTGTTTCACCTTGGACCCATGC18RM134824172000000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG19RM241426,857,374- 26,857,637GTTCATTTCGTGATCTCTGAGCGCAGATTTACAGGTTTGCTAGG20RM41342,212,736- 2,212,814CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 10 | 1001201 | • | 6 574 518 | emeriereeeriororeo | romeenredeenmiteree |
| 11RM142011EMTCOTTOCTOCTOCTOCTOCTOCTOCTOCTOCTOCTOCTOC | 17 | RM 17201 | 4 | 25274544- | GATCGTTGCTGCTTTCAATGAGG | AGTGTTTCACCTTGGACCCATGC |
| 18RM134824172000000CCCTGCTGATGCAACTACGGGCGGATTAGGAGCGTTTGTAGG19RM241426,857,374- 26,857,637GTTCATTTCGTGATCTCTGAGCGCAGATTTACAGGTTTGCTAGG20RM41342,212,736- 2,212,814CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 17 | 1001 17201 | • | 25274570 | | noronneentooneeentoe |
| 19RM241426,857,374- 26,857,637GTTCATTTCGTGATCTCTGAGCGCAGATTTACAGGTTTGCTAGG20RM41342,212,736- 2,212,814CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 18 | RM13482 | 4 | 172000000 | CCCTGCTGATGCAACTACGG | GCGGATTAGGAGCGTTTGTAGG |
| 19RM241420,837,374- 26,857,637OTTCATTTCGTGATCTCTGAGCGCAGATTTACAGGTTTGCTAGG20RM41342,212,736- 2,212,814CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 10 | DM241 | 1 | 26 957 274 | CTTC & TTTCCTC & TCTCTC & CC | CCACATTTACACCTTCCTACC |
| 20,837,03720RM41342,212,736- 2,212,814CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 19 | KW1241 | 4 | 20,037,374- | GITCATTICOTOATCICTOAGC | GCAGATTIACAGOTTIGCIAGO |
| 20RM41342,212,736-CCAATCHTOTCTTCCGGATCTTGCAdataGCCATGGGCGATTCTTGG21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 20 | DM413 | 4 | 20,857,057 | CCAATCTTCTCTTCCCCATCTTCC | A G A T A GCC A TGGGCG A TTCTTGG |
| 21RM1642744,354,814- 4,355,105CTCCTCATGTCGCTGATTCTTGGCCGAGATCTACCTCTTGCTGTCC22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 20 | KWI 415 | 4 | 2,212,730- | CEARCHOICHICEOGRICHIGE | AGAIAGCCATGOGCGATTCTTGG |
| 21RM1042744,353,314CTECTERATOTEGETOATTETTOGCCOAGATETACCTETTOCTOTECCTETACT22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCC 20,519,086AAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGG 25,908,660GTCGGTACTATTCCTGCCATCG AGATAGCCATGGGCGATTCTTGG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 21 | PM16427 | 4 | 2,212,814 A 354 814 | CTCCTCATGTCGCTGATTCTTGG | CCGAGATCTACCTCTTCCTCTCC |
| 22RM142520,518,899- 20,519,086TCTTCCTCTCCACTTCCATTTCCAAGAAGCTCGGGATCTTCACC23RM3486525,908,305- 25,908,660GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 21 | KW110427 | 4 | 4,354,814- | electerioreocioarierioo | economiciaccientocionec |
| 22RM142520,510,057Terrecreterererererererererererererererer | 22 | RM142 | 5 | 20 518 899- | TCTTCCTCTCCACTTCCATTTCC | AAGAAGCTCGGGATCTTCACC |
| 23RM3486525,908,305-GGAGGTCGGCACGTAGTAGAGGGTCGGTACTATTCCTGCCATCG24RM41352,212,736-CCAATCTTGTCTTCCGGATCTTGCAGATAGCCATGGGCGATTCTTGG | 22 | 1001142 | 5 | 20,519,099 | Terreereneeneenteentriee | Mondereddonrerrenee |
| 25,908,660 24 RM413 5 2,212,736- CCAATCTTGTCTTCCGGATCTTGC AGATAGCCATGGGCGATTCTTGG | 23 | RM3486 | 5 | 25 908 305- | GGAGGTCGGCACGTAGTAGAGG | GTCGGTACTATTCCTGCCATCG |
| 24 RM413 5 2,212,736- CCAATCTTGTCTTCCGGATCTTGC AGATAGCCATGGGCGATTCTTGG | 23 | 10015100 | 5 | 25,908,660 | of the second se | Greedeniemiteerdeenied |
| | 24 | RM413 | 5 | 2 212 736- | CCAATCTTGTCTTCCGGATCTTGC | AGATAGCCATGGGCGATTCTTGG |
| 2.212.814 | - ' | | 5 | 2,212,814 | | |
| 25 RM164 5 19.196.472- TCTTGCCCGTCACTGCAGATATCC GCAGCCCTAATGCTACAATTCTTC | 25 | RM164 | 5 | 19,196.472- | TCTTGCCCGTCACTGCAGATATCC | GCAGCCCTAATGCTACAATTCTTC |
| 19.196.736 | | | - | 19,196.736 | | |
| 26 RM169 5 7,497,918- TGGCTGGCTCCGTGGGTAGCTG TCCCGTTGCCGTTCATCCCTCC | 26 | RM169 | 5 | 7,497,918- | TGGCTGGCTCCGTGGGTAGCTG | TCCCGTTGCCGTTCATCCCTCC |
| 7,498,084 | | | | 7,498,084 | | |

