

Supplementary Materials: Genetic Diversity and Association of EST-SSR and SCoT Markers with Rust Traits in Orchardgrass (*Dactylis glomerata* L.)

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Table S1. The primer information for EST-SSR primers in orchardgrass.

| Forward Primer Sequence | | Reverse Primer Sequence | |
|-------------------------|-----------------------|-------------------------|------------------------|
| H37-cn135F | AAGTGAGCACAACGACACGA | H38-cn135R | CGATCCAAAGAAGCAAAGATG |
| H39-cn137F | CTGCCTCGCGTGAAAGATA | H40-cn137R | CCTCCTCGATCTGGATGGT |
| H41-cn139F | TACCTGTGCGGCGATGAAT | H42-cn139R | CAGGAGCAGGAGAACGTGAA |
| H47-cn151F | CTAGGGTTTCCACCTCTCA | H48-cn151R | AATGTCCTTGGCGTTGCT |
| H49-cn153F | CGCAGCAAGTAGGGTTAGGA | H50-cn153R | CCTCGTGGTGGATCTGCAT |
| H53-cn161F | CACGAGTGCAGAGCTAGACG | H54-cn161R | ACAACAACCCGACTGCTACC |
| H55-cn174F | GGCTCGAGCTTAAACCCTA | H56-cn174R | CTCCATCCATTCTTGCCATCT |
| H59-cn1100F | CGTCGTCCTCTGCTGTGAG | H60-cn1100R | AGGTCGTCCATCTGCTGCT |
| H61-cn1101F | GCGGAGGAGAGAAAGCAAT | H62-cn1101R | AGGTCGTCCATCTGCTGCT |
| H63-cn1107F | CCACCAGCTTCCTTCTCTTG | H64-cn1107R | CCCTCAACAAATCGAACTCC |
| H67-cn1115F | CGAGAAGAAGGTGGTGTCTG | H68-cn1115R | AGGTCGTGGAAGGTCTTGG |
| H69--cn1119F | ATCGTCTCCTCCTCCTCCA | H70-cn1119R | ATGCCTCGGTGGACTGGTA |
| H71--cn1130F | AAATGTTGAGCAACGGGAGCT | H72-cn1130R | ACTTCATAGGGCGGAGGTCT |
| H75-cn1144F | AGAAGGCGGCTCAGAAGAAG | H76-cn1144R | GCTCCAACCTCAGAATCAACAA |
| H77-cn1147F | GGCTAGGGTTTCGACTCCTC | H78-cn1147R | AGATGGCGAACTCGACCTG |
| H79-cn1152F | ACAAAGGCTCACCGTGGAA | H80-cn1152R | GTCGGAGGCGATGAACTCT |
| H81-cn1156F | CTCCCTCACCTCAAGCAGT | H82-cn1156R | GACAGCAGCACACCGAGTT |
| H83-cn1158F | CTCATCCACCACCACCAC | H84-cn1158R | CCCTGAAGAAGTCGAACACG |

Table S2. The band information of EST-SSRs in orchardgrass.

| Primer Pairs | Total Number | Number of | Percentage of |
|--------------|--------------|-------------------|-----------------------|
| Bands | of Bands | Polymorphic Bands | Polymorphic Bands (%) |
| H037.038 | 12 | 11 | 91.67 |
| H039.040 | 10 | 10 | 100.00 |
| H041.042 | 8 | 7 | 87.50 |
| H047.048 | 8 | 7 | 87.50 |
| H049.050 | 6 | 5 | 83.33 |
| H053.054 | 7 | 7 | 100.00 |
| H055.056 | 6 | 6 | 100.00 |
| H059.060 | 9 | 8 | 88.89 |
| H061.062 | 7 | 6 | 85.71 |
| H063.064 | 11 | 11 | 100.00 |
| H067.068 | 11 | 9 | 81.81 |
| H069.070 | 14 | 12 | 85.71 |
| H071.072 | 9 | 9 | 88.89 |
| H075.076 | 8 | 7 | 87.50 |
| H077.078 | 10 | 10 | 100.00 |
| H079.080 | 10 | 9 | 90.00 |
| H081.082 | 11 | 9 | 81.81 |
| H083.084 | 7 | 5 | 71.43 |
| Total | 164 | 148 | 90.24 |

Table S3. The information about SCoT primers in orchardgrass.

