**Table S1. Information on SSR primers and their amplification results**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Primers** | **Sequences (5’→3’)** | **repeat motifs** | **fragment size /bp** | **annealing temperature /℃** |
| CUJRA123 | F-TTGGTCTCTTCTTTCCTCTATG | (AC)12 | 198–212 | 53 |
| R-TCGAACGTACAATAACGTACAG |
| CUJRA124 | F-CGTTGCCTGAACAAGTAAGAT | (GT)13 | 165–184 | 58 |
| R-GAAGGAGGCTAACTCCCTATG |
| CUJRA206 | F-GCCGAGAGAGGAAGAGAGACT | (AC)16 | 200–212 | 60 |
| R-CGACTACAGGGACCAATCAAC |
| CUJRB012 | F-ACTCATCAAGATCCCCGACTAC | (AG)15 | 96–118 | 56 |
| R-CCACATCGTCTGGGTTCAT |
| CUJRB103 | F-CATGCTATGGACTACCTCCTC | (TC)17 | 144–174 | 58 |
| R-AAGAGAGACGAACGAAGAGTG |
| CUJRB218 | F-CTAGCGTCGAAGAAGAAGATG | (GA)13 | 168–174 | 58 |
| R-TTGTTTCTCCTCTGTCATGTTT |
| CUJRB220 | F-AGCATGTATAGGCCAATGATG | (TC)25 | 147–196 | 60 |
| R-TCGTTCTATCTACAAGCACTCG |
| CUJRB305 | F-GCTGCTTTATTAGCCATGATC | (GA)19 | 133–179 | 56 |
| R-GGTTCAATGTGCAACAAGAG |
| CUJRB307 | F-CTGGGCTGAAGGAGAATC | (GA)23 | 146–181 | 60 |
| R-TTGGATGTCTGCTTTTTTAGAG |
| CUJRB317 | F-TGCCCACTAACCCTAACC | (TC)17 | 131–151 | 53 |
| R-GAGAAAAAGAATGGCTGTATTG |
| CUJRC310 | F-GCTGTTAGTGGAATCCCAACT | (TTG)7 | 162–172 | 60 |
| R-TAAACGTGATCGAAGTGAAATG |
| CUJRD204 | F-CAGCCAATCTTCTTCTGCTTC | (CTC)4 | 172–174 | 60 |
| R-GAGACCTACGACCACGATCAC |
| JH2753 | F-CAGTTTTGGCCAGCTGCAAT | (GCT)6 | 188–212 | 55 |
| R-TGTGCCCATGCTAAGACTGG |
| ZMZ11 | F-CCAGAACCAGGAGCCAGCAA | (CTG)7 | 162–175 | 55 |
| R-GACCATCGGCCCGAAAGTAA |
| JRE-46 | F-GCCTCTCCTCGTGCTCATTT | (GAA)18 | 190–220 | 60 |
| R-ACTCGCTACTTTTCAGGCCC |
| JSI-15 | F-ATGAGAGCCAGCCAACAGAC | (TC)6 | 180–200 | 60 |
| R-CGAGCGAGCAAGAGAGAGAG |
| JSI-63 | F-TCCGGACAACTCCTCATCCT | (GATCG)5 | 203–208 | 60 |
| R-CTCTCCGCCGAGTCATGTAC |
| JSI-71 | F-AGCTAGCTCTCAAACAACAAGC | (GCAGTA)8 | 135–155 | 60 |
| R-ACAAACATGGCAACCTTCGTG |
| JSI-73 | F-AGCTCAACGGTCAAGGAAGG | (TGCTCG)5 | 160–175 | 60 |
| R-GGAGAGAGAGAGCTCGGCTA |
| R-GGTTCTTCCGCAGTTGGTCT |
| JUG-13 | F-GAAGAGACTCCGTTGCCACA | (GGA)5 | 240–250 | 60 |
| R-ACTCCGTCGTTTCCCTGAAC |