| S. | Markers | Chr | Position | Forward sequence | Reverse sequence |
|-----|----------|-----|---------------------------|---------------------------|---------------------------|
| No. | | No. | (bp) | * | * |
| 27 | RM19341 | 6 | 1764661- 1764696 | GCTACAAATAGCCACCCACACC | CAACACAAGCAGAGAAGTGAAGC |
| 28 | RM340 | 6 | 28,599,181-28,599,319 | GGTAAATGGACAATCCTATGGC | GACAAATATAAGGGCAGTGTGC |
| 29 | RM253 | 6 | 5,425,408- | TCCTTCAAGAGTGCAAAACC | GCATTGTCATGTCGAAGCC |
| 30 | RM111 | 6 | 5,096,744- | CACAACCTTTGAGCACCGGGTC | ACGCCTGCAGCTTGATCACCGG |
| 31 | RM276 | 6 | 6,230,045- | CTCAACGTTGACACCTCGTG | TCCTCCATCGAGCAGTATCA |
| 32 | RM190 | 6 | 6,230,185 1,764,586- | GCTACAAATAGCCACCCACACC | CAACACAAGCAGAGAAGTGAAGC |
| 33 | RM19620 | 6 | 6,212,764- | GCGACGAGGAAGAAGAATTAGTTCG | GCGGCACTTCGAGCAGTACG |
| 34 | RM3431 | 6 | 8,744,950- | AAGGGAACATTCTGGAAGACACG | ACACATTGCGTGTAGTGTGAAGC |
| 35 | RM402 | 6 | 8,745,144 6,399,680- | CATCTCTGCTAGGTGGTGAATGG | CTCAGCTGGCCTATGACAATGG |
| 36 | RM20844 | 7 | 653350- | GAGAGGGAAGGAGTTTCTTAGC | TAGTTTACACGTACCCATGTGC |
| 37 | RM11 | 7 | 19,256,914- | TCTCCTCTTCCCCCGATC | ATAGCGGGCGAGGCTTAG |
| 38 | RM22885 | 8 | 14355643- | ACTGGGTGTGATCCTTTCTGATGC | GTGATCCCAGATACACGATGTAGGG |
| 39 | RM22524 | 8 | 5110402 | GACTTGTGGTTGTTGCTTGTTGG | ACTGCCATATGCATTTCCCTAGC |
| 40 | RM3912 | 9 | 10,826,210- | CACTCAGATTTGGCCGATCC | GCTGATCCAGATCTACCTGACACC |
| 41 | RM296 | 9 | 46135020- | CACATGGCACCAACCTCC | GCCAAGTCATTCACTACTCTGG |
| 42 | RM257 | 9 | 46138300 | CAGTTCCGAGCAAGAGTACTC | GGATCGGACGTGGCATATG |
| 43 | RM24035 | 9 | 9,969,092- | GCTCCAGTTTCTAGTGGGCTTGC | ATGCGGCAGTCAATCAACAGG |
| 44 | RM24085 | 9 | 9,969,522 | CGACGAACTCCTCTACCGTTTACC | CTGCGTGTATCCAATCCCAAGG |
| 45 | RM5095 | 10 | 51,785- | CTATATGACTATGCGAATGG | ACAAATGCAACTAAGGTAGA |
| 46 | RM27962 | 11 | 12,079,770- | GGGAGTCGTGGATTCTGAGACG | ATCCCACGCCAGGAGATAATAAGG |
| 47 | RM 27289 | 11 | 26796502- | ATCGATCGATCTTCACGAGG | TGCTATAAAAGGCATTCGGG |
| 48 | RM206 | 11 | 22,014,679- | CCCATGCGTTTAACTATTCT | CGTTCCATCGATCCGTATGG |
| 49 | RM28607 | 12 | 24,766,329- | AGCATTACAGTGTCCAGGTAGGG | CCTCCCTTCTTATATGCCTTTCC |
| 50 | RM5313 | 12 | 27,139,375- | TCTTCTACTCCTCGTCTTCGTTCG | CATGCAGAGCAGAGACTTCTTGG |
| 51 | RM235 | 12 | 26,107,904- 26,108,004 | AGAAGCTAGGGCTAACGAAC | TCACCTGGTCAGCCTCTTTCT |
| 52 | RM27962 | 12 | 12,079,770- 12,079.904 | GGGAGTCGTGGATTCTGAGACG | ATCCCACGCCAGGAGATAATAAGG |