| Primer Name | Sequences(5'-3') |
|-------------|--------------------|
| SCoT1 | CAACAATGGCTACCACCA |
| SCoT2 | CAACAATGGCTACCACCC |
| SCoT5 | CAACAATGGCTACCACGA |
| SCoT6 | CAACAATGGCTACCACGC |
| SCoT8 | CAACAATGGCTACCACGT |
| SCoT10 | CAACAATGGCTACCAGCC |
| SCoT14 | ACGACATGGCGACCACGC |
| SCoT16 | ACCATGGCTACCACCGAC |
| SCoT25 | ACCATGGCTACCACCGGG |
| SCoT26 | ACCATGGCTACCACCGTC |
| SCoT27 | ACCATGGCTACCACCGTG |
| SCoT34 | ACCATGGCTACCACCGCA |
| SCoT35 | CATGGCTACCACCGGCC |
| SCoT36 | GCAACAATGGCTACCACC |
| SCoT37 | CAATGGCTACCACTAGCC |
| SCoT38 | CAATGGCTACCACTAACG |
| SCoT39 | CAATGGCTACCACTAGCG |
| SCoT40 | CAATGGCTACCACTACAG |
| SCoT41 | CAATGGCTACCACTGACA |
| SCoT42 | CAATGGCTACCATTAGCG |
| SCoT44 | CAATGGCTACCATTAGCC |
| SCoT45 | ACAATGGCTACCACTGAC |

Table S4. The band information of SCoTs in orchardgrass.

| Primer Pairs Bands | Total Number of Bands | Number of Polymorphic Bands | Percentage of Polymorphic Bands (%) |
|-----------------------|--------------------------|--------------------------------|--|
| SCoT1 | 14 | 9 | 64.29 |
| SCoT2 | 17 | 17 | 100.00 |
| SCoT5 | 10 | 10 | 100.00 |
| SCoT6 | 10 | 7 | 70.00 |
| SCoT8 | 20 | 20 | 100.00 |
| SCoT10 | 19 | 17 | 89.47 |
| SCoT14 | 19 | 18 | 94.74 |
| SCoT16 | 10 | 10 | 100.00 |
| SCoT25 | 14 | 14 | 100.00 |
| SCoT26 | 12 | 9 | 75.00 |
| SCoT27 | 10 | 9 | 90.00 |
| SCoT34 | 13 | 13 | 100.00 |
| SCoT35 | 9 | 8 | 88.89 |
| SCoT36 | 8 | 8 | 100.00 |
| SCoT37 | 14 | 14 | 100.00 |
| SCoT38 | 13 | 13 | 100.00 |
| SCoT39 | 17 | 16 | 100.00 |
| SCoT40 | 15 | 15 | 100.00 |
| SCoT41 | 12 | 12 | 100.00 |
| SCoT42 | 20 | 20 | 100.00 |
| SCoT44 | 13 | 13 | 100.00 |
| Total | 289 | 272 | 94.12 |

Table S5. The detailed information about accession code, name, origin, and rust resistance score in orchardgrass.