**Table S2. Diversity of 20 SSR loci in all tested collections of iron walnut in Yunnan**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Loci** | ***N*** | ***H*o** | ***H*e** | ***I*** | ***PIC*** | ***N*m** | ***FIT*** | ***F*IS** | ***F*ST** |
| **CUJRA123** | 13 | 0.42  | 0.69  | 1.37  | 0.67  | 4.03  | 0.40  | 0.40 | 0.06 |
| **CUJRA124** | 21 | 0.27  | 0.43  | 1.04  | 0.49  | 4.90  | 0.37  | 0.38 | 0.05 |
| **CUJRA206** | 18 | 0.25  | 0.60  | 1.44  | 0.70  | 2.51  | 0.54  | 0.59 | 0.09 |
| **CUJRB012** | 19 | 0.50  | 0.56  | 1.28  | 0.64  | 3.63  | 0.08  | 0.11 | 0.06 |
| **CUJRB103a** | 23 | 0.40  | 0.58  | 1.33  | 0.61  | 5.12  | 0.33  | 0.32 | 0.05 |
| **CUJRB218** | 20 | 0.67  | 0.74  | 1.63  | 0.74  | 6.56 | 0.09  | 0.09 | 0.04 |
| **CUJRB220** | 25 | 0.63  | 0.78  | 1.78  | 0.78  | 8.58  | 0.19  | 0.19 | 0.03 |
| **CUJRB305** | 24 | 0.22  | 0.36  | 0.72  | 0.36  | 7.61  | 0.35  | 0.38 | 0.03 |
| **CUJRB307** | 27 | 0.62  | 0.78  | 1.86  | 0.79  | 8.13  | 0.21  | 0.20 | 0.03 |
| **CUJRB317** | 17 | 0.62  | 0.69  | 1.53  | 0.72  | 4.13  | 0.13  | 0.11 | 0.06 |
| **CUJRC310** | 8 | 0.35  | 0.55  | 0.95  | 0.55  | 2.42  | 0.37  | 0.36 | 0.09 |
| **CUJRD204** | 14 | 0.30  | 0.48  | 0.75  | 0.41  | 3.24  | 0.35  | 0.37 | 0.07 |
| **JH2753** | 13 | 0.49  | 0.56  | 0.94  | 0.51  | 8.15  | 0.11  | 0.13 | 0.03 |
| **ZMZ11** | 11 | 0.55  | 0.55  | 0.92  | 0.48  | 6.77  | -0.01  | -0.01 | 0.04 |
| **JUG13** | 17 | 0.18  | 0.26  | 0.55  | 0.22  | 6.66  | 0.30  | 0.29 | 0.04 |
| **JRE46** | 24 | 0.38  | 0.68  | 1.48  | 0.69  | 8.28  | 0.44  | 0.44 | 0.03 |
| **JSI15** | 14 | 0.58  | 0.53  | 0.99  | 0.51  | 11.34  | -0.11  | -0.09 | 0.02 |
| **JSI63** | 11 | 0.75  | 0.54  | 0.85  | 0.45  | 19.72  | -0.42  | -0.41 | 0.01 |
| **JSI71** | 25 | 0.71  | 0.78  | 1.80  | 0.79  | 5.95  | 0.08  | 0.09 | 0.04 |
| **JSI73** | 7 | 0.25  | 0.29  | 0.59  | 0.32  | 6.27  | 0.11  | 0.12 | 0.04 |
| **Average** | 17.55 | 0.46  | 0.57  | 1.19  | 0.57  | 6.70  | 0.19 | 0.20 | 0.05 |
| **Total** | 351 |  |  |  |  |  |  |  |  |

*N*, number of alleles per SSR locus; *H*o, obseved heterozygosity; *H*e, expected heterozygosity; *I*, Shannon’s information index; *PIC*, polymorphic information content; *N*m, gene flow; *F*IT: total population inbreeding coefficient; *F*IS, within-population inbreeding coefficient; *F*ST, among-population genetic differentiation coefficient

**Table S3. Analysis of molecular variance of 14 iron walnut populations**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source of variation** | **df** | **Sum of square** |  **Variance components** | **Percentage of variance (%)** | **P-value** |
| Among populations  | 13 | 984.61 | 0.79 | 5.15 | 0.001 |
| Within populations | 1108 | 16188.91 | 14.61 | 94.85 | 0.001 |
| Total | 1121 | 17173.52 | 15.41 | 100 |  |



**Fig. S1 Capillary electrophoresis spectral diagram of primer CUJRA123 amplified from samples W092 to W095**