Chr = Chromosome

The screening of markers for parental polymorphism among the rice cultivars forms the basis for tagging of the desired resistance gene, fine mapping of the gene in rice chromosome and in subsequent marker assisted selection (MAS) programmes. The polymorphic rice markers can be used in fine mapping for iron and zinc rich micronutrient genes and to study the mapping population of crosses obtained from these parents (Welch and Graham, 2004). The present work gives the first approach in understanding the biofortification of micronutrient such as iron and zinc in cereals like rice. Because trace minerals are important not only for human nutrition but also for plant nutrition.



Plate 1: Representative gel picture of parental polymorphism survey with SSR markers



Plate 2: Representative gel picture of parental polymorphism survey with SSR markers L-Ladder (100 bp), P1 – Parent 1 (Swarna), P2 – Parent 2 (Type 3)

Plant breeding with MAS holds great promise for making a significant, low-cost and sustainable contribution to reducing deficiencies of micronutrients, particularly of minerals, in humans and may have important spin-off effects in increasing farm productivity in developing countries in a way that is environmentally beneficial.

Conclusion: The present study provides a strategy that holds great promise in significantly reducing the recurrent expenditures required for high-cost long-run programmes. The present biofortification strategy may significantly reduce the numbers of people requiring treatment, because staple foods are eaten in large quantities every day by the malnourished poor people. The delivery of enriched staple foods (fortified by the plants themselves during growth) can rely on existing consumer behaviours.

REFERENCES

- Bouis, H.E. 2000. Enrichment of food staples through plant breeding: A new strategy for fighting micronutrient malnutrition. *Nutrition*, **16**: 701-704.
- Cakmak, I. 2002. Plant nutrition research: Priorities to meet human needs for food in sustainable ways. *Plant and Soil*, **247**: 3-24.
- Chen, X., Temnykh, S., Xu, Y., Cho, Y.G and McCouch, S.R. 1997. Development of a microsatellite framework map providing genome wide coverage in rice. *Theoretical and Applied Genetics*, **95**: 553-567.
- Cho, Y.G., Ishii, T., Temnykh, S. and Chen, X. 2000. Diversity of microsatellites derived from genomic libraries and. GenBank sequences in rice. *Theoretical and Applied Genetics*, **100**: 713-722.
- Copenhagen Consensus. 2008. Press Release, Copenhagen Consensus Centre, Copenhagen Business.
- Gangaprasad, C., Ranjitkumar, N., Surapaneni, M., Deborah, D.A., Vipparla, A., Anuradha, G. and Vemireddy, L.R. 2013. Molecular genetic diversity of major Indian rice cultivars over decadal periods. *PLoS ONE*, 8: 66-197.
- Kiranmayi, S.L., Manorama, K., Venkata, V.G.N.T., Radhika, K., Cheralu, C., Roja, V. and Sarla, N. 2014. Identification of markers associated with iron and zinc concentration in recombinant inbred lines of brown rice. *Indian Journal of Genetics and Plant Breeding*, 74: 423-429.
- Phattarakul, N., Rerkasem, B., Li, L.J., Wu, L.H., Zou, C.Q., Ram, H., Sohu, V.S., Kang, B.S., Surek, H., Kalayci, M. and Yazici, A. 2012. Biofortification of rice grain with zinc through zinc fertilization in different countries. *Plant and Soil*, **361**: 131-141.
- Rahman, S.N., Islam, M.S., Alam, M.S. and Nasiruddin, K.M. 2007. Genetic polymorphism in rice (*Oryza sativa* L.) through RAPD analysis. *Indian Journal of Biotechnology*, **6**: 224-229.
- Sambrook, J. 1989. Isolation of high-molecular-weight DNA from mammalian cells. *Molecular Cloning*, **44**: 9-14.
- Shankar, I. and Sarla, N. 2010. Microsatellite marker polymorphism in rice varieties rich in iron and zinc endosperm. *Asian Journal of Experimental Biological Science*, **1**: 751-757.
- Stein, A.J. 2010. Global impacts of human mineral malnutrition. Plant and Soil, 335: 133-154.
- Wang, Y., Xue, Y. and Li, J. 2005. Towards molecular breeding and improvement of rice in China. *Trends in Plant Science*, **10**: 610-614.
- Welch, R.M. and Graham, R.D. 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, **55**: 353-364.
- White, P.J. and Broadley, M.R. 2009. Biofortification of crops with seven mineral elements often lacking in human diets iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist*, **182**: 49-84.
- Xu, Y. and Crouch, J.H. 2008. Marker-assisted selection in plant breeding: From publications to practice. *Crop Science*, **48**: 391-407.