| Accession Code | Accession Name | Origin | Ploidy | Rust Resistance Score in Different Year | |
|----------------|----------------|---|------------|---|------|
| | | | | 2011 | 2012 |
| 1 | 98-102 | United States | Tetraploid | 6 | 5 |
| 2 | 1473 | Australia | Tetraploid | 6 | 5 |
| 3 | 2122 | Animal Husbandry Research Institute, Hubei, China | Tetraploid | 6 | 6 |
| 4 | 947 | Japan | Tetraploid | 6 | 5 |
| 5 | 02-114 | Qujing, Yunnan, China | Tetraploid | 6 | 6 |
| 6 | 02-115 | Deqin, Yunnan, China | Tetraploid | 6 | 5 |
| 7 | 1436 | Jiangshu, China | Tetraploid | 6 | 5 |
| 8 | 1824 | Animal Husbandry Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China | Tetraploid | 6 | 6 |
| 9 | 1993 | Animal Husbandry Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China | Tetraploid | 6 | 5 |
| 10 | PI610830 | Spain | Tetraploid | 6 | 6 |
| 11 | PI610822 | Spain | Tetraploid | 6 | 6 |
| 12 | PI173693 | Turkey | Tetraploid | 6 | 5 |
| 13 | PI237590 | Israel | Tetraploid | 6 | 5 |
| 14 | PI578587 | Portugal | Tetraploid | 5 | 5 |
| 15 | PI325293 | Russian Federation | Tetraploid | 6 | 6 |
| 16 | PI312450 | Former Soviet Union | Tetraploid | 6 | 5 |
| 17 | PI418672 | Italy | Tetraploid | 5 | 5 |
| 18 | PI305497 | Poland | Tetraploid | 5 | 5 |
| 19 | PI384018 | Poland | Tetraploid | 6 | 6 |
| 20 | PI399466 | Finland | Tetraploid | 5 | 6 |
| 21 | PI237268 | Finland | Tetraploid | 5 | 5 |
| 22 | PI598424 | Bulgaria | Tetraploid | 5 | 6 |
| 23 | PI598423 | Ireland | Tetraploid | 6 | 5 |
| 24 | PI231613 | Iran | Tetraploid | 5 | 5 |
| 25 | PI380812 | Iran | Tetraploid | 5 | 5 |
| 26 | PI308794 | India | Tetraploid | 6 | 6 |
| 27 | PI269885 | Pakistan | Tetraploid | 6 | 5 |
| 28 | PI237586 | Tunisia | Tetraploid | 5 | 6 |
| 29 | PI578667 | Egypt | Tetraploid | 6 | 5 |
| 30 | PI231727 | Chile | Tetraploid | 5 | 5 |
| 31 | PI469234 | Australia | Tetraploid | 6 | 5 |
| 32 | 2410 | Xinjiang, China | Tetraploid | 1 | 1 |
| 33 | 79-118 | Netherlands | Tetraploid | 1 | 1 |
| 34 | 2681 | Canada | Tetraploid | 1 | 2 |
| 35 | Anba | Denmark | Tetraploid | 2 | 2 |
| 36 | 2106 | Xinjiang, China | Tetraploid | 1 | 2 |
| 37 | 02-105 | Dazhou, China | Tetraploid | 1 | 2 |
| 38 | 02-106 | Baoxing, China | Tetraploid | 1 | 2 |
| 39 | 90-130 | Maoxian, Sichuan, China | Tetraploid | 1 | 1 |
| 40 | 1819 | Animal Husbandry Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China | Tetraploid | 1 | 1 |
| 41 | Kaimo | Xinjiang, China | Tetraploid | 2 | 2 |
| 42 | PI231517 | Morocco | Tetraploid | 1 | 2 |
| 43 | PI265567 | France | Tetraploid | 1 | 2 |
| 44 | PI277836 | Turkey | Tetraploid | 1 | 2 |
| 45 | PI249732 | Greece | Tetraploid | 2 | 1 |
| 46 | PI231535 | Portugal | Tetraploid | 2 | 1 |
| 47 | PI632497 | Tunisia | Tetraploid | 1 | 2 |
| 48 | PI287804 | Spain | Tetraploid | 1 | 1 |
| 49 | PI302892 | Spain | Tetraploid | 1 | 2 |
| 50 | PI292587 | Israel | Tetraploid | 1 | 1 |
| 51 | PI235281 | France | Tetraploid | 2 | 1 |
| 52 | PI578591 | Portugal | Tetraploid | 1 | 1 |

Table S5. Cont.

| Accession Code | Accession Name | Origin | Ploidy | Rust Resistance Score in Different Year | |
|----------------|----------------|-----------------------|------------|---|------|
| | | | | 2011 | 2012 |
| 53 | PI111536 | Russian Federation | Tetraploid | 2 | 2 |
| 54 | PI345603 | Former Soviet Union | Tetraploid | 1 | 2 |
| 55 | PI311042 | Romania | Tetraploid | 1 | 1 |
| 56 | PI250960 | Serbia and Montenegro | Tetraploid | 2 | 2 |
| 57 | PI231469 | Greece | Tetraploid | 1 | 1 |
| 58 | PI288997 | Libya | Tetraploid | 2 | 2 |
| 59 | PI289003 | Hungary | Tetraploid | 2 | 2 |
| 60 | PI418667 | Belgium | Tetraploid | 2 | 2 |
| 61 | PI237175 | Netherlands | Tetraploid | 2 | 1 |
| 62 | PI196419 | Denmark | Tetraploid | 2 | 2 |
| 63 | PI634311 | Ireland | Tetraploid | 2 | 2 |
| 64 | PI273738 | Argentina | Tetraploid | 2 | 2 |
| 65 | PI250928 | Iran | Tetraploid | 1 | 2 |
| 66 | PI237585 | India | Tetraploid | 2 | 1 |
| 67 | PI419517 | Japan | Tetraploid | 2 | 1 |
| 68 | PI595173 | Xinjiang, China | Tetraploid | 2 | 1 |
| 69 | PI632580 | Afghanistan | Tetraploid | 2 | 2 |
| 70 | PI231480 | Algeria | Tetraploid | 2 | 2 |
| 71 | PI578557 | United states | Tetraploid | 2 | 1 |
| 72 | PI578661 | Colombia | Tetraploid | 2 | 1 |
| 73 | PI285099 | Australia | Tetraploid | 2 | 2 |
| 74 | PI578634 | Morocco | Tetraploid | 2 | 1 |
| 75 | PI578635 | Morocco | Tetraploid | 1 | 1 |